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INTERACTION OF PROSTAGLANDIN E_2 (PGE_2) WITH NORADRENALINE AND ITS ANTAGONISTS IN THE ISOLATED MESENTERIC ARTERY OF RAT

BY

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DEDICATION

This thesis is dedicated to Temitayo Omoteleola whom God has given as consolation for valuable research period lost during transition to marital status.

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CERTIFICATION PAGE

I certify that this work was carried out by Mr. A.S.O. Adeagbo in the Department of Pharmacology and Therapeutics, University of Ibadan, Nigeria.

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ABSTRACT

The effect of PGE_2, PGF_{2a} and PGI_2 on vasoconstriction induced by different mechanisms was studied in the isolated rat mesenteric artery as described by McGregor (1965). Vasoconstriction was induced by mechanisms involving different modes of calcium utilization viz: (i) Pharmacomechanical pathway by low doses of the adrenergic neurotransmitter, noradrenaline acting at α- receptor; (ii) electromechanical pathway by high potassium and (iii) agents which facilitate Ca^{2+} influx e.g. A23187.

The prostaglandins potentiated the vasoconstrictor effect of NA. Potentiation factors calculated from different doses of the prostaglandins showed the effects of the prostaglandins to be dose - dependent and PGE_2 to be significantly more potent $(P>0.005)$ than PGF_{2a} and PGI_2. The prostaglandins failed to potentiate high potassium - induced vasoconstriction. PGE_2 also failed to potentiate NA if the vasoconstrictor effects were evoked in Ca^{2+} - free Krebs solution; but the degree of potentiation increased with increase in the concentration of Ca^{2+} ions in the perfusion fluid. This result suggested strongly that the potentiation was associated with external calcium. Evidence is presented to show that potentiation was not prejunctional since cocaine, bretylium and reserpine pretreatment did not materially alter the effect of PGE_2. It was concluded that prostaglandins potentiated NA vasoconstriction by facilitating Ca^{2+} influx.

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The mechanism of this facilitation is discussed.

NA vasoconstriction was competitively antagonised by adrenoceptor antagonists-phentolamine, tolazoline, yohimbine and phenoxybenzamine (in low concentrations). The blockade caused by these antagonists was reversed by PGE_2. By comparing NA dose-ratios in the presence of antagonist with dose-ratios in the presence of antagonists plus different doses of PGE_2, I showed that the degree of reversal was related to the dose of PGE. For example, the NA dose - ratio for yohimbine (1.28×10^{-6}M) was reduced from 26.6 + 0.9 to 1.7 + 0.1 when PGE_2 (2.8×10^{-8}M) was included in the perfusion fluid with the antagonist. The reversal of antagonism was not due to a change in the binding characteristics of the α- adrenoceptor since pA_2 values for the antagonist were not significantly different ($P < 0.05$) when PGE was included with the antagonists. Evidence is presented which suggests that reversal of antagonism involved utilization of internally bound calcium since reversal of antagonism occured even after the omission of Ca^{2+} from the external medium. In this sense, the mechanism of reversal was different from that of potentiation. Furthermore, the degree of reversal (measured as reversal factor) was quantitatively greater than would be the case if reversal was simply a reflection of the enhanced responsiveness of the vascular muscle to NA.

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In contrast to the "competitive" α- adrenoceptor antagonists, PGE_2 did not reverse the block of NA vasoconstriction caused by phenoxybenzamine (high doses); verapamil, cinnarizine or prazosin. All these agents caused blockade of NA that was not competitive in nature. Since none of the competitive α- adrenoceptor antagonists prevent prostaglandin formation; the point is made that a prostaglandin can reverse NA blockade even if the blockade did not involve inhibition of prostaglandin synthesis.

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CHAPTER ONE

INTRODUCTION

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VASCULAR SMOOTH MUSCLE

The mechanical events responsible for the contraction of vascular smooth muscle are associated with its contractile proteins. These proteins not only develop the mechanical force responsible for the contraction but also act as the enzyme that catalyses the release of energy by which this force is developed. The proteins thus function both as the spark plug and the piston of the contractile machine (Bohr, 1973).

The contractile proteins of vascular smooth muscle are arranged in well organized thick and thin filaments (Burnstock, 1970; Devine & Somlyo, 1971; Somlyo, 1972). The thick filaments, presumably bundles of myosin molecules, average 15.5 nM in diameter and have lateral projections suggestive of cross-bridges extending towards adjacent thin filaments. The thin filaments, presumably fibrous actin, average 5-8nM in diameter appear to be attached to dense bodies that are usually connected to the cell membrane. The most easily interpretable studies of the functions of the contractile proteins are those performed in isolation with the determinants of the enzymatic and physical responses tightly controlled. There is a qualitative similarity between the actomyosin of vascular smooth muscle and the actomysin of skeletal muscle as evidenced by the observation that a hybrid actomysin can be prepared by combining myosin from one of these types of muscles with actin from the other; this

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hybrid provides a functionally active enzyme. Although, the adenosine triphosphatase (ATPase) activity of skeletal muscle actomyosin is many times faster than the activity of vascular smooth muscle actomyosin, the speed of activity of the hybrid proparation is determined by the source of the myosin. This observation correlates with the extensive studies by Barany (1967) showing that a direct parallel exists between the maximum velocity of shortening of a muscle and the ATPase activity of its actin-activated myosin. These observations bear the important implication that the shortening velocity of vascular smooth muscle has the actomyosin ATPase activity as its rate-limiting factor. This slow release of chemical energy is reflected in the slow physical changes in the actomyosin molecule observed in super-precipitation studies (Murphy, Bohr & Newman, 1969) or in studies of contraction velocity of glycerinated fibres (Filo, Bohr & Ruegg, 1965).

Excitation - contraction coupling

A lot of evidence supports the view that Ca^{2+} ions are involved in the coupling of the excitation of the cell membrane with activation of the contractile apparatus in striated, cardiac and smooth muscles (Shane & Wasserman, 1963; Daniel, 1964; Frank, 1964). In the vascular smooth muscle, the functional activity of the actomyosin is measured in terms of its (a) ATPase activity,

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(b) ability to superprecipitate, and (c) ability to contract glycerinated fibres. All these indices have the same low requirements for activator calcium. Half-maximal activity of any of these processes occur at an ionic calcium concentration of about 10^{-6}M (Sparrow, Maxwell, Ruegg & Bohr, 1970). The parallel between the Ca^{2+} requirement for the enzymatic activity and that for the physical change is directly dependent on the enzymatic activity which releases energy from ATP. This calcium concentration is also the concentration required for the activation of the native actomyosin of skeletal muscle.

However, the cellular Ca^{2+} metabolism seems to display marked differences in the various types of muscles. In skeletal muscle, only about 20% of the cellular Ca^{2+} content exchanges with the extracellular $^{45}Ca^{2+}$ at equilibrium (isolated rat diaphragm) (Lahrtz, Lullman & Reis, 1967) whereas, under identical experimental conditions, 50-70% of the Ca^{2+} of the guinea-pig heart muscle exchanges (Klaus, & Lullman, 1964; Hoditz & Lullman, 1964). On the other hand, all the cellular Ca^{2+} of the intestinal smooth muscle exchanges rather quickly with the extracellular Ca^{2+} under comparable circumstances. In skeletal muscle, due to larger size of the cells (surface/volume ratio about 1:15) and the higher speed of shortening, the contractile proteins have to be activated by an intracellular Ca^{2+} release mechanism. On the other hand,

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in smooth muscle, (surface/volume ratio almost 1:1) the actomyosin may be activated sufficiently fast by a simple penetration of Ca^{2+} through the cell membrane, or by a transfer of membrane bound Ca^{2+} towards the contractile proteins.

<u>Evidence for the involvement of Ca^{2+} in the E-C coupling.</u>

One line of evidence supporting the importance of Ca^{2+} in smooth muscle contractility is the need for the cation in the solution bathing the muscle for maintenance of contractility (Daniel, Sehder & Robinson, 1962). Immersion of smooth muscle in Ca^{2+} free solution can cause progressive loss of contractility. The lost contractility is rapidly regained when Ca^{2+} is added back to the environment. Robertson, (1960) observed that 10-12 minutes were required for a major reduction of responsiveness of the rabbit ileum, whereas, significant recovery occurred in 15 seconds after the return to Ca^{2+}. This suggested that Ca^{2+} ions are active at the membrane and that acetylcholine, the stimulant used, increases the permeability of the membrane to Ca^{2+} ions. Burks, Whitacre & Long (1967) observed a non-specific loss of vascular reactivity when isolated mesenteric arteries and small resistance vessels of the dog were perfused with Ca^{2+}-free krebs solution. The responses to noradrenaline, adrenaline, sympathetic nerve stimulation, angiotensin and 5-hydroxytryptamine (5-HT) were all depressed during Ca^{2+} - free perfusion but were

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rapidly restored after reversion to control krebs solution containing 2.5 mM Ca^{2+}. Perfusion of the arteries with a solution containing 1.25 mM Ca^{2+} did not result in reduced vascular reactivity. This could be evidence that external Ca^{2+} does not actually partake directly in the overall effect but replenishes the store from which Ca^{2+} is utilized for contraction.

Secondly, in many studies involving measurement of the Ca^{2+} ion fluxes that accompany smooth muscle contraction, it has consistently been observed that there is a greater entry of Ca^{2+} into the cell, while Ca^{2+} bound intracellularly or in the membrane is released. Several isotope studies have demonstrated that Ca^{2+} influx increases during contraction of smooth muscle (Brigg, 1962; Brigg & Melvin, 1961; Chujyo & Holland, 1962; Robertson, 1960; Sperelakis, 1962). The influx of Ca^{2+} ions during contraction is not dependent on membrane potentials. This is evident from the followings: (a) it is as prominent in the depolarized muscle as it is in intact muscle (Sperelakis, 1962); (b) the dependence of the magnitude of the response on the external Ca^{2+} concentration is equivalent in the normal and depolarized muscle (Durbin, & Jenkinson, 1961; Edman & Schild, 1962; Waugh, 1962), infact, the depolarized muscle is more responsive to elevations in Ca^{2+} concentration.

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Stimulating agents, in addition to increasing Ca^{2+} influx, are also capable of activating or releasing bound Ca^{2+} from its bound sites. The free Ca^{2+} ion thus released is capable of triggering the contractile process. This observation is supported by the finding that the response to stimulating agents persists for a period after the smooth muscle is transferred to a Ca^{2+} free medium and secondly that repeated stimulation in a Ca^{2+} free solution accelerates the decline of the response (Barr & Alpern, 1963; Edman & Schild, 1962; Okpako & Oladitan, 1979); each stimulus seems to add to the exhaustion of the Ca^{2+} complex. Portzehl, Caldwell & Ruegg (1964), using perfused crab muscle fibre demonstrated that an intracellular concentration of 1uM Ca^{2+} would induce contraction. By isotopic labelling of the fibre Ca^{2+} using intracellular perfusion, subsequent immersion of the fibre in high K^{+} saline produced a marked increase in $^{45}Ca^{2+}$ efflux (Caldwell, 1964) - which strongly supports the view that stimulants release Ca^{2+}. Schatzmann (1961) found that nearly half of the Ca^{2+} in the intestinal smooth muscle is present in an electrochemically inactive form. The amount of this cation in a bound form, and its changes from a bound to a free form constitute a most important aspect of contraction and relaxation of smooth muscle. Bound Ca^{2+} in the smooth muscle cell membrane acts to alter cell membrane permeability to Na^{+} and hence to

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influence cellular excitability (Bulbring & Kuriyama, 1963; Burnstock, Holman, & Prosser, 1963).

Forms of activator Ca^{2+}

The manner in which the excitatory action of different agents depends upon cellular Ca^{2+} has been found to differ in longitudinal smooth muscle from guinea-pig ileum (Weiss & Hurwitz, 1963); in rat ventral tail artery (Hinke, 1965); and in uterine smooth muscle (Van Breemen & Daniel, 1966). In studies of the effects of ethanol and Ca^{2+} ions on smooth muscle, Weiss & Hurwitz, (1963) postulated three different Ca^{2+} ion sites: (i) Ca^{2+} ions bound to sites involved in acetylcholine-induced increase in K^{+} efflux - accessible to attack by ethanol and also EDTA; (ii) Ca^{2+} ions bound to tissue sites which restrict the outward movement of K^{+} ions from the muscle - only partly accessible to inhibitory effects of ethanol and (iii) Ca^{2+} ions bound to sites which activate or enhance K^{+} - induced increase in K^{+} efflux. This Ca^{2+} site equilibrates slowly with Ca^{2+} in the external medium and it is insensitive to the inhibitory effect of ethanol. Hudgins & Weiss (1968) studied the manner in which noradrenaline, histamine and K^{+} depend upon Ca^{2+} ions to elicit contractile responses in rabbit aortic strips. They used Ringer containing 1.5mM Ca^{2+} (normal Ringer); Ringer with no added Ca^{2+} and Ringer with no Ca^{2+} but with 0.1mM EDTA. From the experiments the authors concluded that K^{+}, histamine and NA

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differed in the degree to which their action was dependent upon extracellular Ca^{2+}, Ca^{2+} in loose association with the cell membrane, and Ca^{2+} which is firmly bound (or sequestered). K^{+} ions seem to initiate contractile response by inducing the influx of extracellular Ca^{2+} (Briggs, 1962) and depolarization of the cell membrane (Su & Bevan, 1965). This view is supported by the fact that K^{+} induced permeability changes is rapidly lost by removal of extracellular Ca^{2+} (Weiss & Hurwitz, 1963; Hudgin & Weiss, [illegible]). Histamine utilizes extracellular Ca^{2+} since it affects permeability changes and produces depolarization (Su & Bevan, 1965) and in addition interacts with loosely bound Ca^{2+}. This is evident from the observation that responses of vascular smooth muscle perfused with Ca^{2+}- free solution although reduced was not abolished until additional fraction of Ca^{2+} bound to membrane was removed by EDTA. Responses to NA on the other hand were sustained, though reduced by removal of extracellular Ca^{2+} or inclusion of EDTA in the bathing medium suggesting that NA - induced contractions are at least partially dependent upon sequestered Ca^{2+} stores. Recent experimental evidence have however shown that alpha-adrenergic stimulation uses Ca^{2+} from both the intracellular and extra-cellular sources. According to Sitrin & Bohr, (1971); Steinsland, Furchgott &

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Kirpekar, (1973); and van Breemen, Farinas, Casteels, Gerba, Wuytack & Beth, (1973); the initial fast response following NA administration appears to be caused by Ca^{2+} of intracellular origin, and a maintained slow response appears to be caused by Ca^{2+} from the extra-cellular pool. Most stimulatory agents therefore, are in some manner dependent upon the presence of an adequate concentration of ionized intracellular Ca^{2+}. This ionized Ca^{2+} is derived from the tissue bound sites which is maintained by the extracellular Ca^{2+}.

Sites of cellular Ca^{2+}

There are three possible storage sites for cellular calcium-sarcoplasmic reticulum, mitochondria, and plasma membrane with its surface vesicles, In recent years three types of studies have furnished evidence about these possible sources of activator Ca^{2+}: (1) electron microscope studies, (2) cell fragment studies, and (3) studies employing Ca^{2+} - blocking agents. Devine, Somlyo and Somlyo (1972), have demonstrated, by electron microscopic studies, that the sarcoplasmic reticulum occupies an appreciable part of the vascular smooth muscle cell, ranging from over 5% of the volume in the aorta and the main pulmonary artery to approximately 2% in the portal, anterior mesenteric vein and the mesenteric artery. These authors made the interesting corollary observation that

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smooth muscles containing the larger amounts of sarcoplasmic reticulum maintain their contractions better in a Ca^{2+} - free environment than do muscles with relatively little intracellular sarcoplasmic reticulum. They showed that the extracellular markers, ferritin and lanthanum, do not enter the sarcoplasmic reticulum but that the sarcoplasmic reticulum does accumulate the bivalent cation marker, strontium. These authors also observed that the mitochondria of the vascular smooth muscle cell accumulate barium and strontium, and, therefore, are possible sequestration sites for the divalent cation calcium. Both the sarcoplasmic reticulum and the mitochondria make close contact with the plasma membrane and the surface vesicles. The latter are differentiated from the sarcoplasmic reticulum by the fact that they are open to the extracellular markers. The mitochondria and the microsomal fractions from the vascular smooth muscle have been shown to be capable of energy dependent sequestration of Ca^{2+} (Fitzpatrick, Landon, Debbas & Hurwitz, 1972; Baudouin, Meyer, Fermandjian & Morgat, 1972). Although, details of the control of activator Ca^{2+} is still obscure nevertheless, the studies of the uptake and the release of Ca^{2+} from cell fragments promises valuable insight, since, for instance, angiotensin in physiologically active concentrations accelerates the release of bound Ca^{2+} from the microsomal fraction (Baudouin et al, 1972),

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Agents which effectively decrease membrane permeability to Ca^{2+} have been valuable tools for studying the source of activator Ca^{2+}. At least four such agents have been extensively studied: cinnarizine (Godfraind & Kaba, 1969), SKF 525A (Kalsner, Nickerson & Boyd, 1970), verapamil (Haeusler, 1972; Peiper, Gri[illegible] & Wende, 1971; Golenhofen & Lammel, 1972); and lanthanum (van Breemen et al, 1972). Vascular smooth muscle incubated in a Ca^{2+} - free potassium sulphate depolarizing solution can be caused to contract by the addition of low calcium concentrations to the muscle bath. This response presumably occurs because the added Ca^{2+} passes through the membrane which has been made highly permeable by the potassium sulphate - induced depolarization. Various Ca^{2+} blocking agents eliminate or greatly reduce this contractile response. These agents are much less effective in eliminating the response produced by agonists such as adrenaline, noradrenaline and angiotensin, suggesting that the contractile responses initiated by these agonists are less dependent on the passage of extracellular Ca^{2+} through the plasma membrane than is the contractile response initiated by potassium sulphate - induced depolarization. Van Breemen et al (1972) demonstrated that lanthanum blocks the passage of Ca^{2+} across the cell membrane of vascular smooth muscle and that it replaces all Ca^{2+} bound to extracellular

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structures. By measuring the Ca^{2+} remaining in segments of rabbit aorta, these researchers estimated intracellular Ca^{2+} content. Using this technique, they observed that there were parallel rates of increase in tension and in intracellular Ca^{2+} content in response to activation by potassium sulphate - induced depolarization or by lithium substitution for sodium. However, NA (10^{-5}M) caused a maximum increase in tension with no increase in intracellular Ca^{2+} concentration, which again indicates that the activator Ca^{2+} involved in contraction in response to alpha-adrenergic stimulation must have its origin primarily in an intracellular pool. When this pool is discharged by NA, adrenaline or angiotensin after the muscle has been in a Ca^{2+} - free lanthanum containing medium for 30 minutes, a single contraction occurs, but the muscle will not respond subsequently to stimulation with any of the three agonists. Therefore, each of the three agents appears to use the same Ca^{2+} pool. However, in spite of the enormous biochemical, histological and pharmacological evidence about the three candidates for the site of the cellular calcium pool, their relative significance as sources of Ca^{2+} for the physiological response of adrenaline, angiotensin and NA remains unclear.

Electrical and non-electrical activation

In mammalian fast striated muscle fibres, the initiation of contraction by a chemical stimulus (acetylcholine) is mediated

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by the action potential which triggers the release of Ca^{2+} from the sarcoplasmic reticulum raising free myoplasmic Ca^{2+} to a critical level required for activation of the actomyosin system (Davies, 1963; Sandow, 1965). In smooth muscle systems, the mechanism by which various stimulatory agents initiate contractile responses appear to be diverse. Bozler (1946); Burnstock et al (1963), using intestinal smooth muscle for their studies have established a compelling case for a primary spike - mediated mechanism of excitation - contraction coupling, which differ only in minor details from that of striated muscle. Marshall (1962) demonstrated a similar behaviour for uterine smooth muscle. However, the case for the action potential being the sole E-C coupling process of visceral smooth muscle was weakened by the demonstration by Evans, Schild & Thesleff (1958) that drugs can produce contraction of depolarized smooth muscle.

The contraction of the vascular smooth muscle may be initiated by either electrical or non-electrical activation. The classical model used for study of electrical activation is the portal vein. Smooth muscle in this vessel has spontaneous action potentials which cause the delivery of activator Ca^{2+} into the myoplasm, and, hence, cause contraction of the muscle. However, this muscle and other vascular smooth muscles can be made to contract in response to NA and other agonists when the

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cell membrane has been completely depolarized by isotonic potassium sulphate (Somlyo & Somlyo, 1968). Under these conditions, the response to the constrictor agonist occur in the absence of a change in membrane potential, and therefore, is reasonably called non-electrical activation - also termed pharmacomechanical coupling (Somlyo & Somlyo, 1968). Conversely, physiological responses that involves depolarization of the cell membrane or brings about changes in membrane potential are defined as Electrical activation (or electromechanical coupling Somlyo & Somlyo, 1968). Microelectrode studies support the possibility that NA usually causes constriction of smooth muscle in large arteries without the occurrence of action potentials. Haeusler (1972) reported that in over 200 microelectrode penetrations of at least 10 seconds each in the smooth muscle cells of the rabbit pulmonary artery contracting in response to NA (10^{-6}M), he observed no action potentials. He did find that NA reduced membrane potential from a mean of 58.4 mV to 43.5mV. Mekata & Niu, (1972), using lower concentrations of adrenaline on the common carotid artery of the rabbit, observed contraction of this vascular smooth muscle without action potentials or a change in membrane potential. These studies agree with the earlier observations by Somlyo & Somlyo (1968) that the same pattern of inequality of maximal responses to adrenaline, angiotensin and

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vasopressin is obtained whether the smooth muscle is polarized or depolarized. This observation indicates that the inequality in response in the polarized state is not due to differences in the electrical phenomena of the membrane and that factors which determine the magnitude of non-electrical activation are important when the cell is polarized.

Electrical and non electrical modes of activation have been explained in terms of differential pathways of Ca^{2+} leakage into the contractile proteins. In their review article Rasmussen & Goodman, (1977) noted that the regulation of the free Ca^{2+} ion concentration in the cell cytosol is achieved by pump-leak systems at both the plasma membrane and at the inner mitochondrial membrane. It is the view of these authors that there is an inward leak of Ca^{2+} down its concentration gradient and an outward pump in the plasma membrane. At least, two separate channels by which Ca^{2+} leak into the cell has been described. The first is a relatively specific Ca^{2+} channel that is independent of the membrane potential and that may well be altered when certain hormones interact with their receptors but do not lead to membrane depolarization (pharmacomechanical pathway). The second is a potential -dependent Ca^{2+} permeability channel (electromechanical pathway). This process is also referred to as the "late Ca^{2+} channel". In addition to these two pathways, some

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Ca^{2+} may also enter the cell after membrane depolarization, via the sodium channel and is called "early Ca^{2+} channel" in nerve. The relative importance of these two (or three) different processes by which the entry of Ca^{2+} into the cell is regulated varies from one cell to another, and different ones are of major importance in the activation of particular cells by extracellular messangers. Evaluation of the relative importance of the channels can be obtained by the use of agents that block specific ion channels. The early Ca^{2+} entry via the Na^{+} channel can be blocked by tetrodotoxin and the late, potential-dependent entry of Ca^{2+} can be blocked by verapamil, D600 (a methoxy derivative of verapamil) or Mn^{2+}.

ANTAGONISTS OF NORADRENALINE - INDUCED E - C COUPLING

2 - substituted imidazolines

The 2 - substituted imidazolines have a wide range of pharmacological actions including adrenergic blocking, antihypertensive, sympathomimetic, antihistaminic, histamine-like, and cholinomimetic; slight changes in structure may make one or another of these properties dominant. At the present time, members of the series are marketed for each of the first four properties listed. The structural formulas of tolazoline (2-benzyl-2-imidazoline) and phentolamine are shown in fig. 1a and b respectively.

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FIGURE 1

Structural formulas of tolazoline (A); phentolamine (B) and phenoxybenzamine (C) as redrawn from the pharmacological basis of therapentics. (Eds) L.S. Goodman and A. Gilman pps 550 and 559.

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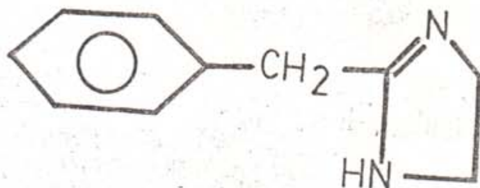
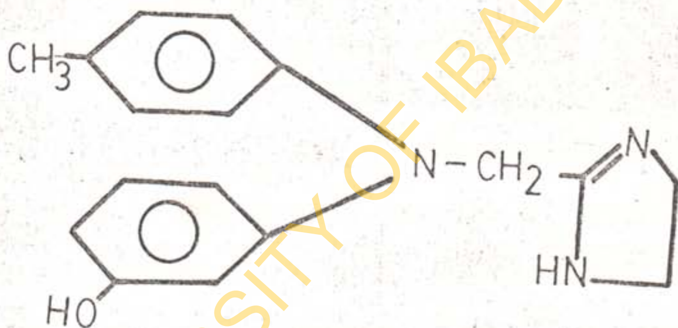
FIG 1

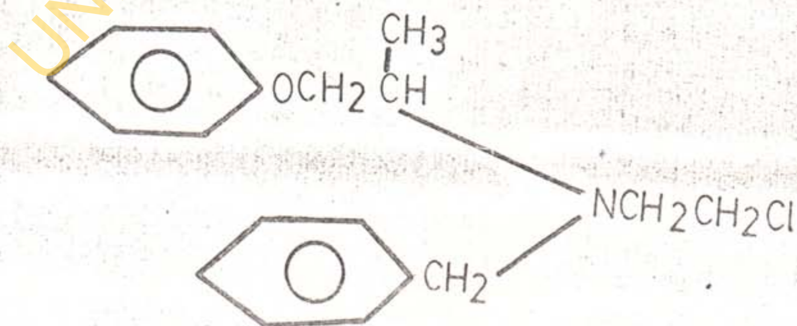
(a)



(b)



(c)



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Tolazoline and phentolamine produce a moderately effective competitive alpha-adrenergic blockade that is relatively transient. That is, the reaction of the antagonist with specific receptors usually exhibit mass-action characteristics, and the blockade produced is a measure of competition between the agonist and antagonist for receptor occupancy. Thus, both phentolamine and tolazoline can correctly be referred to as classical competitive antagonists (Nickerson, 1959). Apart from the receptor blocking effect, both phentolamine and tolazoline have important direct actions on cardiac and smooth muscle that may be divided into three classes: (1) "sympathomimetic" including cardiac stimulation, (2) parasympathomimetic, including gastrointestinal tract stimulation that is blocked by atropine; and (3) histamine-like, including stimulation of gastric secretion and peripheral vasodilatation (Ahlquist, Huggin & Woodbury, 1947; Nickerson, 1949). Both phentolamine and tolazoline were found to be ineffective against the metabolic effects of adrenaline.

Yohimbine

Yohimbine is an alkaloid obtained from a West African tree, yohimbehe. It is closely related chemically to the rauwolfia alkaloids. Despite the fact that yohimbine's adrenergic blocking action has been known since 1925 (Raymond-Hamet), it has had only limited use as a laboratory tool and has not been employed therapeutically as a blocking agent. However, following about

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a decade old postulate that the adrenergic neurotransmitter, NA, in the peripheral nervous system, regulates its own release through a negative feedback mechanism mediated by prejunctional α-adrenoceptors (Langer, Enero and Stefano, 1971; McCulloch, Rand and Story, 1972; Rand, Story, Allen, Glover and McCulloch, 1973), yohimbine has been recognised as a predominant presynaptic α-adrenoceptor antagonist. The evidence for this is based on selective antagonism of presynaptically acting α-agonists like clonidine, naphazoline and oxymetazoline by yohimbine (Drew, 1976, 1977). Post synaptically acting α-adrenoceptor agonists like phenylephrine and methoxamine were not antagonized by yohimbine or any other relatively selective presynaptic α-antagonist. Thus, yohimbine, will cause an overflow of neurotransmitter substance by shutting up the auto-regulatory pathway of release mediated by presynaptic α-adrenoceptors. Yohimbine is a classical competitive antagonist of the presynaptic α-adrenoceptor site. However, it is not devoid of post-synaptic antiadrenergic activity.

Phenoxybenzamine (PBZ)

Phenoxybenzamine is a member of the B-haloalkylamine blocking agents. It differs from dibenamine (an older member of the group) only in the replacement of one benzyl group by a phenoxyisopropyl moiety. Fig.1(c) represents its structural formula. The haloalkylamine adrenergic blocking agents are closely related chemically to the nitrogen mustards; like the latter, the tertiary amine

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cyclizes to form a reactive ethylenimonium intermediate. The molecular configuration directly responsible for blockade is probably a highly reactive carbonium ion formed when the three-membered ring breaks (Belleau & Triggle, 1962). The relatively slow onset of action, even after intravenous injection, is probably due to the time required for the formation of these reactive intermediates, which then act as alkylating agent (Harvey & Nickerson, 1954). It has been shown (Nickerson, 1956) that PBZ (its reactive intermediate) combines with the same receptors as the agonist, as in classical competitive antagonism, but then reacts with the receptor or some adjacent group(s) to form a relatively stable chemical bond. Such a reaction precludes further mass-action "competition" and effectively reduces the number of available receptors. PBZ has thus been described as a NONEQUILIBRIUM antagonist. This type of blockade has also been referred to as "irreversible competitive" (Furchgott, 1955) and "unsurmountable" (Gaddum, Hameed, Hathway & Stephens, 1955). The exact nature of the groupings on or near α-adrenergic receptors with which the haloalkylamine react has not been determined. It was anticipated that a labelled haloalkylamine could be used in combination with agonist protection of specific receptors to identify and characterize the α-adrenergic receptors. However, experiments of this type have been beset by numerous complications, and the only valid conclusion that has been drawn

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to date appears to be that these receptors are protein in nature (Lewis & Miller, 1966; Yong & Marks, 1969). This is in agreement with the experimental observation that trypsin can reverse the blockade of α-adrenergic receptors by dibenamine (Graham & Al Katib, 1966).

In addition to producing α-adrenergic blockade, PBZ is also thought to exert important effects on catecholamine metabolism. Dibenamine and PBZ increase the amount of NA released in the venous effluent per stimulus delivered to the splenic nerves at low but not at high frequencies. This view was first attributed to blockade of α-adrenoceptors and later to inhibition of NA inactivation. Prevention of access to inactivating enzymes was thought to be the most important component of this effect but prevention of NA uptake is also known to be involved (Brown & Gillespie, 1957; Kalsner & Nickerson, 1969). A more up-to-date explanation has been presented. This view states that the increase in NA overflow obtained in the presence of the α-blocker PBZ or phentolamine, represents an actual increase in transmitter release and is not due to blockade of sites of loss for the released transmitter. The reason for this is that, these effects are obtained with concentrations of the drug which do not inhibit neuronal or extraneuronal uptake (Starke, Montel & Schumann, 1971; Langer, 1974; Alder-Graschinsky & Langer, 1974). Further evidence in support of the view that α-receptor

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blocking agents actually increase transmitter release during nerve stimulation was obtained by Cubeddu, Barnes, Langer & Weiner (1974). These authors found an increase in the release of dopamine-B-hydroxylase when the sympathetic nerves of the perfused spleen were stimulated in the presence of the α-receptor blocking agent. Farah & Langer, (1974) have concluded that phentolamine and PBZ increase transmitter release by acting on the same prejunctional α-adrenoceptors.

Furthermore, PBZ and other haloalkylamines can inhibit responses to 5-HT, histamine, and acetylcholine. Blockade of these other types of agonist has the same general pharmacological characteristics as does the adrenergic blockade. Effective blockade of responses to acetylcholine usually require relatively high dose of haloalkylamines. Owing to this non-selectivity in the type of receptor antagonism by PBZ, the latter has also been thought to act at a point beyond the receptor level but which is common to all the agonists (probably a specific process between receptor activation and the contractile response). PBZ is thought to inhibit Ca^{2+} fluxes across cell membranes. This has been supported by the observation that PBZ also antogonized the contractile response induced by potassium chloride. Such contraction is exclusively said to be due to calcium influx from the extracellular sources (Bevan & Su, 1965; Hudgin & Weiss, 1968; Kalsner & Nickerson, 1969).

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Prazosin

Compounds having the quinazoline ring system have been reported to have diverse biological activities (Armarego, 1963). One of these compounds, prazosin, was found to possess significant antihypertensive effect which was believed to be due to direct relaxation of the arteriolar wall and to blockade of α-adrenoceptors in the peripheral vascular wall (Constantine, 1973).

As an α-antagonist, prazosin exhibits properties which are not typical of conventional α-blocking agents. Thus, it lowers blood pressure without causing tachycardia, does not cause renin release and reverses the sustained tachycardia induced by hydralazine therapy in hypertensive dogs (Constantine, McShane, Scriabane & Hess, 1973; Constantine, 1974; Massingham & Hayden, 1975).

Cinnarizine and Verapamil

Cinnarizine (1-benzhydrxl-4-cinnamyl piperazine dihydrochloride) (stugeron) is an antihistamine (H_1-receptor antagonist) and also has vasodilating properties. It antagonizes adrenaline, angiotensin and 5-HT. It is used clinically for the treatment of peripheral arterial disease and disorders of balance. Cinnarizine inhibits the contractile response to Ca^{2+} and induces relaxation of depolarized muscle previously contracted by Ca^{2+}. Its antagonism of adrenaline responses occurs only in polarizing solution and not in

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Ca^{2+}-free depolarizing solution. Based on these observations, the two most likely sites for cinnarizine action in arterial smooth muscle would seem to be the membrane of the muscle fibril (where the drug would reduce the availability of Ca^{2+} ions to the contractile mechinery of the depolarized muscle) or the contractile machinery itself (where it would interfere with the binding of Ca^{2+}). The latter mechanism is unlikely since cinnarizine did not affect adrenaline induced contractions in Ca^{2+}-free depolarizing solution (Godfraind & Kaba, 1969, 1972). Cinnarizine has been shown to be about four times more potent than chlorpromazine as Ca^{2+} antagonist and several times more potent than each of the other Ca^{2+} antagonists (procaine, papaverine and manganese ions). However, the potency of these agents as Ca^{2+} antagonists is usually determined by the extent to which they antagonise calcium chloride - induced contractions.

Verapamil (iproveratril) is another example of antagonist of excitation - contraction coupling. Its mode of action is similar to that of cinnarizine and has therefore been used in laboratories as a Ca^{2+} antagonist (Golenhofen & Lammel, 1972; Godfraind & Kaba, 1972). This agent has been shown to exhibit some selectivity in its mode of Ca^{2+} antagonism. According to Rasmussen & Goodman (1977) verapamil antagonises specifically the Ca^{2+} influx induced as a result of membrane depolarization (i.e. it blocks the electromechanical pathway).

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Indomethacin and sodium meclofenamate

These two compounds belong to the same group of drug generally classified as non steroidal anti-inflammatory drugs (NSAIDS). They have been most extensively studied and used to investigate the functional role of the prostaglandins in physiological and pathological systems. Both indomethacin and sodium meclofenamate have been shown to reduce or abolish responses to NA and angiotensin in the rat mesenteric vascular bed (Horrobin, Manku, Karmali, Nasser & Davies, 1974; Malik & McGiff, 1974; Mtabaji, Manku & Horrobin, 1976). The effects of these drugs could not be ascribed specifically to endogenous prostaglandin synthesis inhibition This idea is widely based on the non-specific nature of the antagonism. For instance, in the rat mesenteric artery preparation, indomethacin inhibited the pressor stimuli to adrenaline, calcium chloride and barium chloride (Northover, 1968, 1971, 1972); and to vasopressin and angiotensin (Horrobin et al, 1974; Manku & Horrobin, 1976). Similarly, contractile responses of the intestinal smooth muscle to prostaglandins are also inhibited by indomethacin (Sorrentino, Capasso, & Dirosa, 1972). It has thus been thought that indomethacin and other NSAIDS may be affecting a common process crucial to the manner in which all the agonists cause contraction (i.e. the excitation-contraction coupling process). Evidence in support of this view is as follows: (a) the observation by Northover, (1968) that indomethacin was equally effective in blocking adrenaline vasoconstriction in arteries perfused with normal salt, against

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vasoconstriction produced by Ca^{2+} or Ba^{2+} in the artery perfused with a high K^+ depolarizing solution. This will suggest that the antagonist (indomethacin) was not preventing Ca^{2+} entry; (b) the observation that indomethacin rapidly relaxed the spasm observed in an artery previously perfused for a long time with Ca^{2+} - depolarizing solution i.e. in a situation in which Ca^{2+} ion must have already gained entrance to the vicinity of the contractile proteins (Northover, 1968).

The contractile proteins of vascular smooth muscle are thought to respond to Ca^{2+} ions as a result of the activation of an adenosine triphosphatase enzyme (Somlyo & Somlyo, 1968; 1970) and since indomethacin did not reduce creatine phosphate and adenosine triphosphate (ATP) content of the muscle (Northover, 1971), it was concluded that the contraction inhibiting effect of indomethacin was due to some other disturbance of the excitation-contraction coupling. Two other suggestions advanced by Northover (1971) are that NSAIDS could either cause (a) failure of Ca^{2+} ions to reach the contractile proteins or (b) failure of the contractile proteins to respond to Ca^{2+} ions. Based on the observation that indomethacin did not inhibit contraction of glycerinated smooth (i.e. smooth muscle preserved for 1 week at 0^oC in a solution of glycerol) elicited by a mixture of ATP, Ca^{2+} ions and Mg^{2+} ions, it was concluded by exclusion, that indomethacin distrupts e - c coupling mainly by reducing the availability of Ca^{2+} ions within the muscle. Direct evidence in support of this conclusion was obtained by measuring the Ca^{2+} content of strips of smooth muscle subjected to electrical stimulation in vitro. The rise in Ca^{2+} content is reduced by treatment with indomethacin in concentrations similar to those

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required to inhibit inflammation (Northover, 1971, 1972). It has also been shown (Flower, 1974) that indomethacin in concentrations higher than 5 ug/ml can distrupt metabolic processes including inhibition of oxidative phosphorylation. Other effects of indomethacin at high concentrations include inhibition of proteolytic enzymes and interference with various biological membranes (Famaey, Fontaine & Reusse, 1977).

SUPERSENSITIVITY TO SYMPATHOMIMETIC AMINES:

In his review article of 1963, Trendelenburg outlined physiological and pharmacological procedures that can induce supersensitivity to sympathomimetic amines in the cat nictitating membrane as follows:-

(i) administration of cocaine
(ii) chronic pretreatment with reserpine
(iii) decentralization and
(iv) denervation.

Procedures (i) and (ii) are characteristically pharmacological in nature while (iii) and (iv) are physiological in nature.

The sensitizing effect of cocaine seems to be restricted to sympathomimetic amines. Acetylcholine is, for instance, not potentiated by cocaine. 5- hydroxytryptamine which has been shown to be potentiated by cocaine was also shown to stimulate the adrenal medulla (Lecomte, 1955) and the superior cervical ganglion (Trendelenburg, 1956) in addition to its direct stimulant action on the muscle of the nictitating membrane. Notwithstanding, 5 -HT is only potentiated by cocaine when resting tension rises - an observation compatible with the view that the endogenous noradrenaline exerts an additive effect. It was thus concluded by Trendelenburg (1963) that the sensitizing action

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of cocaine on the nictitating membrane is highly selective and seems to be especially pronounced for all sympathomimetic amines possessing a phenolic hydroxyl group in the meta-position. The basis for this conclusion was derived from the experiments of Trendelenburg et al (1962) where they compared the ED50 of sixteen different sympathomimetic amines before and after the administration of cocaine, in acutely reserpinized animals. Acute reserpinization eliminated the indirect component of the action of these amines.

The effect of pretreatment with reserpine depends on the schedule employed for the pretreatment. Short-term pretreatment (24 hours) causes depletion of the NA stores of the cat's nictitating membrane with no concomitant supersensitivity to NA (Fleming & Trendelenburg, 1961). However, when a small amount of reserpine (0.1 mg/kg) was injected daily for 3, 7 and 14 days, supersensitivity of the nictitating membrane to NA was first observed after 7 days and becomes more pronounced after 14 days. Fleming (1963) in his own studies extended the pretreatment period to 28 days and found no further increase in response to NA after 28 days. These observations indicate that pretreatment with reserpine is able to cause supersensitivity to NA but that time is essential for the development of this supersensitivity. The time factor varies from organ to organ. For example, supersensitivity of the cardiovascular system develops after 2 days of pretreatment with 2.5 - 5 mg reserpine

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per kilogram per day (Burn & Rand, 1958) and also within 3 days of long-term pretreatment whereas supersensitivity of the nictitating membrane developed only after 7 days (Fleming & Trendelenburg, 1961). Quite different results have been reported after acute injections of reserpine into spinal cats. A short time after such an injection, the response to NA was enhanced and was observed to remain so for a few hours (Innes, 1960; Nakamura & Shimamoto, 1960; Schmitt & Schmitt, 1955). The time course of the development of this potentiation was found to be similar to the time course of the elevation of the plasma level of catecholamines after intravenous injections of reserpine (Muscholl & Vogt, 1957). This observation was explained as being due to an addition of the effects of endogenous NA (increased in the proximity of the receptors because of the releasing action of reserpine) to the action of injected sympathomimetic amines - a view that was supported by the finding that tyramine is not only effective but actually more effective than normally, on the nictitating membrane during the first few hours after the intravenous injections of reserpine. Other explanations are (1) that the reserpine - induced release of NA saturated the non-specific receptor sites (as defined by Koelle, 1959) and that consequently, a larger proportion of the injected amines reaches their respective sites of action

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and (2) that the increased response to tyramine was due to the summation of the releasing action of reserpine and that of tyramine.

Decentralization, otherwise known as chronic preganglionic denervation, is well known to cause supersensitivity of the nictitating membrane to various substances. It causes a characteristic type of supersensitivity which is (a) of moderate degree, (b) not accompanied by a loss of NA stores of the nictitating membrane, (c) non-specific in that it is as prominent with acetylcholine as it is with NA, and (d) not associated with subsensitivity to any of the sympathomimetic amines. The non-specificity is striking, since supersensitivity after decentralization is of similar magnitude for directly and for indirectly acting amines, and it is of similar magnitude for m - OH compounds and their p - OH analogues (e.g. phenylephrine and synephrine). According to the conclusion of Trendelenburg & Weiner (1962), the most important factor responsible for the development of this type of supersensitivity seems to be a prolonged functional interruption of the pathway between the central nervous system and the nictitating membrane. Thus, procedures or pharmacological agents which produce this effect also produce this type of supersensitivity. This view agrees with the extensive studies of Emmelin (1961) who reached similar conclusions on the basis of experiments on the salivary

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glands of the cat. This type of supersensitivity is termed "pharmacological denervation" (Emmelin, 1961) or "pharmacological decentralization" (Trendelenburg 1963). Supersensitivity after decentralization develops slowly and reaches its maximum about two weeks after the section of the preganglionic nerve (Hampel, 1935); this characteristic time course is similar to that for the development of supersensitivity after long-term pretreatment with reserpine. Slow development of supersensitivity has also been observed by Emmelin and his group (1961) in their studies of the supersensitivity of the salivary glands of the cat. In order to obtain more information about this time factor, Trendelenburg & Weiner (1962) determined dose - response curves on the nictitating membrane of the spinal cat for three test substances (NA, tyramine and acetylcholine) in normal preparations as well as after various pretreatment schedules designed to interrupt the normal pathway between the higher centres and the nictitating membranes. These pretreatments were: (a) 7 days of denervation; (b) 7 days of decentralization; (c) 7 days of ganglion block (produced by two daily injections of chlorisondamine); (d) 7 days of prevention of the release of NA from its peripheral stores (produced by daily injections of TM 10); (e) 7 days of depletion of the NA stores (produced by daily injections of a small amount, 0.1 mg/kg, of reserpine); (f) 7 days of blockade of the NA receptors

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(produced by daily injections of phenoxybenzamine). After some of these prolonged pretreatments, the catecholamine content of the nictitating membrane was determined fluorimetrically. Short-term pretreatment with chlorisondamine, TM10, or reserpine (agents c, d and e above) had no effect on the sensitivity of the nictitating membrane to NA and acetylcholine, but long-term pretreatment with these drugs caused uniformly a moderate supersensitivity to both substances similar to that observed after decentralization. Denervation, on the other hand, caused the well-known pronounced supersensitivity to NA, but its sensitizing effect in regard to acetylcholine was not much stronger than that of decentralization. It was thus concluded that there is a correlation between the development of supersensitivity and the time factor. No correlation was found between the development of supersensitivity to NA and the NA content of the nictitating membrane; however, the response to tyramine clearly depends on the presence of these stores.

The similarity of the sensitizing effect on the nictitating membrane of chronic post ganglionic denervation (i.e. denervation) and of the administration of cocaine is very pronounced and has been observed by numerous workers. However, this supersensitivity develops rather slowly after denervation and reaches its maximum after about 2 weeks (Hampell, 1935), whereas

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the sensitizing effect of cocaine is observed within a few minutes after the intravenous injection of this substance (Trendelenburg, 1959). The close similarity is convincingly demonstrated on the nictitating membrane by a quantitative study of the influence of denervation (Fleckenstein & Burn, 1953) and of cocaine (Fleckenstein & Bass, 1953; Fleckenstein & Stockle, 1955) on the dose - response curves of a large number of sympathomimetic amines. Fleckenstein & Bass (1953), concluded from this study that the administration of cocaine causes "pharmacological denervation". Fleckenstein & Stockle (1955) on their own part found the correlation between the effects of cocaine and of denervation to extend to their desensitizing effects towards amines with indirect actions. Also, the sensitization of the nictitating membrane caused by denervation exhibits specificity in the sympathomimetic amines as was found for cocaine (Trendelenburg et al, 1962). However, as closely similar as the effects of cocaine and denervation appear to be, they have been classified as different forms of supersensitivity based on the substantial differences between the two types. In two successive publications, Trendelenburg (1962), and Trendelenburg et al (1962) came to the conclusion that chronic - denervation - induced type of supersensitivity is not only different from cocaine - induced type, but that it is actually

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equal to the sum of the sensitization caused by decentralization, plus the highly selective supersensitivity similar to that produced by cocaine, plus the effect of depletion of the NA stores. The evidence for this includes the fact that (i) administration of cocaine fails to cause supersensitivity of the nictitating membrane to acetylcholine whereas denervation does cause supersensitivity to this substance; (ii) denervation shifted the dose - response curves of all of thirteen directly - or indirectly - acting sympathomimetic amines more to the left than by cocaine. This effect was proved to be a qualitative one since cocaine interaction with indirectly - acting sympathomimetic amine e.g. tyramine or ephedrine is antagonistic and moreover since cocaine did not potentiate the direct component (obtained by pretreatment with reserpine) of the action of ephedrine. A plot (on log - scales) of the shifts of the dose - response curves of thirteen sympathomimetic amines as observed after denervation of the nictitating membrane (ordinates) against the shifts due to the administration of cocaine (abscissa) though gave regression co-efficient of nearly unity (1.095) but the regression line was displaced in such a way that at no point on the line were the values for cocaine and denervation the same. This finding was taken to be consistent with the view that supersensitivity caused by denervation also comprises a moderate and non-specific type of supersensitivity similar to that produced by decentralization.

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In his concluding remark, Trendelenburg (1963) identified two separate and distinct forms of supersensitivity in adrenergically innervated structures denervated post ganglionically. The terms presynaptic and post synaptic (Trendelenburg, 1966) or prejunctional and postjunctional (Fleming, McPhillips & Westfall, 1973) have been used to describe the two forms of supersensitivity. Prejunctional supersensitivity does not involve a change in the ability of the target cells to respond. Rather, it is the loss of some process, such as neuronal amine transport, which normally diverts specific drugs away from their site of action. Such supersensitivity is characterised by rapid onset and high specificity. Sensitization of the same characteristics as stated above was classified "type 1 sensitizations" by Kalsner (1974). In contrast, postjunctional supersensitivity is a true change in the sensitivity of the target cells. It usually comes on slowly (days to weeks, depending on the type of target cells) and is generally quite non-specific, the sensitivity being increased to a variety of unrelated drugs and ions. Kalsner (1974) described supersensitivity of this nature "type II sensitizations". The characteristics of this type II sensitizations are similar to those described for post junctional supersensitivity. Other terms used to denote postjunctional type of supersensitivity include non-

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specific supersensitivity (Fleming, 1963) and disuse supersensitivity (Sharpless, 1965). These terms have their set backs. For instance, supersensitivity cannot yet be correctly classified on the basis of specificity since no one is sure that all types of postjunctional supersensitivity have been tested for specificity. Also, the question of whether "disuse" (i.e end organ inactivity) or loss of trophic factor released by nerves is responsible for supersensitivity after interruption of the innervation has not been adequately explored in smooth muscles. Furthermore, under certain circumstances, extraneuronal uptake (including uptake into the effector cells themselves) and subsequent intracellular metabolism divert a portion of the administered dose of a drug from its site of action. Inhibition of these processes can produce supersensitivity (Kalsner & Nickerson, 1969). This phenomenon does not readily fit into the classification of pre- and post - junctional. This is because, its location is postjunctional (i.e. in the effector cells) but its mechanism (i.e. an increase in the percent of administered drug that reaches the receptors) is essentially the same as prejunctional supersensitivity. However, in a recent symposium on supersensitivity in smooth muscle, Fleming, (1975) described as premature any effort to choose the best terms by which types of supersensitivity may be classified.

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He classified smooth muscle supersensitivity into two as follows:-

I "Deviation" supersensitivity or sensitivity due to changes in uptake and/or metabolism of the drug i.e. functionally similar to prejunctional supersensitivity.

(A) Decreased adrenergic neuronal uptake of specific sympathomimetics (cocaine, desmethylimipramine, destruction of adrenergic neurones).

(B) Decreased cholinesterase activity (supersensitivity to cholinesterase sensitive choline esters, as caused by cholinesterase inhibitors or destruction of cholinergic neurones).

(C) Decreased extraneuronal uptake and metabolism.

II "Non-deviation" supersensitivity or enhanced responsiveness of the target cells. This term infers that there is no change in the portion of an administered dose of a drug that reaches the receptors. Rather, the responsiveness of the target cells is increased.

MECHANISMS OF SUPERSENSITIVITY

Cannon and Rosenblueth (1949) were the first to demonstrate genuine insight into the mechanism underlying the phenomenon

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of supersensitivity. These authors raised the following important points : (a) that there must be more than one mechanism underlying supersensitivity, (b) that one of these mechanisms probably involved changes in the removal, by metabolism or storage, or normal transmitters, and (c) that in view of the non-specific characteristics of some examples of supersensitivity, another mechanism may involve a change in the physiological characteristics of the responding cells, such as an altered permeability of the membrane to ions.

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Supersensitivity and metabolizing enzymes:

The potentiation of catecholamine effects by cocaine is accompanied by raised blood levels of the catecholamines, (Trendelenburg, 1959; Muscholl, 1961). This is an indication that cocaine probably inhibited the inactivation of catecholamines. Philpot (1940) provided an experimental evidence that cocaine inhibits the enzyme monoamine oxidase (MAO). This enzyme, found in almost all body tissues (Blaschko, 1952) has been shown in vitro to oxidise the side chain of many amines, for example adrenaline, NA, tyramine and 5 - HT. For some time, the enzyme was thought to be mainly responsible for inactivation of catecholamines in the body. It was suggested that the enzyme played a role at the sympathetic nerve endings analogous to that of acetylcholinesterase at cholinergic nerve endings (Burn, 1953). Burn & Robinson (1953) demonstrated a diminution

in the amount of amine oxidase after denervation and degeneration of the sympathetic nerve fibres. This observation was later confirmed by Stromblad (1965) in studies of the enzyme content of salivary glands. Burn & Hutcheon and Stromblad (1952) independently found a correlation between the fall in amine oxidase content and the development of supersensitivity and suggested that the supersensitivity was due to the fall in MAO content. Burn & Rand (1958, 1959) abandoned this suggestion on the grounds that reduction in enzyme level was not sufficient to change the response of the membrane so greatly as denervation does. In 20 out of 25 experiments of Burn & Hutcheon (1952) the amine oxidase in the denervated nictitating membrane was not reduced below 53% of the amount in the normal membrane. In addition, reduction in enzyme activity fails to explain the increased sensitivity of the denervated structure to agents other than the chemical mediator. Burn & Rand (1958, 1959) therefore concluded that the supersensitivity to NA and adrenaline which develops in sympathetically innervated tissues after degeneration of these nerves is attributed to the disappearance of stores of NA in these tissues. This conclusion was justified by the observation that there was a simultaneous decline of the NA stores of the iris and the spleen and increased sensitivity to NA after degeneration of the sympathetic nerves. Furthermore,

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administration of the potent amine oxidase inhibitors, iproniazid to animals failed to cause potentiation of adrenaline, NA, and 5 - HT (Griesemer *et al*, 1953; Foster, Ing & Veragic, 1955; Corne & Graham, 1957; Vane, 1959). This finding has been widely supported by other workers (Brown & Gillespie, 1957; Hertting & Axelrod, 1961; Kopin, 1964). Evidence is therefore lacking that MAO plays a major role in the inactivation of adrenaline and NA. Consequently, inhibition of this enzyme is unlikely to be the major cause of supersensitivity to these amines.

The discovery of derivatives of adrenaline and NA methylated in the hydroxyl position of the phenyl ring (Armstrong et al, 1957; Axelrod, 1957; Axelrod *et al*, 1959) led to a detailed study of the fate of the amines in man and of the methylating enzyme. The involvement of the enzyme, catechol-o-methyl-transferase (COMT), in the catabolism of injected NA led to the postulate that this enzyme terminates the active life of NA and that blockade of its activity could explain the phenomenon of supersensitivity. Muscholl (1960, 1961), Stromblad & Nickerson (1961) have independently demonstrated an appreciable accumulation of NA and adrenaline in the rats heart and salivary glands by using fluorimetric assay techniques. Lavollay (1941) showed that pyrogallol, quercitin and similar compounds, found later to be COMT inhibitors would potentiate slightly the biological

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effects of adrenaline and NA. Pyrogallol (Wylie, Archer & Arnold, 1960) and 4 - methyltropolone (Murnaglan & Mazurkiewicz, 1963) also prolonged the pressor effects of injected catecholamine in the cat only slightly. But, Lund (1951); Celander,(1954) showed that intravenously administered adrenaline and NA disappear very rapidly from the plasma and this rapid inactivation process was not markedly retarded by inhibition of either MAO or COMT or even by the simultaneous inhibition of the two enzymes (Crout, 1961). COMT inhibition as the sole mechanism of supersensitivity was further turned down on the following additional grounds : (a) that cocaine (an agent that can cause supersensitivity) fails to block catechol-o-methyl-transferase (Wylie et al, 1960) and (b) that cocaine produces supersensitivity to amines which are not substrates of COMT e.g. phenylephrine (Axelrod & Tomchick, 1958).

Supersensitivity and NA content:

Denervation is known to cause a considerable fall in the NA content of the denervated organ (Burn & Rand, 1959; Cooper, Gilbert, Bloodwell & Crout, 1961; Kirpekar, Cervioni & Furchgott, 1962; Stromblad, 1960). Fleckenstein & Bass (1953) observed that cocaine prevented the release of NA from nerve terminals following postganglionic stimulation. On the strength of this observation, these authors postulated that cocaine, by virtue of its local anaesthetic action can block the postganglionic

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fibres and thus cause "pharmacological denervation" since cocaine and denervation caused very similar types of supersensitivity. Macmillan, (1959) likewise postulated that cocaine interferes with the release of NA from the stores. However, explanation of the phenomenon of supersensitivity based on this concept is untenable since (a) there is evidence that cocaine does not impair the release of NA on nerve stimulation (Kirpekar & Cervioni, 1962; Trendelenburg, 1959); (b) compounds known to block the release of NA from nerve endings on nerve stimulation (such as TM 10 and bretylium) caused only a very slight supersensitivity to NA, which is quite different from that produced by either denervation or the administration of cocaine (Exley, 1957; Boura, Green, McCoubrey, Lawrence, Moulton & Rosenheim, 1959); and (c) other compounds which are at least as potent local anaesthetics as cocaine do not cause the typical cocaine - like supersensitivity.

When reserpine became available as a pharmacological tool for depleting the NA stores, experiments seemed to indicate that pretreatment with reserpine (like denervation and cocaine) always led to supersensitivity to NA, when depletion of the stores was achieved. Therefore, Burn & Rand, (1959) postulated that the sensitivity of an organ is inversely related to its NA content. However, this hypothesis is untenable since the following experimental observations cannot be explained on its basis:

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(a) decentralization, a process known not to deplete the stores (Kirpekar et al, 1962; Kohn, 1958) caused supersensitivity to NA; (b) short-term pretreatment with reserpine causes depletion of the NA stores without concomitant supersensitivity to NA (Crout et al, 1962; Fleming & Trendelenburg, 1961; Krayer, Alper, Paasonen, 1962; Trendelenburg, 1961; Krayer, Alper, Paasonen, 1962; Trendelenburg & Weiner, 1962); (c) prolonged ganglion block causes supersensitivity to NA without reducing the NA content of the tissues (Trendelenburg & Weiner, 1962); (d) prolonged treatment with TM 10 or bretylium causes supersensitivity to NA (Emmelin & Engstrom, 1961; Trendelenburg & Weiner, 1962) with very little change in the NA content of the tissue (Boura et al, 1959); (e) prolonged pretreatment with very small amount of reserpine causes supersensitivity to NA although the stores of NA are not completely depleted, as judged by the reduced (but by no means abolished) response of the nictitating membrane to nerve stimulation (Fleming & Trendelenburg, 1961). Apparently in favour of this hypothesis of an inverse relationship between the sensitivity of an organ and NA content is the observation that an infusion of NA into a reserpine pretreated preparation not only increases its response to tyramine but also reduces its sensitivity to NA (Bejrablaya, Burn & Walker, 1958; Burn & Rand, 1958). This pehnomenon has been interpreted as a "normalization" in so far as the infusion of NA refills the

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originally depleted stores and reduces to normal the originally increased sensitivity of the organ. However, repetitive - stimulation, of the post ganglionic fibres of previously decentralized nictitating membrane also caused a reduction of the supersensitivity of this organ to injected NA (Wolff & Cattell, 1937); although decentralization is known not to affect the NA content of this organ (Kirpekar et al 1962). Moreover, observations with the isolated guinea-pig atria likewise do not support this hypothesis. After short-term pretreatment with reserpine the NA content of the atria was only 1% of normal, and they failed to respond to tyramine, but their sensitivity to NA was normal; although exposure to NA then restored their response to tyramine to 70% of normal, their sensitivity to NA remained unchanged after the "refilling" of the stores (Crout et al, 1962).

Deviation of transmitter substance from its site of loss to the receptors

The site of loss of NA, may be taken to include uptake of the transmitter into the NA stores, temporary binding to proteins ("silent receptors"), enzymatic catabolism and diffusion from the receptor. By far the most popular hypothesis concerned with the phenomenon of supersensitivity are those which consider supersensitivity to be the result of a deviation of liberated or injected NA from its usual "site of loss" to the pharmacological receptor. This concept implies that the supersensitive effector organ is not

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changed at all but that the effective concentration of the injected agonist at the receptor is increased by the sensitizing agent or procedure. According to the uptake theory, the sensitivity of an effector organ is determined by the rate with which uptake into the *nerve terminals removes the NA from the neighbourhood of the receptors.* In the presence of normal uptake, the concentration of injected NA at the receptors remain low, under such conditions, the sensitivity to NA is low. Thus, blockade of the "site of loss" (uptake system for adrenergic transmitter) results in a large number of NA (or other catecholamine) molecules in the vicinity of adrenergic receptors to produce the exaggerated and prolonged pharmacological responses of the effector organ i.e. in supersensitivity. This mechanism of action was first proposed by Macmillan (1959). The supporting experimental evidence includes the demonstration that cocaine delayed the disappearance of injected NA from the circulation and that the drug inhibited uptake of NA into various peripheral tissues (Whitby, 1960). Dengler & Titus (1961) confirmed the ability of cocaine to inhibit NA uptake in tissue slices incubated with H^3-NA. The same effect of cocaine has been demonstrated by Lindmar & Muscholl (1962, 1964) in perfused rabbit and rat heart where the drug more than doubled the overflow of NA following sympathetic nerve stimulation. In addition, Muscholl (1961) found a quantitative correlation between the changes in uptake and in sensitivity.

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This author determined (i) the response of the blood pressure to a small dose of L-NA before and after the intravenous injection of 10-20 mg/kg of cocaine; (ii) the NA content of the heart after an i.v. infusion of 20 ug of NA and found an inverse linear correlation between the NA content of the heart and the sensitivity of the blood pressure to this amine. In other words, the more pronounced the impairment of the uptake of NA, the more pronounced was the increase in sensitivity of the blood pressure to NA - a correlation that is consistent with the uptake theory. Furthermore, administration of cocaine (or denervation) was observed to sensitive an effector organ much more to an amine that is taken up rapidly than to an amine that is taken up slowly. For example, the relative rates of uptake of the d - isomers of NA and adrenaline are : 1 - NA 1 - adrenaline d - isomers (Iversen, 1963; Maickel, Beaven, & Brodie, 1963) and cocaine was found to increase the sensitivity of the nictitating membrane of the spinal cat to these amines by factors of 23, 5 and 2.5 respectively (Trendelenburg, 1965). Also, the amine, isoprenaline is not uptaken by the heart of the rat (Hertting, 1964) and neither cocaine nor denervation caused supersensitivity to its effects on blood pressure (Maxwell, Daniel, Sheppard & Zimmerman, 1962); and nictitating membrane (Smith, 1963).

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Supersensitivity due to neuronal uptake blockade has been substantiated yet in another way. It was recently postulated that the release of NA by nerve stimulation is regulated through a negative feedblack control mechanism mediated by presynaptic a - adrenoceptors (Langer, Alder, Enero & Stefano, 1971; Starke, 1972; McCulloch, Rand & Story, 1972; Enero & Langer, 1973, 1974). Since cocaine increases the concentration of NA at the neuroeffector junction, it was suggested to enhance the presynaptic inhibition of the transmitter released by nerve stimulation (Langer, 1974; Dubocovich & Langer, 1974). Compatible with this view is the observation that cocaine 10^{-8} - 10^{-6}M increased moderately the overflow of NA while it reduces the overflow of dopamine - B - hydroxylase elicited by nerve stimulation in the perfused cat spleen (Cubeddu et al, 1974). The conclusion of Langer & Enero (1974) still supported and maintained the hypothesis that supersensitivity by cocaine is due predominantly to inhibition of neuronal uptake of the transmitter (NA).

As fascinating as the uptake blockade concept of explaining supersensitivity is, it is not devoid of setbacks. Firstly, the available evidence concerning the supersensitivity observed after decentralization does not fit into the concept. There is no known reason why chronic section of the preganglionic axon should impair the uptake mechanism in the postganglionic nerve

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terminal, since decentralisation does not lead to any loss of NA from the tissue (and, therefore, presumably does not damage the stores). The alternative hypothesis, that decentralization and the consequent absence of tonic impulses from the central nervous system leave the relevant compartment of the nerve terminal so full of transmitter substance that it cannot take up anymore, is inconsistent with the finding that short-term pretreatment with reserpine (which depletes the stores of the decentralized nictitating membrane (Kirpekar _et al_, 1962) does not abolish the phenomenon of decentralization supersensitivity (Trendelenburg _et al_ 1962). Secondly, although the uptake concept would account for the immediate sensitizing effect of cocaine, it completely fails to explain the phenomenon of slow development of supersensitivity after prolonged pretreatment with reserpine. Thirdly, neither denervation nor the administration of cocaine causes pronounced supersensitivity to the p- OH compounds (e.g. tyramine) although they induce supersensitivity to their m - OH (e.g metaraminol) analogues. It is hard to imagine the possibility that the p - OH analogues are not taken up into the neurones. Many of the p - OH analogues have a strong indirect action, thus the possibility of not being taken up would run counter to the available evidence that indirectly acting substances are taken up into the stores. Furthermore, in a study of the effect of cocaine on the magnitude

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of responses to several biologically active amines and on their rates of inactivation in strips of rabbit thoracic aorta in vitro, Kalsner & Nickerson (1969) presented results which were incompatible with neuronal uptake inhibition as the sole explanation for cocaine - induced supersensitivity. The authors made 2 important conclusions :(i) that supersensitivity and inhibition of amine inactivation reflect two largely independent actions of cocaine in vascular smooth muscle and probably other organs, and (ii) that supersensitivity is a generally unreliable criterion of the blockade of processes inactivating sympathomimetic amines. These conclusions were on the following grounds : (a) although cocaine both potentiated responses to NA, adrenaline and phenylephrine and slowed their inactivation, the correlation between these two parameters under experimental conditions was poor, and in all cases, the delay in intrinsic inactivation was found to be inadequate to account for the observed potentiation; (b) potentiation of responses to NA by cocaine was found to be little decreased in strips stored at 6°C for 10 days although responses to low doses of tyramine was abolished. Cocaine was also observed to potentiate responses to NA for at least 28 hrs at 37°C, at which time, responses to NA alone were markedly decreased; (c) cocaine was also observed to potentiate responses to phenylephrine after 60 min. as well as after 10 min. exposure to the amine in strips in which all intraneuronal disposition of this amine had been

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eliminated by treatment with reserpine and iproniazid; (d) cocaine which was never found to alter the tissue inactivation of histamine but that of 5 - HT, potentiated the former and had a slight variable effect on responses to 5 - HT and (e) procaine, observed to slow amine inactivation in the same way and to the same extent as did cocaine, did not potentiate responses or affect potentiation produced by cocaine added in its presence.

Mechanisms involving the post synaptic sites:

Maxwell, Plummer, Povalski, Schnider & Coombs (1959) observed that cocaine reversed the blocking action of surmountable antagonists of adrenaline and NA (phentolamine) but did not affect the blocking action of an unsurmountable and irreversible antagonist (Dibenamine). From these experimental observations, which were made on the blood pressure of the dog, it was concluded that the site of action of cocaine was postsynaptic, probably at the a - adrenoceptor. It was thought that cocaine might produce its action by a change in the configuration of the receptor i.e. "deform" the receptor. The above mentioned results do not prove such a postsynaptic site of action as potrayed. It is possible that cocaine, by delaying the inactivation of injected NA would increase the local concentration of the injected drug at the receptor. Then, one would expect what actually has been observed : a reduction of the surmountable (competitive) type of antagonism (phentolamine),

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and no change of the unsurmountable (irreversible) type of receptor block. Another explanation for postjunctional supersensitivity is that cocaine (or any other agent that can induce this types of supersensitivity) act to increase the number of receptors or to alter, by an allosteric conformational change, either the intrinsic activity of the receptor, the receptor - agonist affinity or binding (Carrier & Holland, 1965, Maxwell, et al, 1966; Nakatsu & Reiffenstein, 1968; Innes & Karr, 1971). This hypothesis has been questioned by Green & Fleming (1968) who reasoned that if cocaine altered the sensitivity of receptors, then a change in affinity of NA for the receptor should be detectable by determination of the pAx values for agonist - antagonist interaction. No such changes in the affinity of adrenoceptors for phentolamine were observed in the supersensitive cat nictitating membrane (Green & Fleming, 1967); cat spleen (Green & Fleming, 1968; Innes & Mailhot, 1973) or aortic strips (Taylor & Green , 1971).

Post junctional supersensitivity has further been explained on electrophysiological grounds. Increased responsiveness to NA has been said to be a result of facilitated synchronisation of contraction i.e. enhancement of cell to cell communication (Westfall, McClure & Fleming, 1962). Consistent with this hypothesis is the observation that denervation increases the number of cell to cell contacts in rat vas deferens (Westfall et al, 1975). The increase in the number of cell to cell contacts may develop in parallel with membrane depolarization, since reduction in membrane bound Ca^{2+} has been

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shown to cause depolarization and initiate membrane fusion (Poste & Allison, 1973). This idea has been questioned on histological grounds (Paton, Buckland, Nicks & Johns, 1976) and instead, it has been suggested that spontaneous activity is associated with membrane depolarization arising from a decrease in Ca^{2+} bound to the cell membrane (Fleming & Westfall, 1975) or to change in tissue content of ATP since these are increased by procedures which produce postjunctional supersensitivity (Westfall et al, 1975).

In studies on the rabbit aortic strips, Maxwell et al (1966) observed cocaine to produce a concentration dependent reduction in total uptake, binding rate and in diffusible NA. They noted that in the range of 30 - 70% reduction in binding rate, the response to NA is an increasing function of the percent reduction in binding produced by these drugs. However, since these authors also observed that cocaine and guanethidine can produce considerable additional increase in response without additional reduction in binding rate, it was concluded that decrease in the binding rate of NA may not be an important determinant of the supersensitivity produced by cocaine, guanethidine and methyphenidate in rabbit aortic strips. An action in the smooth muscle cells was suggested to be responsible for the supersentivity. Kasuya & Goto (1968) in their studies of the mechanism of supersensitivity to NA induced by cocaine in rat isolated vas deferens concluded that this agent

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potentiates NA by a postsynaptic direct action which is non-specific and related to improvement of intracellular Ca^{2+} in addition to its presynaptic inhibition of NA uptake. This conclusion was based on the observation that cocaine potentiated responses to predominantly postsynaptically acting agonists such as K^{+}, Ba^{2+}, acetylcholine and angiotensin. Cocaine has also been reported to potentiate the contractile response of artery segments to isoprenaline and histamine (Gay, Rand & Wilson, 1967). However, K^{+}, angiotensin, acetylcholine have been shown to release catecholamines from the terminal nerve endings of adrenergic nerves (Douglas & Rubin, 1964; Kiran & Khairallah, 1969). Therefore, cocaine - induced potentiation of the maximum responses to KCl and angiotensin could be a result of enhancement of the component of the response to these agonists mediated by the neurotransmitter whose reuptake back into the neurone has been blocked. This observation is well buttressed by the observed inability of cocaine to facilitate the responses to KCl, angiotensin and ACH in vas deferens depleted of their catecholamines stores by reserpine (Greenberg & Long, 1971). Conversely, these latter authors observed the ability of cocaine to enhance responses of the vas deferens to Ca^{2+} and Ba^{2+} when the amine content of this tissue has been depleted by reserpine. These authors hypothesized that cocaine enhanced the permeability of the smooth muscle membrane to extracellular Ca^{2+} ions as a result of the ability of cocaine to

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weaken the binding between Ca^{2+} and its tissue binding sites. Recently, Summer & Tillman (1979) proposed a more specific explanation for cocaine - induced supersensitivity. It is these authors' view that cocaine enhances the influx of Ca^{2+} across the cell membrane during responses of agonists that utilize the extracellular pool of Ca^{2+} and that this effect is responsible for a large part of the observed supersensitivity. They could not explain supersensitivity to NA after cocaine in the cat spleen strips by a presynaptic mechanism for the following reasons : (a) that cocaine produced an increase in maximum response as well as a shift to the left of the dose - response curve to NA. According to Kalsner (1974), an increase in maximum response is not associated with inhibition of uptake but suggests a post junctional action; (b) cocaine potentiates responses to oxymetazoline. This a - adrenoceptor agonist is not a substrate for neuronal uptake (Birmingham, Paterson & Wojcicki, 1970); (c) desmethylimipramine and chlorpheniramine known to be potent neuronal uptake inhibitors did not cause supersensitivity of cat spleen strips to NA. Evidence in support of the post - synaptic mode of action of cocaine is as follows : firstly, that cocaine had no effects in the cat spleen strips, on responses to angiotensin indicating no effect on intracellular Ca^{2+} metabolism. Angiotensin had been shown to initiate contraction almost exclusively by metabolizing the Ca^{2+} firmly bound to the intracellular membrane (Kalsner *et al*, 1970;

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van Breemen, et al, 1972). Secondly, SKF 525A prevented the enhancement of the response to NA by cocaine. This is supported by the findings of Shibata, Hattori, Sakurai, Mori & Fujiwara (1971) who showed in rabbit aortic strips that NA is not potentiated by cocaine in Ca^{2+} - free media or in the presence of Mn^{2+} or Co^{2+} which are thought to inhibit specifically Ca^{2+} influx (Hagiwara & Nakajima, 1966; Geduldig & Junge, 1968; Shibata, 1969). Be that as it may, the effect of cocaine on influx of Ca^{2+} as an explanation for supersensitivity to NA is by no means generally accepted (Barnett, Greenhouse & Taber, 1968; Kasuya & Goto, 1968; Greenberg & Innes, 1968; 1976; Pennefather, 1976). In particular, in cat spleen strips, it has been suggested that cocaine potentiates the response to NA by making the tightly bound intracellular Ca^{2+} store available for contraction (Greenberg & Innes, 1976). Evidence for this suggestion is that cocaine potentiates the response to NA in a Ca^{2+} free medium and in strips which have been previously depleted of Ca^{2+} by treatment with the chelating agent disodium edetate (EDTA). Similarly, since the work of Burn and Rand in 1958, in which they reported the potentiation of smooth muscle (vascular, splenic, nictitating membrane) responses to catecholamines in reserpinized cats, much research has been done to uncover the mechanism of supersensitivity in reserpinized animals. Apart from the proposal that reserpinization results in increased receptor

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affinity and also population, a more general mechanism is associated with Ca^{2+} fluxes that is, that reserpine causes supersensitivity by enhancing Ca^{2+} influx (Green & Fleming, 1968; Taylor & Green, 1971; Carrier (Jnr.) 1975; Carrier (Jnr.) & Hester 1976).

In summary, although there is a large body of evidence in favour of prejunctional site of action in supersensitivity induced by cocaine and reserpine; more recent studies suggest at least a contribution for postjunctional site involving Ca^{2+} movement.

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DIVALENT CATION IONOPHORE A23187:

A23187 is produced by the fermentation of *Streptomyces chartnensis*. It has the molecular formula $C_{29} H_{37} N_3 O_6$ with the structure shown in Fig. 2. It is an ionophore in the sense that it has the ability to carry ions across lipid barriers including artificial and biological membranes. The ionophore catalyses transport by the following processes : (a) enveloping an ion at a membrane interphase with a consequent dehydration of the ion; (b) diffusion across the membrane as a cation complex; (c) releasing the ion which undergoes concomitant rehydration at the opposite interphase and diffusing back uncomplexed, to the original interphase to complete the catalytic cycle. Ionophores enfold their complexed cation in a three dimensional cage. The relationship between the conformational constraints of the ionophore cage and the size of the captured cation, together with the free energy of ion

FIGURE 2

Structural formula of the divalent cation ionophore, A23187. Obtained from the manufacturer's manuals (Elli Lilly, U.K.).

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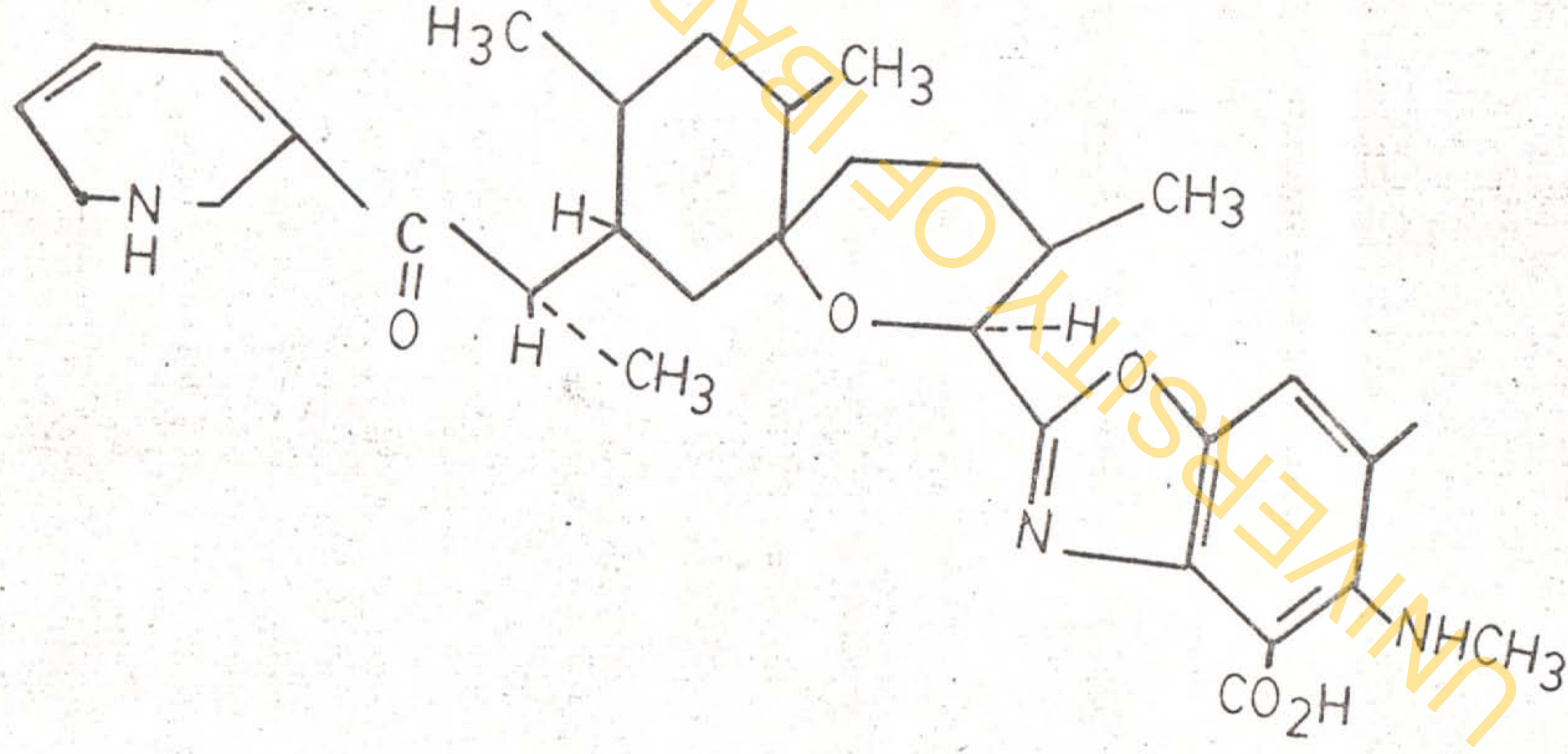
FIG 2



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desolvation and ion-dipole interaction during complex formation, combine to produce a remarkable degree of ion selectivity. The affinity series for A23187 has been reported by Reed (1972) to be $Ca^{2+} > Mg^{2+} > Sr^{2+} > Ba^{2+}$. The ionophore A23187 has thus proved to be a valuable tool in the study of the role of Ca^{2+} in physiological processes. In the presence of Ca^{2+}, it causes the release of histamine from most cells (Foreman, Mongar & Gomperts, 1973), stimulates fluid secretion from fly salivary gland (Prince, Rasmussen & Berridge, 1973), and causes an increased release of serotonin from platelets. It is able to facilitate the movement of divalent cations across the membranes of mitochondria and sarcoplasmic reticulum via a process that is said to be primarily electroneutral (Case, Vanderkooi & Scarpa, 1975).

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PROSTAGLANDINS : Formation

Prostaglandins (PGs) are a family of unsaturated fatty acids first detected by von Euler in the seminal fluid. Although, they were thought originally to be secreted by the prostate gland (hence the name), these biologically active substances have now been found in almost every animal tissue and organ, and even in plants.

PGs are not stored in tissues but formed in response to a variety of stimuli including antigen-antibody reactions, irritants, tissue damage, nerve stimulation, and stretch. The initial step in the formation of PGs is the cleavage of arachidonic acid from phospholipid by the action of the enzyme phospholipase A_2. Arachidonic acid is then rapidly converted to PGs in a cascade of enzyme reactions and intermediate products. The rate limiting step appears to be the action of cyclo-oxygenase. (Fig. 3 shows the biosynthetic pathway of prostaglandins E_2, F_{2a} and I_2). Cyclooxygenase is responsible for catalyzing the conversion of arachidonic acid to PGG_2 (the hydroperoxy analogue of PGH2). Cyclooxygenase can be inhibited by a group of drugs called prostaglandin synthetase inhibitors such as aspirin and indomethacin.

Site and mode of release:

Prostaglandins are released from sympathetically innervated organs as a result of both nerve stimulation and administration of

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FIGURE 3

Prostaglandin biosynthetic pathway. Redrawn from prostaglandins 14 (6) pp.1058.*

*SUN, F.F.; CHAPMAN, J.P. & MCGUIRE, J.C. (1977) Metabolism of prostaglandin endoperoxides in animal tissues
Prostaglandins 14: 1055 - 1074.

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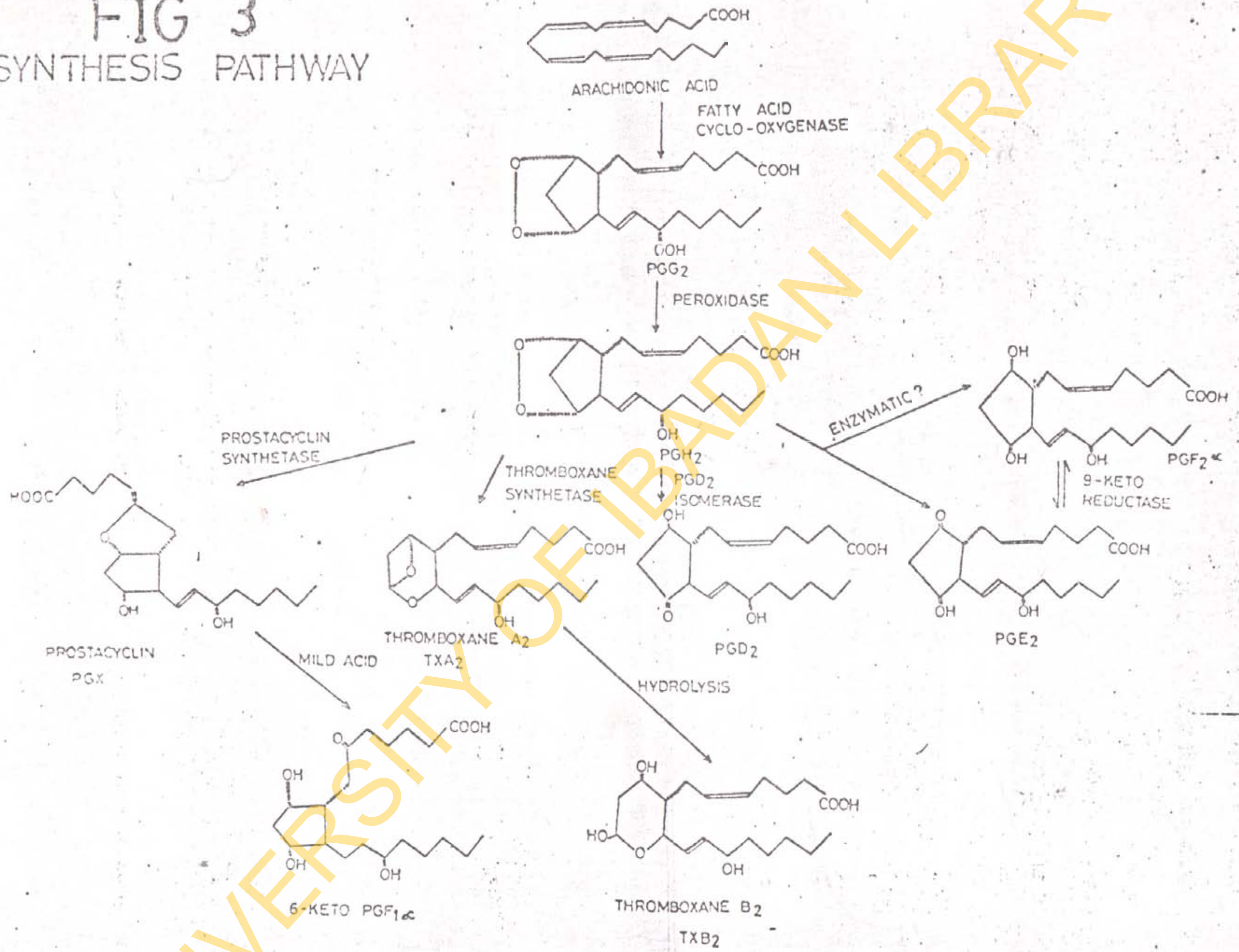

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vasoactive substances. Ferreira & Vane (1967) identified PGs in dog splenic venous blood when the spleen contracted. Gilmore, Vane & Wyllie (1968) and Davies, Horton & Withrington (1968) independently detected PGs in the effluent blood from the dog spleen after splenic nerve stimulation; and also during adrenaline - induced contraction. PGF_{2a} and PGE_2 were identified in the perfusate by thin layer chromatography and bioassay techniques. The PGs so released were not of nervous origin because : (a) adrenaline also induced release, (b) - adrenoceptor antagonists prevented splenic contraction and adrenaline - induced prostaglandin release, and (c) the quantities of PGE_2 and PGF_{2a} released during adrenaline stimulation of denervated and innervated preparations were not significantly different (Gilmore _et al_, 1968).

Release of prostaglandins have also been reported from many other tissues of various animal species. Shaw & Ramwell (1968) reported prostaglandin release from rat epididymal fat pad on nervous and humoral (NA) stimulations; Dunham & Zimmerman (1970), Mcgiff, Crowshaw, Terragno, Malik & Lonigro (1972) demonstrated a basal release of material identified as PGE from the dog kidney. It was observed that the release was markedly enhanced during periods of renal vascular constrictions induced by either nerve stimulation or noradrenaline (NA) infusion. In 1972, Davis & Horton, reported the mass spectrometric identification of PGE_2 and PGF_{2a} in rabbit renal venous blood. This basal output was greatly

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increased by renal nerve stimulation. Indomethacin (10 mg/kg) injected intravenously reduced the basal PG output into renal venous blood and also prevented the increase in output in renal nerve stimulation.

The extent to which vascular smooth muscle wall participates in the generation of PG detected in the outflow from organs treated with vasoconstrictor substances has stimulated much interest in recent years. Hedqvist (1972) observed PG in the venous effluent of blood perfused hindlimb of the cat after NA infusion and sympathetic nerve stimulation and proposed a possibility. of an intramural generation of prostaglandins by contracting blood vessels. Aikens (1974) made a similar observation. Gryglewski & Korbut (1975) reported that the vasoconstriction induced by NA in the perfused rabbit ear is accompanied by a release of prostaglandin-like substance. Since exogenous prostaglandin antagonized the vasoconstriction action of NA, the authors concluded that the peripheral function of the PG was to attenuate NA. In preparations pretreated with indomethacin (3 ug/ml), NA infusion did not release any substance capable of contracting biological assay tissues. Also, - receptor blocking drug (phenoxybenzamine) completely blocked the pressor response to NA and also the release of prostaglandin like substance into the effleunt.

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Grodzinska, Panczenko & Gryglewski (1976) demonstrated simultaneous increase in perfusion pressure and release of PGE - like material after infusing NA into the mesenteric vascular preparation of rabbit. Indomethacin prevented, whereas arachidonic acid (0.2 ug/ml) augmented the NA - evoked PG release. The authors noted no prostaglandin output from perfused or superfused rabbit arteries unless the small distal vessels were left in the vascular preparation and therefore proposed the arteriolar wall or precapillary vessels as the site of prostaglandin generation. Other site at which PG - like substances are released in response to nerve stimulation or humoral substances include rat diaphragm (Ramwell, Shaw & Kucharski, 1965; Laity, 1969); frog skin (Ramwell & Shaw, 1970); guinea-pig vas deferens (Ambache & Zar, 1970) and cat spleen (Hedqvist, Sjarne & Wennmalm, 1971). The ubiquity of prostaglandin is therefore not in doubt. Despite this ubiquitous nature however, PGs have not been proved to be transmitters at nerve endings although they are known to modify synaptic functions. Their release, especially from muscles, is thought to be transynaptic in mechanism i.e. as a secondary but simultaneous action of the humoral substances.

<u>Prostacyclin</u> (PGI_2)

Prostacyclin is formed by microsomes of several organs such as pig and rabbit aorta (Gryglewski, Bunting, Moncada,

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Flower & Vane, 1976); rat stomach fundus, pig mesenteric arteries, and venous tissues incubated with PG endoperoxides. The PG endoperoxides are then enzymically converted to an unstable product (PGX) which relaxes arterial strips and prevents platelet aggregation. Microsomes from rat stomach corpus, rat liver, rabbit lungs, rabbit spleen, brain, kidney medulla, ram seminal vesicle as well as particulate fractions of rat skin homogenenate transform PG endoperoxides to PGE and PGF rather than PGX (Gryglewski et al, 1976). Prostacyclin is the primary metabolite of arachidonic acid in blood vessels of all species so far tested including man (Bunting, Grygl wski, Moncada & Vane, 1976). This prostaglandin, originally called PGX, was later identified as 5Z - 5, 6 - didehydro-9-deoxy-6-9 - epoxyprostaglandin F_1 and renamed Prostacyclin by Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whitacre, Bunting, Salmon, Moncada & Vane (1976) or simply PGI_2.

Smooth muscle actions of PGE's and PGI_2.

Prostaglandins are known to produce two kinds of effects on smooth muscle. First, the direct effect which may manifest as contraction or relaxation (Hall & Pickles, 1963; Strong & Bohr, 1967; Mark, Schmid, Eckstein & Wendhing,1971). Secondly, they may indirectly inhibit or enhance responses to other agonists. For instance, PGE_1 and PGE_2 can induce a persistent non-specific

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increase in sensitivity to agonists such as adrenaline, NA, tyramine, acetylcholine and serotonin in rat and guinea-pig seminal vesicles (Elliason & Risley, 1966), in rat uterus and guinea-pig ileum (Clegg, Hall & Pickles, 1966; Hall & Pickles, 1963). Also, PGE_1 enhances the responses of the rat stomach fundus to acetylcholine (Coceani & Wolfe, 1965). Prostaglandins E_1 and E_2 have been shown to enhance contractile responses to nerve stimulation and catecholamines in the guinea-pig vas deferens while having no effect on responses in the cat and actually depressing responses to both nerve stimulation and catecholamines in the rabbit vas deferens (Graham & Al Katib, 1967; Naimzada, 1969 a, b). Euler & Hedqvist, (1969); Hedqvist & Euler, (1972) reported that low and high doses of PGE inhibited and potentiated respectively the contractile response of the guinea-pig vas deferens to post-ganglionic nerve stimulation. Exogenous NA was also shown to be consistently potentiated by high dose of the PGEs. They concluded that the potentiation by high doses of PGE_1 was post-junctional since it was greatly or completely antagonized by compound SC 19220 (a PG receptor antagonist). The inhibitory effect, on the other hand has been shown to be a prejunctional effect where the postaglandin modulates the release of the adrenergic transmitter (Hedqvist, 1972, 1974).

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The effect of postaglandins on the isolated vascular smooth muscle in vitro varies according to the animal species, the vascular tissue under study, the existing tone of the tissue and the concentration of prostaglandins used in the study. PGE_1 potentiates vasoconstrictor responses to exogenous NA in the rabbit aorta and mesenteric artery in vitro (Strong & Chandler, 1972; Tobian & Viet, 1970). PGE_2 on the other hand has been shown to facilitate the vaso-constriction induced by nerve stimulation without affecting the responses to NA in the dog mesenteric arteries and veins (Kadowitz, Sweet & Broody, 1976). However, in the anaesthesized rat, PGE_s and PGI_2 are hypotensive in vivo. According to Armstrong, Lettimer, Moncada & Vane (1978), intravenous PGI_2 produced hypotension which is 4 - 8 times and about 128 times more than that produced by PGE_2 and 6-oxo-PGF_{1a} respectively in rats. Also, the potency of PGI_2 measured by its hypotensive effect was 2 times that of PGE_2 and about 250 times more potent than 6-oxo-PGF_{1a} in rabbit.

It has been hypothesized (Horton, 1979) that PGs act on receptors that can be distinguished from those for other agonists by actions of selective antagonists. In support of this is the fact that the actions of PGs on smooth muscle are not blocked by atropine, methysergide, mepyramine, propranolol, phenoxybenzamine or hexamethonium. Three groups of PG receptor antagonists have been described and are represented by (i) polyphloretin phosphate, (ii) the

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dibenzoxazepine, SC 19220, and (iii) the 7 - oxaprostaglandins. None of these antagonists is either very potent or highly selective (Eakins & Sanner, 1972). Pickles (1967) had put forward evidence for the existence of more than two prostaglandin receptors. Many smooth muscle actions of PGs have also been associated with a change in tissue levels of one of the cyclic nucleotides (i.e. either adenylate cyclase or guanylate cyclase systems). Furthermore, events at the cellular (including cell membrane) level have received some attention. Contraction of smooth muscle by PGs is associated with depolarization alone (e.g. main pulmonary artery, Kitamura, Suzuki & Kuriyama, 1976) or with depolarization and with increased frequency and length of action potential bursts (e.g. rat uterus, Kuriyama & Suzuki, 1976). Relaxation of smooth muscle on other hand, has been described as associated with hyperpolarization of the membrane (Stomach, Hischima & Kuriyama, 1976). An increase in membrane conductance has been found both during excitatory and during inhibitory smooth muscle responses to prostaglandins. According to Reiner & Marshall (1976), it appears there is an action potential - dependent mechanism of tension generation which can be triggered by PGs(and by other stimulants) which can be blocked by Ca^{2+} antagonist verapamil and a more sustained contractile component which does not involve action potential and is resistant to blockade by verapamil. Thus, as elaborate as the volume of the literature on smooth muscle actions of PGs is, very little

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measure of consistence has been attained as to the mechanism of the various actions.

AIM AND SCOPE OF PRESENT WORK

The present investigation is aimed at finding out how PGE_2, PGF_{2a} and PGI_2 interact with adrenergic neurotransmitter substance, NA, and agents that antagonize its vasoconstrictor action. The isolated rat mesenteric artery was chosen for this investigation because the aim was to study the interaction between prostaglandins, NA and NA antagonists in arterial smooth muscle in which NA produces a purely vasoconstrictor effect. The preparation as it is made completely excludes the venous side.

The study was prompted by the finding that prostaglandins of the E series can potentiate agonist action on smooth muscle by facilitating Ca^{2+} influx (Pickles, 1966, Eagling, et al 1972). Prostaglandins are therefore potentially useful agents for investigating NA supersensitivity mechanisms which occur entirely postjunctionally since prostaglandins do not appear to influence uptake or other inactivating mechanisms of NA (Adeagbo & Okpako, 1980). This kind of study seems worthwhile because it has been suggested that prostaglandins mediate or amplify the vasoconstrictor action of NA in mesenteric arteries (Horrobin et al, 1974; Coupar & McLennan, 1978; Coupar, 1980).

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The interaction of PGE_2 with antagonists of NA was also investigated. For the purpose of the study, antagonists of NA have been classified into three as follows:-

a) α- adrenoceptor antagonists - phentolamine, tolazoline, ychimbine, phenoxybenzamine and prazosin;

b) Non-steroidal anti-inflammatory drugs - indomethacin and sodium meclofenamate.

c) antagonists interfering with Ca^{2+} ions - cinnarizine and verapamil.

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CHAPTER TWO

MATERIALS AND METHODS

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MATERIALS AND METHODS

Animals

Albino rats (sprague Dawley strain) and guinea-pigs (bred from a strain developed in Vom, Nigeria) were used throughout this study. All animals were bred locally in the departmental animal house. The house was adequately ventilated. Rats were fed on standard livestock cubes (Pfizer Nigeria Ltd.) while guinea-pigs were fed on standard livestock pellets supplemented with green grass. All animals had free access to water.

Physiological solutions used

The physiological solutions used and their composition were as follows:-

(a) Krebs solution (mM per litre):

Sodium chloride, 113.0; potassium chloride, 4.7; calcium chloride, 2.5; sodium dihydrogen phosphate, 1.2; magnesium chloride, 1.2; sodium bicarbonate, 25.0 and glucose, 11.5.

(b) Calcium - free Krebs solution

This solution has the same composition as Krebs solution described above except that calcium chloride was completely omitted.

(c) Depolarizing krebs solution

This solution has the same composition as Krebs solution described in (a) above except that sodium chloride and sodium bicarbonate were replaced by potassium sulphate and potassium

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bicarbonate respectively. Depolarizing Krebs solution used in this study is of the following composition (in mMol per litre):- potassium sulphate, 92; potassium chloride, 10; sodium dihydrogen phosphate, 1.2; potassium bicarbonate, 10; magnesium chloride, 1.2 and glucose, 11.5. When calcium chloride was to be used as agonist, it was omitted in the preparation of the depolarizing solution.

(d) Tyrode solution (mMol/litre).

Sodium chloride, 136.8; potassium chloride, 2.7; calcium chloride, 1.8; sodium dihydrogen phosphate, 0.3; sodium hydrogen carbonate, 11.9; magnesium chloride, 0.9 and glucose, 5.6.

Krebs solution was gassed with 5% CO_2 and 95% O_2 mixture and Tyrode solution gassed with air.

Preparation of mesenteric artery for perfusion

Surgical operations were carried out according to the method of Mcgregor (1965). Adult male rats weighing 250g, and above were anaesthesized by diethyl ether or chloroform. The abdomen was opened and the pancreatico-duodenal, ileo-colic and colic branches of the superior mesenteric artery were all tied off. The dorsal aorta was ligated a few millimeters anteriorly and posteriorly from its junction with the superior mesenteric artery. The latter was then isolated by cutting round the intestinal borders of the mesentery (Fig. 4). Thereafter, the whole preparation was quickly transferred

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FIGURE 4

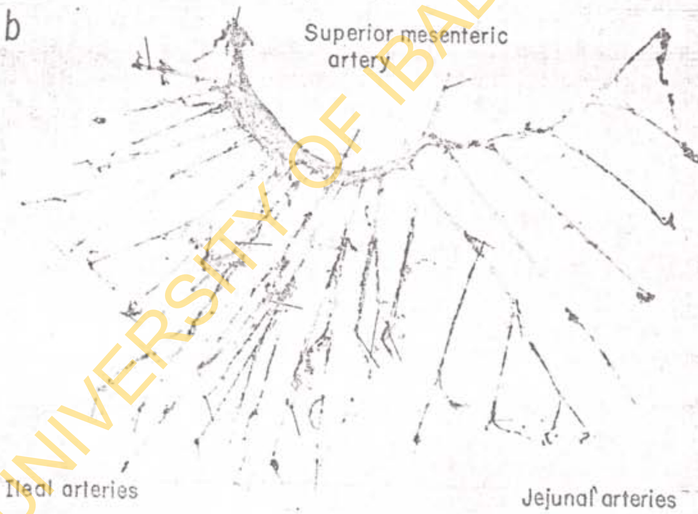
The preparation of 'mesenteric arteries' stained with sudan black. The preparation consists of superior mesenteric, jejunal, and ileal arteries. (a) adipose tissue attached. (b) adipose tissue and veins removed. (Reproduced from "Blood vessels" 13 : 279 - 292)*

*J. WEI: R.A. JANIS & E.E. DANIEL (1976) Isolation and characterization of plasma membrane from rat mesenteric arteries Blood vessels (1976) 13:279-292.

FIG 4

a

b



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unto the surface of a conical flask containing water at 37°C. The artery was cannulated and perfused with Krebs solution whose composition is given above. The solution was bubbled with 5% Co_2 and 95% O_2 mixture and was perfused through the tissue at a constant flow rate of 4 ml min^{-1} with a Watson-Marlow constant flow inducer (type MHRE-88). The preparation was carefully arranged on a blotting paper moistened with krebs solution which was placed on the surface of a 250 ml conical flask in which water at 37°C circulated. The preparation was lightly covered with moist cotton wool which was periodically wetted with warm Krebs solution from a pipette. An angle poised lamp arranged above the flask ensured that the whole preparation was maintained at 37°C throughout the experiment. Changes in perfusion pressure were recorded by a Bell & Howell pressure transducer (type 4-327-L -223) on a devices M.19 recorder. All arteries set up were allowed to equilibrate for at least 30 min. before the commencement of the experiment.

Experimental Procedure

Drugs were injected through a pressure tubing placed just before the constant flow inducer in volumes not exceeding 0.2ml and at 5 min. intervals. In order to ensure that the tissue responses are reasonably constant, two or three dose responses were recorded following injections of geometrically increasing doses of the agonist. The time interval between agonist injection was 5 min. and responses

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obtained in this manner are termed 'control responses'. In cases where the artery was perfused with prostaglandin or antagonist or any other test drug or vehicle, the Krebs solution was prepared to contain the desired concentrations of the drugs. In such a medium, the artery was allowed to equilibrate for at least 10 min. before the next addition of the agonist.

Ascertaining the potency of prostacyclin (PGI_2)

The half-life ($t\frac{1}{2}$) of PGI_2 at pH 7.48 is between 3.5 min and 10.5 min. at 25°C depending on buffer concentration, but with shorter $t\frac{1}{2}$ at higher buffer concentration. At pH 12, $t\frac{1}{2}$ is approximately 6 days at 25°C (Cho & Allen, in the manufacturers mannual). The biological activity was sustained at pH 9.37 (o.05M tris-buffer) for 48 hrs. when stored at 0°C. Under our working conditions, 2% sodium carbonate solution pH 10 was used to dissolve PGI_2. In view of the intermediate position and owing to the relative unstable nature of PGI_2, it was important to ascertain on a daily basis the retention of potency of an aliquot of the prostacyclin before use. The model used for this purpose was the inhibition by PGI_2, of the arachidonic acid-induced human platelet aggregation. Platelets obtained from the blood of voluntary donors in the University Teaching Hospital, Ibadan, aggregated in the presence of low concentrations of arachidonic acid (AA). The aggregometer used was Payton module made by Payton Associates, Scarborough, Canada. Low concentrations of PGI_2 would prevent

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this action, and the ability to do so is taken as a means of ascertaining the potency of various aliquots of the prostacyclin. A particular PGI_2 aliquot is assumed to have lost potency when it can no longer prevent AA - induced human platelet aggregation or when the concentration at which it does so has become considerably high.

Metabolism of prostaglandins in rat mesenteric and guinea-pig pulmonary vasculature

In order to determine whether the effects of the infused prostaglandins was due to the primary prostaglandins or their metabolites, the following experiments were performed:

(a) Inactivation of prostaglandins on perfusion through the rat mesenteric artery

Different concentrations of PGE_2 and PGF_{2a} were perfused through the rat mesenteric artery. The effluent was collected and its biological activity determined using a 3-point assay technique on the rat stomach strips (Vane, 1957) or rat colon (Rogeli & Vane, 1964) superfused with Tyrode solution containing a combination of antagonists: atropine 0.1, cyproheptadine 0.1, propranolol 0.2, and phenoxybenzamine 0.5 (ug/ml) (Vane; Gilmore *et al*, 1968). The inclusion of these antagonists makes the assay system more specific for prostaglandins. Contractions of the tissues were registered mechanically by auxotonic levers (Paton, 1957) with a magnification of 6 and an initial load of 1g. Percentage inactivation was

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calculated from the difference between the concentration of PG in the effluent and the concentration of the parent solutions.

$$\frac{P_A - E_A}{P_A} \times \frac{100}{1}$$

where P_A = physiological activity of the parent prostaglandin

E_A = " " " prostaglandin in the effluent.

(b) Inactivation of prostaglandins in the pulmonary vascular bed of the guinea-pig lung

Guinea-pigs of either sex weighing between 300g and 500g were killed by neck fracture and exsanguinated. The lungs were isolated and set up for perfusion through the pulmonary artery as described by Okpako (1971). When the effluent was free of PG - like activity, different concentrations of PGE_2 and PGF_{2a} were perfused through the lungs. In the concentrations used (10^{-8} and 10^{-7} gml^{-1}), the effluent showed no detectable activity on the rat stomach strips or rat colon showing that there was near complete inactivation of the prostaglandins on passage through the pulmonary vasculature. The effluent was presumed to contain metabolites inactive on the assay tissues. The effluent was used to study a possible effect of metabolites on NA - induced vasoconstriction in the rat mesenteric artery.

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Investigations on a possible influence of endogenous NA on prostaglandin action

Investigations of this sort are important in the light of the findings by Furness & Marshall (1974) that the principal and small arteries and also the terminal arterioles of the rat mesenteric vascular bed are innervated by a meshwork of adrenergic nerves. The procedure involves the pretreatment of rats with a large dose of reserpine (10 mg/kg) daily for 1 or 2 day(s) before the mesenteries were dissected out and prepared as described above. The reserpine was administered intraperitoneally. In some cases, smaller dose of reserpine (5 mg/kg) was injected i.p. daily for 4-5 days in order to find out if reserpinization affects vasoconstrictor action of NA. In some other experiments, the adrenergic neurone blocking drug, bretylium was included in the Krebs solution perfusing the mesentery. This also was geared towards finding out the influence of this drug on NA vasoconstriction and the possible involvement of endogenous catecholamine in the prostaglandin.actions with agonists.

Effect of prostaglandins on NA uptake

The effect of PGE_2 and PGF_{2a} on the vaso constrictor responses to methoxamine was investigated. This drug is not a substrate for the uptake process (Iversen, 1967; Trendelenburg, Maxwell & Pluchino, 1970) and thus constitutes a useful probe for the uptake

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process. The procedure for the investigation was by constructing dose - response curves to graded doses of methoxamine during perfusion with plain krebs solution, after which a calculated dose of prostaglandin making the final concentration in the perfusion medium 10^{-8} g ml^{-1} was added. The tissue was subsequently allowed to equilibrate in the prostaglandin containing krebs for 10 min. before another dose - response curve to methoxamine was constructed. A similar procedure was followed when A23187 was tested as NA uptake antagonist.

The effect of prostaglandins on NA uptake was studied yet in another way. This involved the interaction of prostaglandins with a novel uptake inhibitor - cocaine. The procedure was as follows:-
(a) obtaining the maximum potentiating effect of cocaine by constructing dose-response curves to a wide range of cocaine concentrations (The concentration at which increase in cocaine concentrations produced no further increase in the degree of potentiation was taken as the maximum potentiating concentration);
(b) constructing dose-response curves to the maximum potentiating concentration of cocaine, and the submaximal potentiating concentration of prostaglandin separately and in combination on the same preparations.

Measurement of the degree of potentiation

An estimate of the degree of potentiation was obtained by measuring the quantity known as 'potentiation factor' (P.F.). The procedure for measuring this parameter consisted of constructing

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dose-response curves to NA before and in the presence of different concentrations of prostaglandin or any other potentiating agent. In the presence of any potentiating agent, the NA dose-response curve was shifted leftwards but parallel. The P.F. was the ratio:

$$\frac{\text{Dose of NA causing 50\% apparent maximum response before the potentiating agent}}{\text{Dose of NA causing 50\% apparent maximum in the presence of the potentiating agent.}}$$

Examination of the shift in the dose-response curve at the ED50 level is important since according to Trendelenburg (1963), a meaningful measurement of the sensitizing effect of drug or procedure can be obtained only by the determination of the horizontal shift of the dose-response curve.

Potentiation factors measured in this way can attain any value greater than unity.

Determination of the degree of antagonism

Degrees of antagonism were determined according to the method of Arunlakshana & Schild (1959). The method consisted of obtaining dose-ratios (DR) for different concentrations of competitive antagonists against NA at the level of 50% of the maximum response pA_2 was determined from a plot of the Log_{10}(dose ratio -1) as ordinate versus Log_{10} molar concentration of the antagonist as abcissa. The point at which the straight line graph cut the "x-axis" corresponded to the pA_2 value. pA_2 determinations made in this way and in normal

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Krebs solution were termed "control pA_2" values. Other determinations were made in krebs solution containing a particular concentration of a potentiating agent.

<u>Mesenteric artery preparation as a model for studies on sources of Ca^{2+} used during vascular muscle contractions.</u>

Rasmussen & Goodman (1977) have suggested the occurrence of at least two distinct channels by which Ca^{2+} can leak into the cell. Firstly, there is a channel that is independent of the membrane potential and which is altered when hormones interact with their receptors without leading to membrane depolarization. Secondly, there are potential - dependent Ca^{2+} permeability channels. These two channels correspond respectively, to the pharmacomechanical and the electromechanical pathways of evoking vascular muscle contractility earlier described by Somlyo & Somlyo (1968). Mesenteric vascular muscle contractions evoked by NA represents the former pathway while K^+ - induced vasoconstriction represent the latter. On this basis, agents not found to antagonize NA competitively were tested against K^+-induced contractions. The procedure consisted of: (a) obtaining 3 or 4 regular responses to a particular dose of KCl in normal Krebs solution (control responses); (b). changing perfusion medium to Krebs containing a particular concentration of the antagonist being tested, followed 15 min. later, by a record of the responses to K^+ as in (a) above. These procedures

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were repeated until about 4 or 5 different concentrations of the antagonist have been tested. Each test dose is preceeded by a recording of the control responses. The following agents were so tested: cinnarizine, indomethacin, phenoxybenzamine, prazosin and verapamil.

Drugs used, sources and methods of preparation and storage

The drugs used in the study include:- (-) Noradrenaline base (Sigma & Co. Ltd., London); Methoxamine Hcl, (Wellcome & Co., London); Potassium chloride (Analar Chem. & Co., London); Prostaglandins E_2, F_{2a} and I_2 (Upjohns Ltd., Kalamazoo); A23187 (Elli-Lilly, U.K); Reserpine (serpasil, ciba); Cocaine Hcl (E. Coburn Ltd); Bretylium Hcl (Wellcome & Co. London); aspirin (Graesser Salicylates Ltd., U.K.); Indomethacin (Merck, Sharp & Dohme, U.S.A.); sodium meclofenamate (Parke Davis & Co., Pontypool); phentolamine Hcl (ciba); tolazoline Hcl, (Ciba Geigy); yohimbine Hcl (Sigma & Co. U.K.); phenoxybenzamine (Smith, Kline, & French Ltd.); Cinnarizine (Janssen Pharmaceuticals Ltd.) and verapamil Hcl (Cordilox ampoules, Abbot Laboratories Ltd.); atropine sulphate (BDH Laboratory Chemicals); cyproheptadine Hcl (BDH biochemicals); Mepyramine maleate (May & Baker); propranolol Hcl (I.C.I.); and arachidonic acid (Sigma & Co. Ltd., London).

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NA stock solution (10 mg/ml) was prepared in 0.1M Hcl while stock solutions of methoxamine and bretylium were made in normal saline. Arachidonic acid, phentolamine and reserpine were present as injectable solutions. Stock solutions of aspirin, indomethacin, sodium meclofenamate and PGI_2 were made in 2% sodium carbonate solution while those of PGE_2 and PGF_{2a}(1mg/ml) were made in 95% ethanol. Cinnarizine was dissolved in 50% methanol and phenoxybenzamine in acidified ethanol according to Benfey & Grillo (1963). Prazosin was dissolved in 25% methanol.

All stock solutions were stored at $-20^{\circ}C$ and diluted fresh in distilled water or physiological salt solution just before use. Stock concentrations are expressed per unit base or salt.

Statistical analysis

Results are expressed as mean $\pm$ standard error of the mean where 'n' represents the number of observations in the group. Where appropriate, comparison between paired group were made using students 't' test or by analysis of variance. The difference between the groups is taken to be significant when $P \geq 0.05$.

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CHAPTER THREE

RESULTS

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RESULTS

Vasoconstrictor responses of the rat mesenteric artery to NA in normal Krebs

Effective doses of NA caused an increase in perfusion pressure, which was taken to indicate arterial vasoconstriction. As shown by Mcgregor (1965), the vasoconstrictor effect of NA was clearly dose dependent and rapidly reversible (Fig. 5). NA threshold dose was about 0.1ug injected into the perfusion fluid and the highest recordable response was achieved with about 1.6ug. In the range of sensitivity used, the maximum response recorded was 200 mmHg. The maximum pressure output of the arteries was of the order of 220 mm Hg recordable at a lower sensitivity range. The higher sensitivity range was used in these studies so that NA could be studied in physiological doses. When first set up, the responses to a given dose of NA tended to increase with time (Fig. 6). It was therefore necessary to inject NA repeatedly until the response had become stable. This required a period of 30-45 min. Thereafter, dose-response curves were constructed to NA. In each experiment, at least three responses were recorded for each dose of NA before the introduction of a modifying drug.

Interactions of exogenously administered PGE_2, PGF_{2a} and PGI_2 with NA

Control dose-response curves to NA were established by bolus injections of graded doses. The responses were repeated in Krebs solution containing a wide range of PGE_2 concentrations (10^{-9}, 10^{-8}, 10^{-7} and 10^{-6}g ml^{-1}). A similar procedure was adopted for PGF_{2a} and PGI_2. PGE_2 and PGF_{2a}, in concentrations which did

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FIGURE 5

Perfused rat mesenteric artery preparation : vasoconstrictor responses noradrenaline (A); methoxamine (B) and potassium chloride (C). The dots indicate the doses in ug for A and B; and mg for C.

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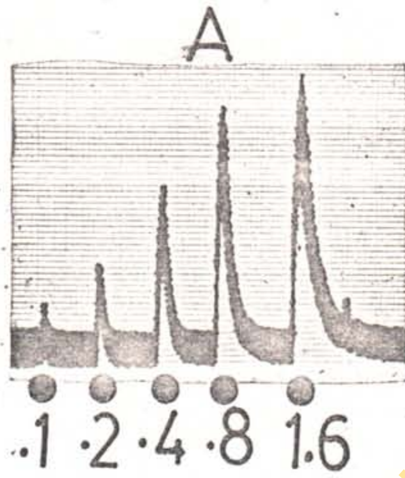
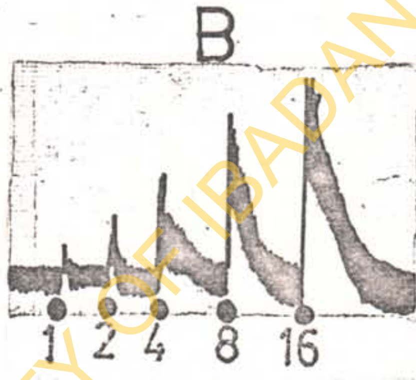
Fig. 5

A



B



C

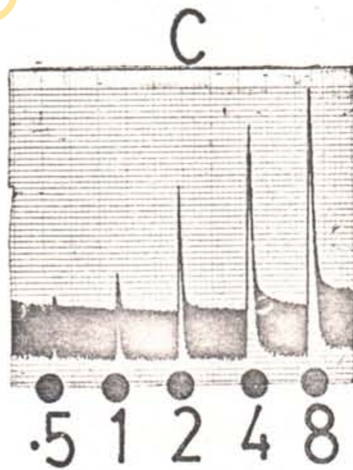


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FIGURE 6

Variation with time, of the responses of isolated rat mesenteric artery to noradrenaline. (O); (X) and (●) represent responses to NA at 15, 30 and 45 min respectively after setting up the mesenteric artery. Each point on the graph represents the mean of 8 - 10 experiments.

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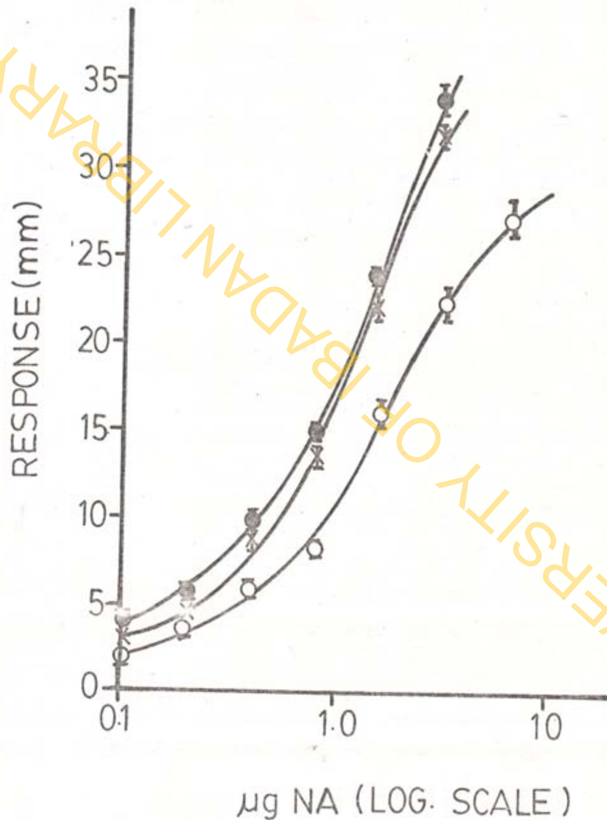
FIG. 6



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not show any direct vasocontrictor or dilator effects greatly potentiated the responses to NA. The potentiating effect of PGI_2 on the other hand was very small. The extent of potentiation in each case was measured as potentiation factor (PF.). The determination of this parameter has been described under methods. The results are summarized in Table 1. It can be seen from the table that the maximal potentiation of NA by PGE_2 was obtained at 10^{-7}g ml^{-1}.

Table 1

The effect of prostaglandin dose on the degree of potentiation of NA caused by PGE_2, PGF_{2a} and PGI_2

Concentration of prostaglandins	Mean potentiation factors ± S.E.M.		
g ml^{-1}	PGE_2	PGF_{2a}	PGI_2
10^{-9}	2.2± 0.1	2.4 ± 0.3	1.4 ± 0.1
10^{-8}	*5.0 ± 0.2	**2.2 ± 0.1	1.6 ± 0.1
10^{-7}	*10.2 ± 0.7	**4.0 ± 0.1	2.2 ± 0.2
10^{-6}	9.4 ± 0.6	5.2 ± 0.2	2.2 ± 0.2

PGE_2 was significatly more potent in enhancing NA vasocontrictor responses than PGF_{2a}.

* Statistically significant $p > 0.005$

** Statistically significant $p > 0.005$

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FIGURE 7

Prostaglandin - induced potentiation of noradrenaline vasoconstrictor responses in relation to prostaglandin dose, in rat mesenteric artery. (O) prostaglandin E_2 (PGE_2); (O-----O) PGF_{2a} and (●) prostacyclin (PGI_2). Each point is a mean of measurements from 8 separate preparations. Vertical bars are s.e mean.

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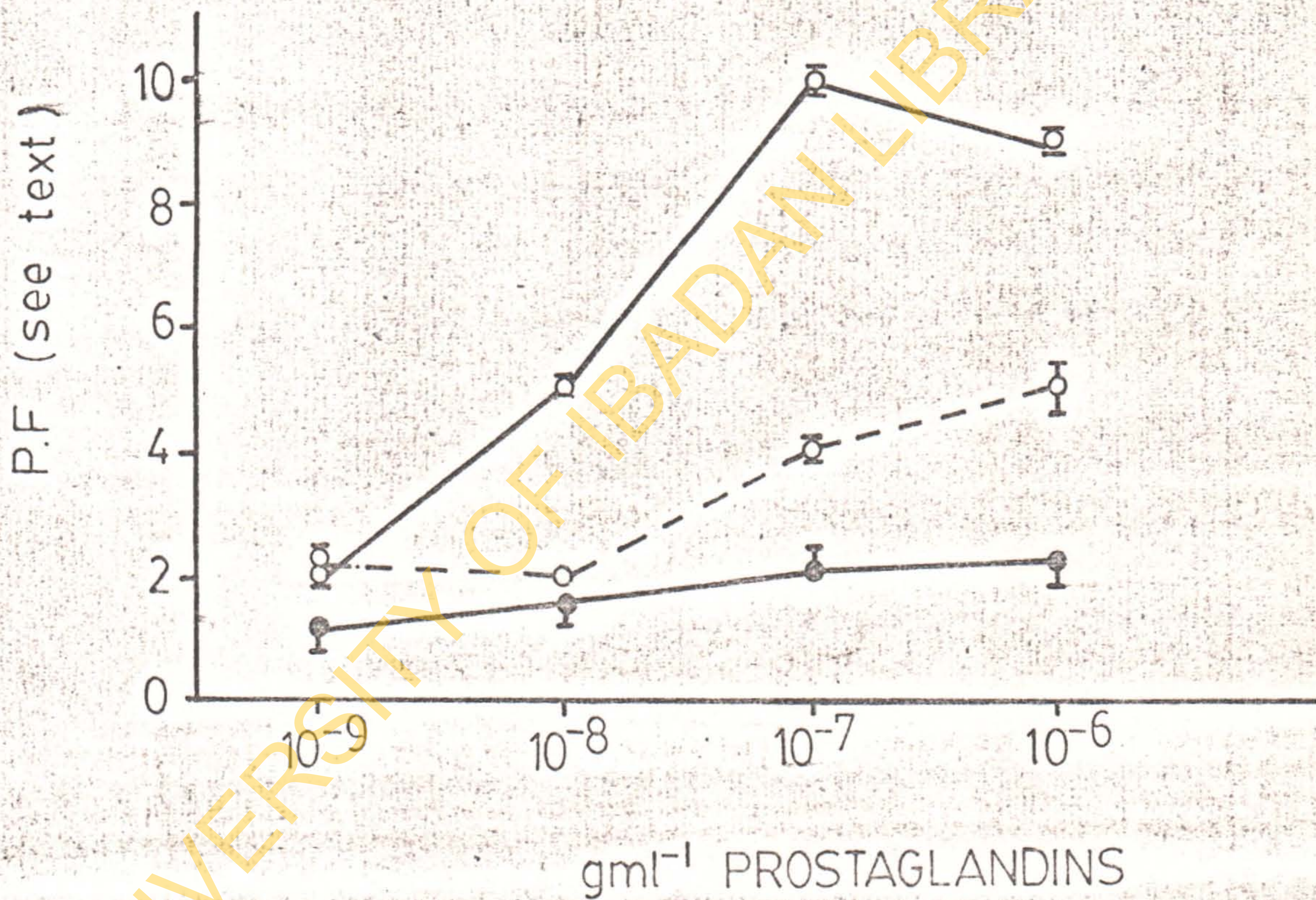
FIG. 7



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Anti-aggregatory action of PGI_2

Platelet rich human plasma is turbid and almost opaque in nature. When caliberating the aggregometer, the zero margin was set by slotting a micro tube containing high concentration of platelets while the upper (100%) margin on the aggregometer chart was set using clear tris buffer solution. Following addition of low concentration of arachidonic acid (AA) there was a gentle rise of the recording pen towards 100% level - an indication of aggregation. This was preceeded by a short latent period. Addition of PGI_2 before AA prevented the aggregation (Fig. 8). The anti-aggregatory property was due to the PGI_2 and not the vehicle ($Na_2\ Co_3$ solution) since the latter, added before AA did not prevent platelet aggregation.

Inactivation of prostaglandins in rat mesenteric vascular bed

It was important to know whether the effects of the prostaglandin seen in these experiments was due to the primary prostaglandins or their metabolites since it was possible that the potentiating effect of the prostaglandins was caused by metabolites. PGE_2 or $PGF_{2\alpha}$ at a concentration of 10^{-8} g ml^{-1} was therefore perfused through the preparation and the effluent assayed against the parent solution as described in methods. Percentage inactivation of the prostaglandins was calculated as follows:-

$$\frac{(\text{PG concentration infused}) - (\text{PG concentration in perfusate})}{\text{PG concentration infused}} \times \frac{100}{1}$$

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FIGURE 8

Effect of prostacyclin(PGI_2) on arachidonic acid (AA) - induced aggregation of human platelet - rich plasma. Platelets were incubated in an aggregometer at 37°C with constant stirring for 2 min. with either tris buffer (pH 7.4) (control) or tris buffer to which prostacyclin (13.5 mM) has been added. The dose of AA used to induce platelet aggregation was 0.5 mM.

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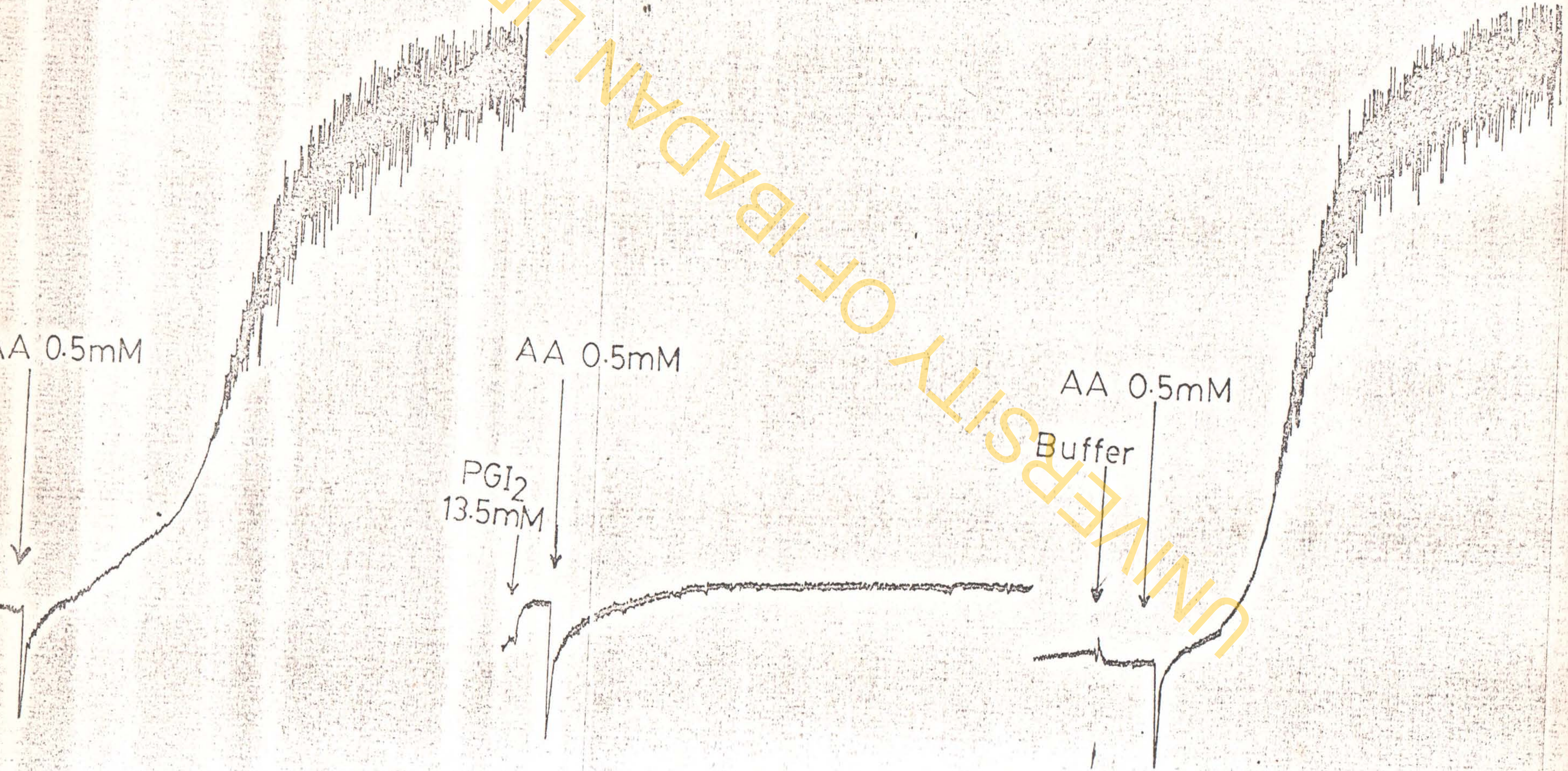
FIG 8



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The results are summarized in Table 2. It can be seen from the table that only about 20% of PGE_2 and PGF_{2a} was inactivated during perfusion.

Table 2

Inactivation of PGE_2 and PGF_{2a} in rat mesenteric artery

Conc. of prostaglandin in perfusion medium	Mean % inactivation of added prostaglandin ± S.E.M.	
$g\ ml^{-1}$	PGE_2	PGF_{2a}
10^{-8}	20.6 ± 3.1 (n = 8)	23.0 ± 3.9 (n = 8)
10^{-7}	19.0 ± 2.1 (n = 6)	20.0 ± 2.5 (n = 6)

This degree of inactivation was considered substantial enough. It was therefore desirable to establish whether the metabolites of PGE_2 and PGF_{2a} had NA potentiating actions in the mesenteric artery. Thus, concentrations of PGE_2 and PGF_{2a} causing NA potentiation were inactivated by passage through the guinea-pig lung pulmonary vasculature, and the effect of the perfusate effluent on the vasoconstrictor responses to NA investigated. When 10^{-8} and 10^{-7} $g\ ml^{-1}$ PGE_2 and PGF_{2a} were perfused through guinea-pig lungs, no activity in the effluent was detected on the assay tissues

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(rat stomach strips for PGE_2 and rat colon for PGF_{2a}). The sensitivity of the assay tissues was good enough to detect 0.5 ng ml^{-1} (Fig. 9). The effect of the effluent used immediately after collection on NA vasoconstrictor responses in the rat mesenteric artery are shown in table 3. Only the effluent from 10^{-7} g ml^{-1} showed slight potentiation.

Table 3

The effect of two concentrations of PGE_2 and PGF_{2a} after passage through guinea-pig pulmonary circulation on vasoconstrictor responses to NA in the rat mesenteric artery

Conc. of prostaglandin in Krebs solution perfusing the lungs	Mean P.F. due to lung perfusate	
g ml^{-1}	PGE_2	PGF_{2a}
10^{-8}	1.1 ± 0.2 (n = 8)	Not done
10^{-7}	1.4 ± 0.3 (n = 6)	1.3 ± 0.2 (n = 8)

Interactions of prostaglandins with cocaine and methoxamine

Cocaine is an established inhibitor of neuronal uptake of the adrenergic transmitter substance (Iversen, 1967). In order to find out whether prostaglandins potentiate NA by inhibition of uptake, maximal potentiating concentration of cocaine was obtained as described under methods; and was used separately and in combination

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FIGURE 9

Assay of guinea-pig lung perfusate on rat stomach strip (RSS) and rat colon (RC). The biological activities of PGF_{2a} and PGE_2 were detected more specifically by RC and RSS respectively. Doses under the dots are in ng while 'E' in either case represents the guinea-pig lung effluent.

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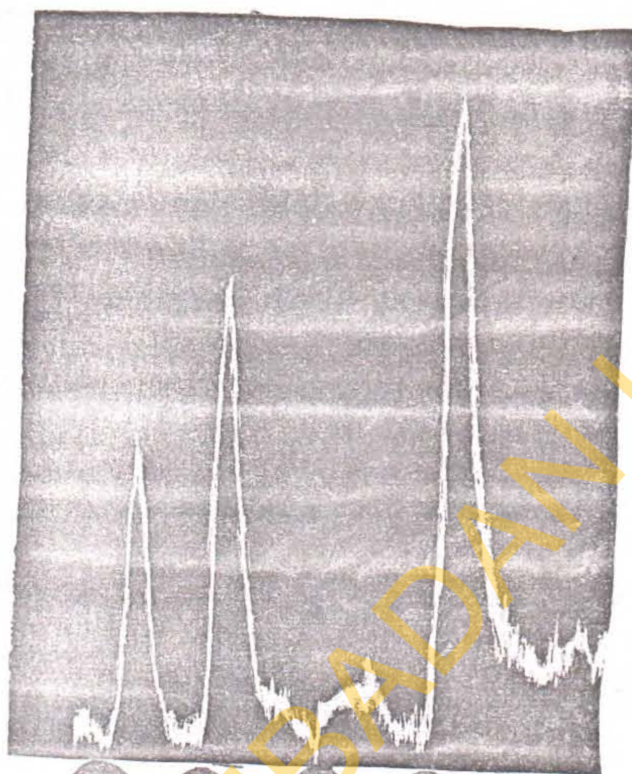
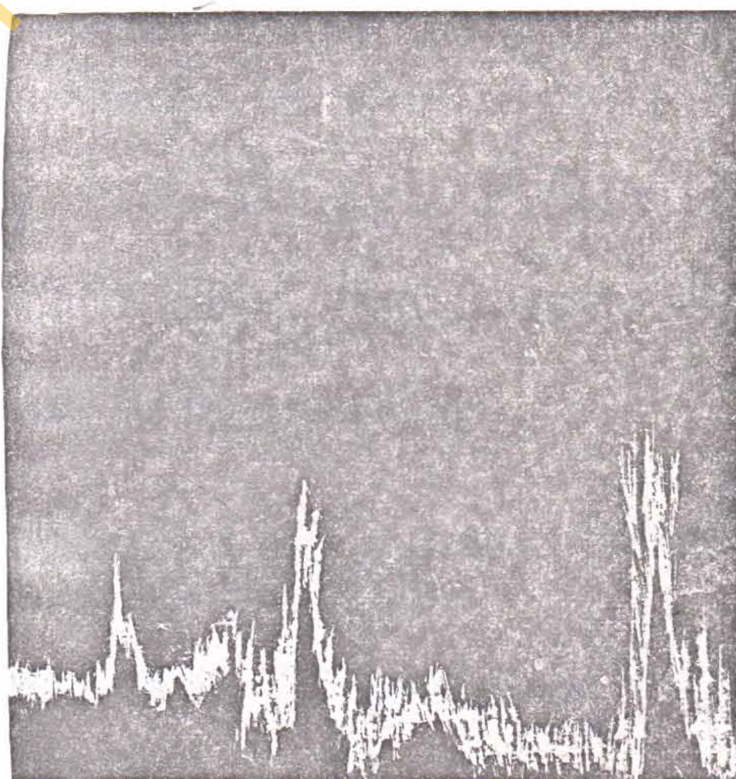
FIG 9

RSS



RC



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with submaximal potentiating concentration of PGE_2 (found to be 10^{-8} g ml^{-1}) on the same preparations. The principle of this test is based on the idea that if PGE_2 is producing potentiation by blocking uptake (as assumed for cocaine), then it should not work in a situation where uptake is completely blocked e.g. by a dose of cocaine which produces maximum potentiation. The results are presented in (fig. 10). From the figure, the P.F. value due to the submaximal potentiating concentration of PGE_2(A) was 5.2 ± 0.1 (n=6) and that due to the maximal potentiating concentration of cocaine (B) 5.6 ± 0.1 (n = 6) while that due to the combination of the former procedures (C) was 5.9 ± 0.1 (n = 6). B and C are significantly different $p < 0.05$.

Methoxamine caused vasoconstriction of the rat mesenteric artery similar to vasoconstrictor response of NA (although less potent than the latter) (see Fig. 5). Dose - response curves for methoxamine were obtained in the absence and presence of PGE_2 and PGF_{2a} and results compared (Fig. 11). Methoxamine which is not a substrate for the uptake process (Iversen, 1967; Trendelenburg et al, 1970) was potentiated by both prostaglandins.

Interactions of prostaglandins with NA in reserpinized rats and in Krebs solution containing bretylium

The mesenteric artery is innervated by a meshwork of adrenergic neurones (Furness & Marshall, 1974). In order to examine whether Prostaglandin - induced supersensitivity was mediated by release

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FIGURE 10

Perfused rat mesenteric artery: effect of cocaine and PGE_2 on NA vasoconstrictor responses. Shown in the figure are the dose - response curves to NA (X) alone; (●) in the presence of 10 ng/ml PGE_2; (O) in the presence of 100 ng/ml cocaine; and (△) in the presence of 10 ng/ml PGE_2 and 100 ng/ml cocaine combined. Standard errors are small and completely masked by the symbols. Each point on the graph represents a mean of 6 - 8 experiments.

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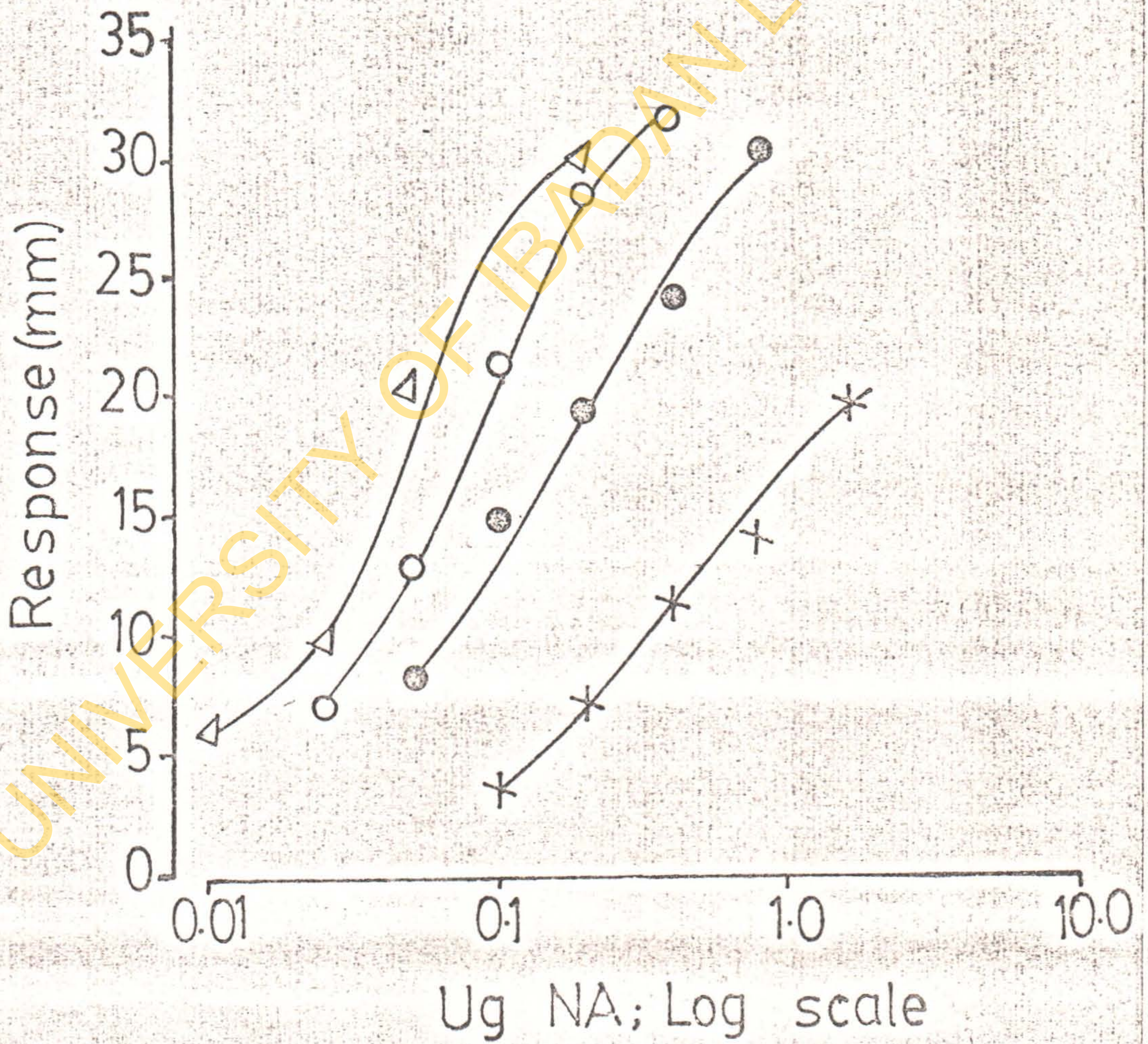

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FIGURE 11

Potentiation of methoxamine - evoked vasoconstrictor responses by prostaglandin E_2 (PGE_2) and PGF_{2a} in rat mesenteric artery. The first set of responses in each panel are control responses. PGE_2 (A) or PGF_{2a} (B), in the concentrations shown was introduced at the arrow. Methoxamine responses were repeated 5 min. after adding a prostaglandin. The doses of methoxamine under the dots are in ug. (A) and (B) are from separate mesenteric artery.

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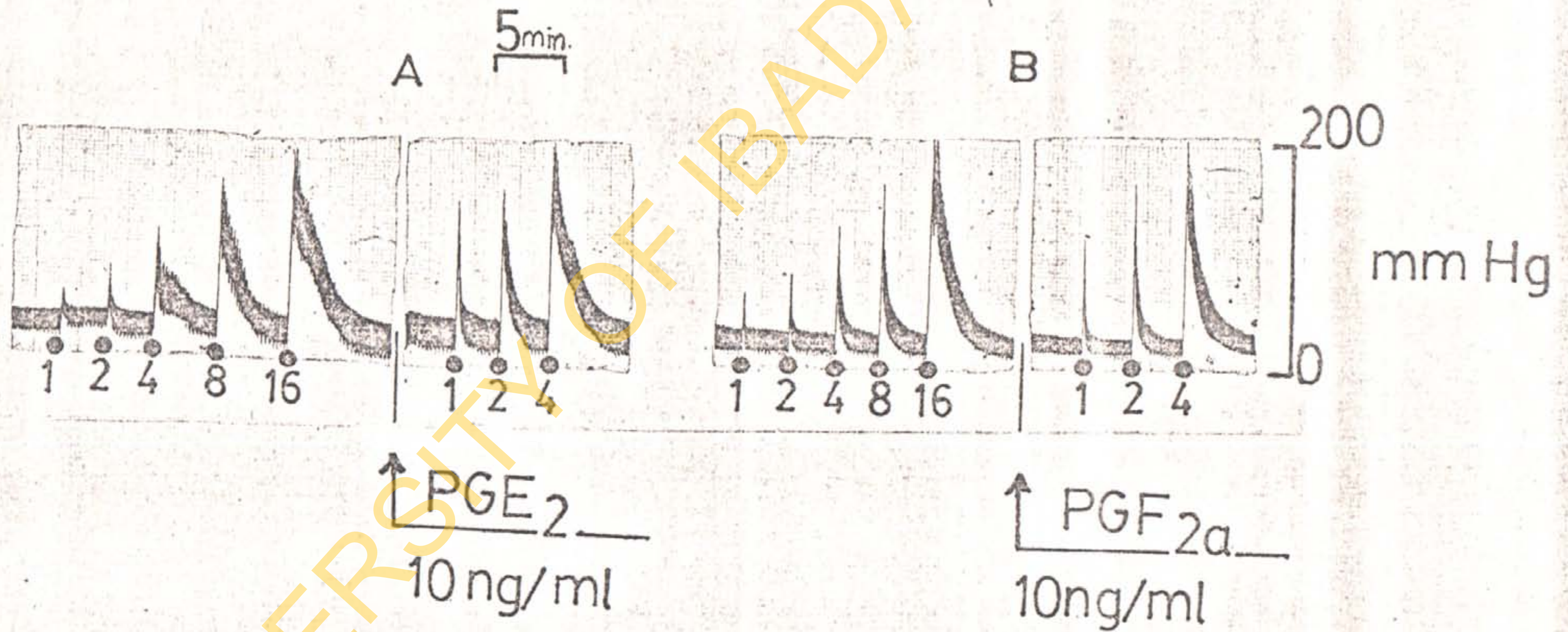
FIG 11



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of transmitter substance, from the adrenergic nerve endings, 6 rats were given a daily dose of reserpine (10 mg/kg) for 3 days before isolating and setting up the artery as described earlier. The potentiation factors due to PGE_2 and PGF_{2a} (10^{-8}g ml^{-1}) in each case on the vasoconstrictor action of NA in control preparations were 5.0 ± 0.9 (n = 6), and 2.2 ± 0.1 (n = 6) respectively. In reserpinized rats, P.F due to PGE_2 was 9.6 ± 1.1 (n = 6), a value that is significantly higher than control (analysis of variance $p < 0.005$). Fig. 12 shows a comparison of ED_{50} to NA obtained in the control and reserpinized rats. It can be observed from the figure that animals pretreated consecutively for 3 days with reserpine were more sensitive to exogenous NA. No such effect was observed in mesenteries isolated from rats pretreated for 1 or 2 days before setting up or when reserpine was administered in vitro (i.e. added to the perfusion medium).

In preparations perfused with Krebs solution containing bretylium (10^{-5}g ml^{-1}), the P.F. values for PGE_2 and PGF_{2a} were 3.0 ± 0.6 and 1.7 ± 0.6 respectively (n = 6) in either case). These values are significantly lower than the controls (i.e. the potentiation induced by the two prostaglandins were reduced in the presence of bretylium).

NA antagonism by phentolamine, tolazoline, yohimbine and phenoxybenzamine (PBZ

As would be expected, phentolamine (3.2×10^{-8} - 25.2×10^{-8}M); tolazoline (2.5×10^{-6} - $4.0\ 10^{-5}$M) and yohimbine (5.0×10^{-8} - 12.8×10^{-7}M) blocked NA in a competitive manner; (a) the NA dose -

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FIGURE 12

Comparison of the ED_{50} of NA in control (A) and reserpinized rats. (B). In B, x——x, 0----0; and 0——0 represent responses to NA 24, 48 and 72 hours respectively after reserpine pretreatment.

ED_{50} in control rats = 0.38 ug

" after 24 hrs of reserpinization = 0.4 ug

" " 48 hrs " " = 0.36 ug

" " 72 " " = 0.20 ug.

ED_{50} in control rats were statistically higher than those obtained in the rats 72 hours after reserpinization ($p < 0.005$: analysis of variance).

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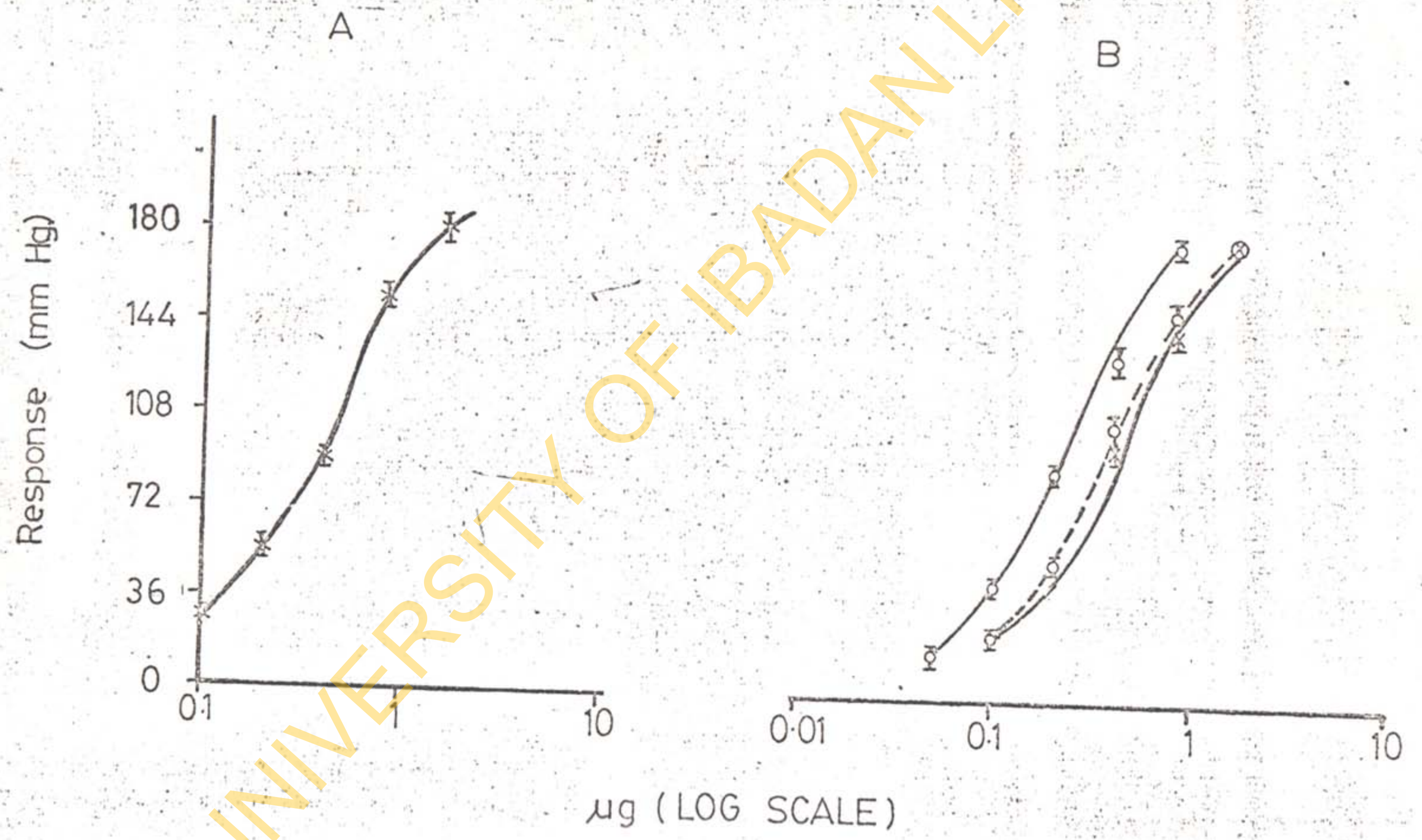
FIG. 12



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response curve in the presence of the antagonist was shifted rightwards parallel to the control curve; and (b) the slopes of the A - S plots 0.96 $\pm$ 0.04 (phentolamine); 1.03 $\pm$ 0.04 (tolazoline) and 0.85 $\pm$ 0.02 (yohimbine) (n = 8 in each case) were not significantly different from 1 ($p > 0.05$).

In the dose range (7.4×10^{-11} - 2.9×10^{-10}M), PBZ block of NA was surmountable by increasing the dose of NA (Fig. 13) and the block exhibited characteristics of competitive antagonism viz, parallel rightward shift of the NA dose-response line; the slope of the A-S line plotted with dose ratios obtained in the presence of three different concentrations of PBZ was 0.87 $\pm$ 0.01. This value was not significantly different from 1. Furthermore, when pA_2 was calculated from the equation $pA_2 = \log(DR-1) - \log(I)$ where (I) = concentration of PBZ, the values obtained with three different concentrations of PBZ were not significantly different from one another as indicated by zero regression of the plot of pA_2 versus Log(I) (Fig. 14). This suggests competitive antagonism (Mackay, 1978). At higher concentrations of PBZ, antagonism of NA vasoconstriction was not competitive. In concentrations higher than 2.9×10^{-10}M, the block of NA could not be overcome by increasing the dose of NA.

Effects of PGE_2 on α- adrenoceptor blockade

Antagonism of NA vasoconstriction caused by phentolamine, tolazoline and yohimbine were consistently reversed by PGE_2 (Figs 15 a & b

FIGURE 13

The block of NA vasoconstriction by phenozybenzamine in rat mesentery. The first set of responses in each panel are the control responses. The second set were obtained in the presence of the stated doses of PBZ. Doses indicated by dots are in ug. (A), (B) and (C) are from separate artery preparations.

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FIG 13

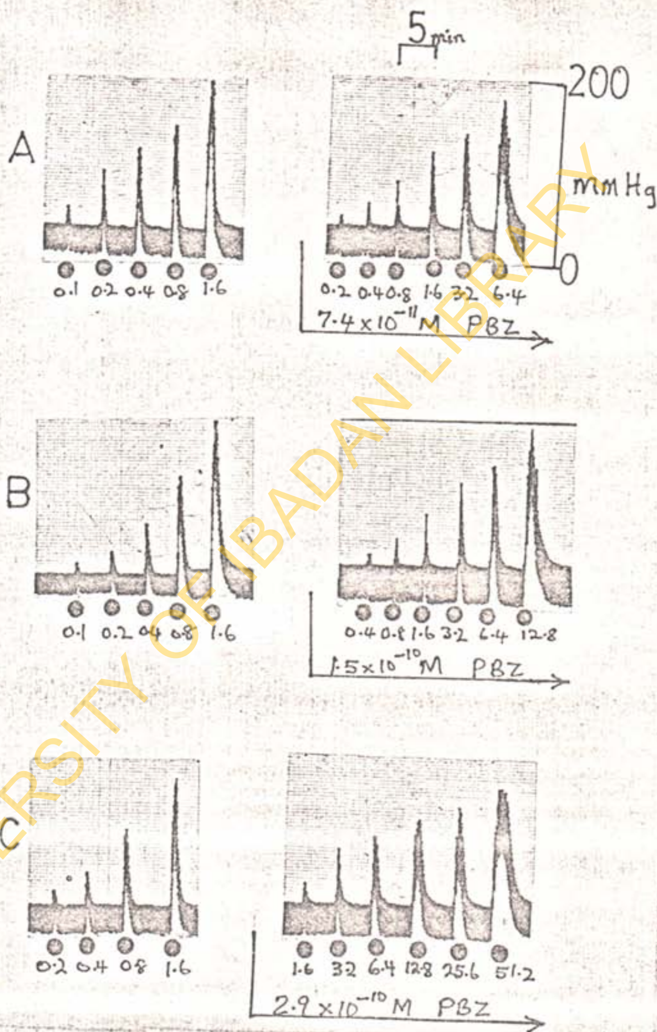

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FIGURE 14

The figure shows a plot of pA_2 against antagonist concentration. The pA_2 values were calculated from the equation pA_2 = Log (DR-1) - Log (I) where (I) = conc. of PBZ (according to Mackay, 1978). Each point on the graph represents the mean of 6 - 8 experiments. Vertical bars represent s.e mean.

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FIG 14

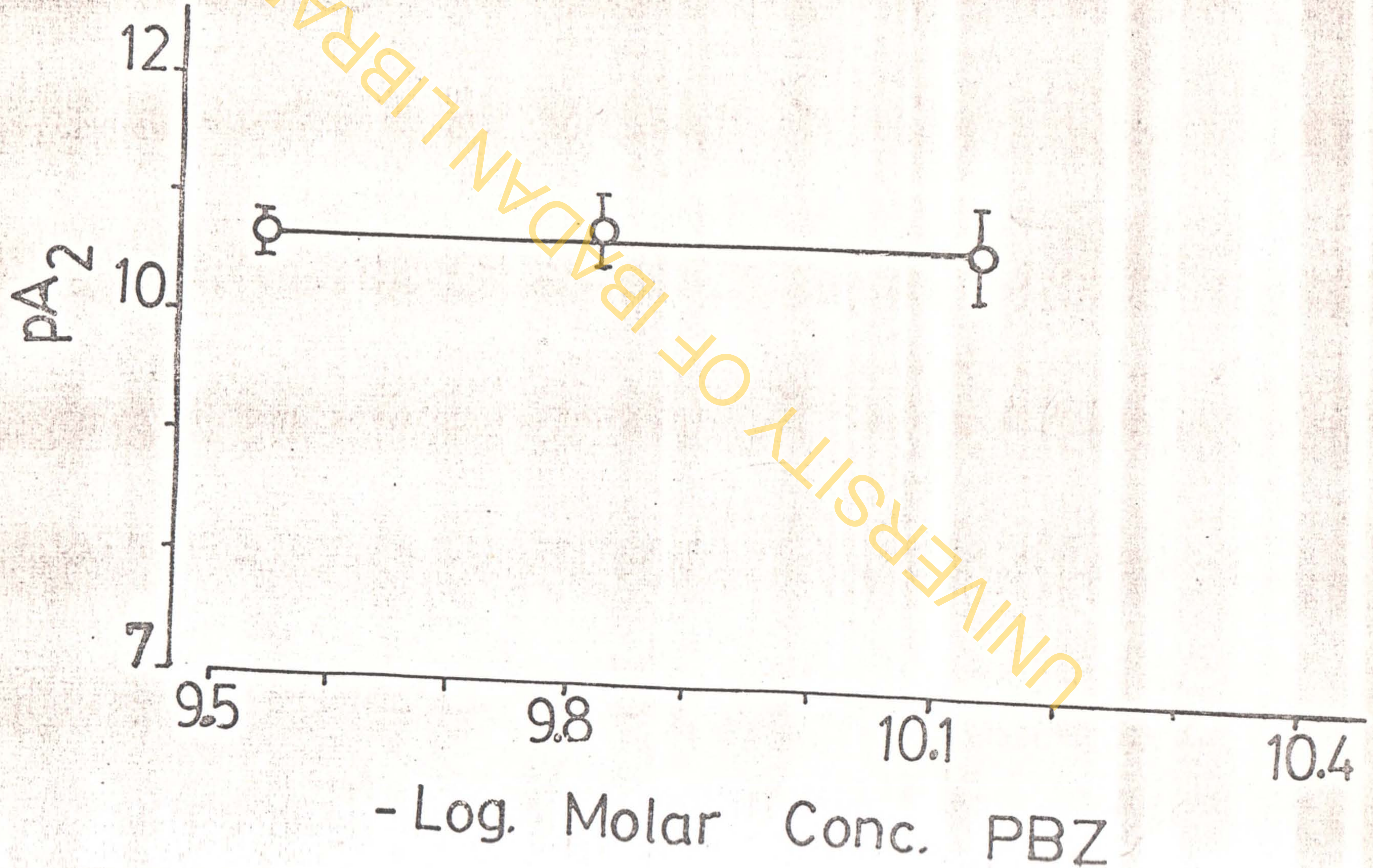

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FIGURE 15a

Reversal of phentolamine antagonism on NA vasoconstrictor responses by PGE_2 in rat mesenteric artery preparation. Doses of NA indicated by the dots are in Ug. Responses in the second panel were obtained in the presence of 3.2×10^{-8}M phentolamine while those in the third panel were in the presence of phentolamine and PGE_2 (2.8×10^{-8}M) combined.

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FIG 15a

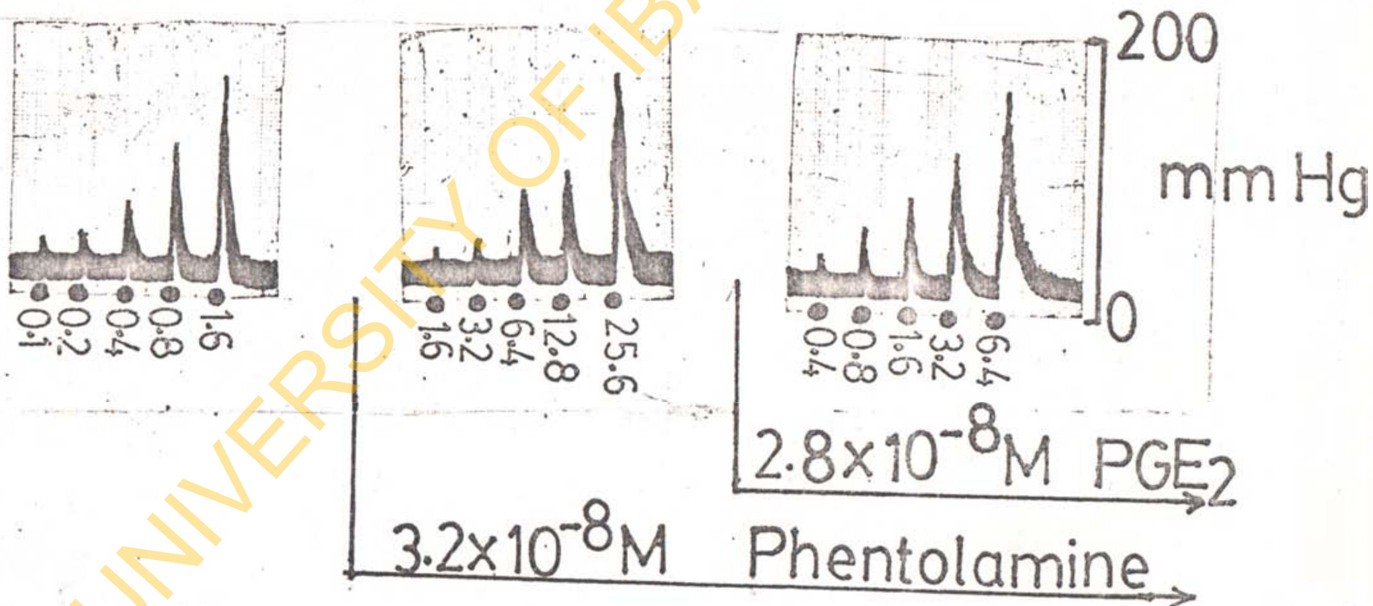

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FIGURE 15b

Reversal of phentolamine (3.2×10^{-8} M); phenoxybenzamine (2.9×10^{-10} M); tolazoline (4×10^{-5} M) and yohimbine, (12.8×10^{-7} M) antagonism on NA vasoconstrictor responses by PGE_2 in rat mesenteric artery preparation. (●) control responses to NA, (O) responses to NA in the presence of each of the antagonists and (O------O) responses to NA in the presence of each of the antagonists and 2.8×10^{-8} M PGE_2. Each point is a mean of 10 - 12 experiments and vertical bars represent S.E.M.

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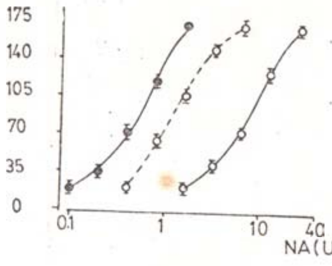
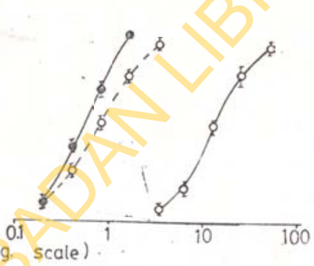
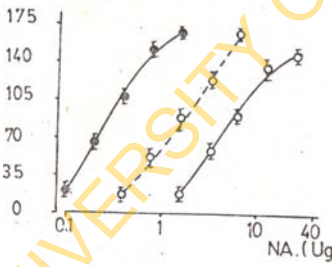
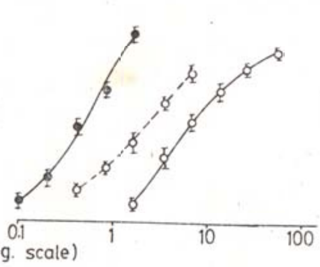
Response (mm Hg)





NA (Ug. Log. scale)





NA. (Ug. Log. scale)

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the degree or reversal being directly proportional to the concentration of the prostaglandin. Antagonism of NA vasoconstriction by PBZ in the concentration range ($7.4 \times 10^{-11} - 2.9 \times 10^{-10}$M) in which the block was competitive, was also reversed by PGE_2 (Fig. 16). Block by PBZ at higher concentration were not reserved by PGE_2.

The degree of reversal by PGE_2 of the block by each of the antagonists was estimated by determining dose-ratios with the antagonist before and in the presence of different concentrations of PGE_2. The results are shown in Table 4. Concentrations of PGE_2 ranging from $2.8 \times 10^{-9} - 2.8 \times 10^{-7}$M greatly reduced the NA dose ratios obtained with the four antagonists.

The ability to PGE_2 to reverse competitive type block of α- adrenoceptor may be taken to suggest that PGE_2 interferred with antagonist - receptor interaction. If this were so, different binding characteristics for the antagonist might be expected. To test this possibility, pA_2 for each antagonist was determined against NA before and in the presence of 2.8×10^{-8}M PGE_2. The results, presented in Table 5, show that PGE_2 does not reduce the affinity of antagonist for the receptor. Indeed, in the case of phentolamine, and tolazoline, PGE_2 significantly increased the pA_2 values. PGF_{2a} was tried against phentolamine block and was found not to cause any change in pA_2 value of the antagonist although reversed its blockade of NA effect.

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FIGURE 16

Reveral of phenoxybenzamine antagonism on NA vasoconstriction by PGE_2 in rat mesenteric artery. Doses of NA indicated by the dots are in ug. Responses in the second panel were obtained in the presence of 2.9×10^{-10}M PBZ while those in the third panel were in the presence of PBZ and PGE_2 (10 ng/ml) combined.

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FIG 16

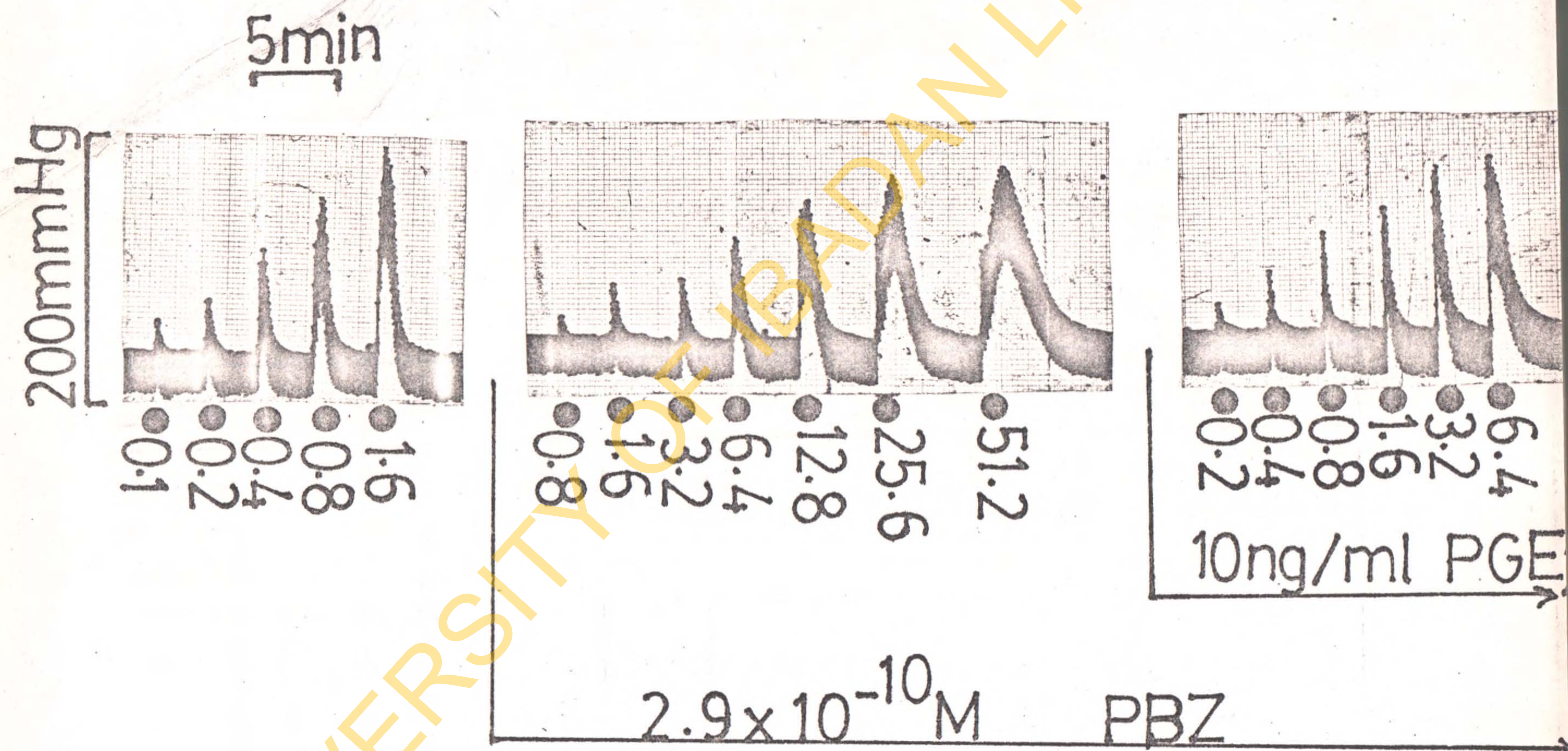

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Table 4

Effect of PGE_2 (2.8×10^{-8} M) on NA blockade caused by various α - adrenoceptor antagonists in isolated perfused rat mesenteric artery. The mean values are derived from 6 - 9 experiments.

ANTAGONIST	Concentration of antagonist	NA dose-ratio in the presence of antagonist alone (DR_A)	NA dose - ratio in the presence of antagonist + PGE_2 (DR_{APG})	$DR_A - DR_{APG}$ ("Reversal factor")	NA dose - ratio in the presence of PGE_2 (DR_P)
PHENTOLAMINE	3.2×10^{-8} M	13.9 ± 1.0	2.5 ± 0.1	11.4 ± 0.8	5.0 ± 0.2
TOLAZOLINE	4.1×10^{-5}	22.0 ± 1.0	5.9 ± 0.4	16.1 ± 0.9	
YOHIMBINE	1.28×10^{-6} M	26.6 ± 0.9	1.7 ± 0.1	24.9 ± 0.9	
PHENOXYBENZAMINE	2.9×10^{-10} M	17.9 ± 1.0	8.4 ± 0.8	9.5 ± 0.7	

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Table 5

Effect of PGE_2 on α - adrenoceptor antagonism in isolated rat mesenteric artery

ANTAGONIST	NORMAL KREBS		DURING PGE_2 2.8 x 10^{-8}M PERFUSION	
	PA_2	SLOPE OF A - S PLOT	PA_2	SLOPE OF A - S PLOT
PHENTOLAMINE	8.58 ± 0.11 (n = 8)	0.96 ± 0.04	* 8.95 ± 0.14 (n = 8)	** 0.92 ± 0.02
TOLAZOLINE	5.69 ± 0.01 (n = 8)	1.03 ± 0.04	* 6.15 ± 0.01 (n = 8)	** 0.97 ± 0.03
YOHIMBINE	7.48 ± 0.07 (n = 8)	0.85 ± 0.02	** 7.54 ± 0.04 (n = 8)	** 0.83 ± 0.01
PHENOXYBENZAMINE (PBZ)	10.84 ± 0.01 (n = 6)	0.87 ± 0.01	** 10.55 ± 1.10 (n = 6)	** 0.83 ± 05

* Significantly higher than controls ($P < 0.005$).

** Not significantly different from controls ($P < 0.05$).

Interactions of indomethacin and prazosin with NA and the effect of PGE_2 on the NA block produced

The inclusion in the perfusion fluid, of indomethacin $2.8 \times 10^{-7}M - 2.8 \times 10^{-5}M$ or prazosin ($1.3 \times 10^{-10}M - 5.3 \times 10^{-10}M$) greatly inhibited the vasoconstrictor action of NA. The threshold dose was not appreciably changed in the presence of each of these antagonists. The magnitude of block in either case increased with time (Fig. 17). Also, the usual characteristics of the response to NA (i.e. quick contractile and relaxant phases) was changed by indomethacin and prazosin. The relaxant phase became very prolonged (Fig. 18). Thus, the drugs increased the duration of action of each dose administered. The block was rapidly overcome by washing out the antagonists. PGE_2 $2.8 \times 10^{-8}M$ reversed completely the indomethacin block due to low doses $2.8 \times 10^{-7}M$ and $2.8 \times 10^{-6}M$. However, with $2.8 \times 10^{-5}M$ indomethacin, reversal by PGE_2 was inconsistent, occuring only in 17 out of every 25 experiments (Figs 19 and 20). Prazosin block of NA on the other hand, was consistently irreversible by PGE_2 (Fig. 20) even up to concentration of $2.8 \times 10^{-6}M$. The block produced by $2.8 \times 10^{-7} - 2.8 \times 10^{-6}M$ indomethacin and $1.3 \times 10^{-10}M - 2.6 \times 10^{-10}M$ prazosin, was surmountable by increasing the dose of NA. However, there was little reduction of the maximal response (to about 80% level) when indomethacin and prazosin concentrations were increased to $2.8 \times 10^{-5}M$ and $5.3 \times 10^{-10}M$ respectively. The suppression was more severe when the concentration of the antagonists was further increased. pA_2 values

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FIGURE 17

Perfused rat mesenteric artery preparation: Increasing NA dose ratio in the presence of 2.8 x 10^{-5}M indomethacin (o) and 2.3 x 10^{-10}M prazosin (●). Each point or the graph is a mean of 6 experiments on separate tissue preparations. Vertical bars are s.e mean.

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FIG 17

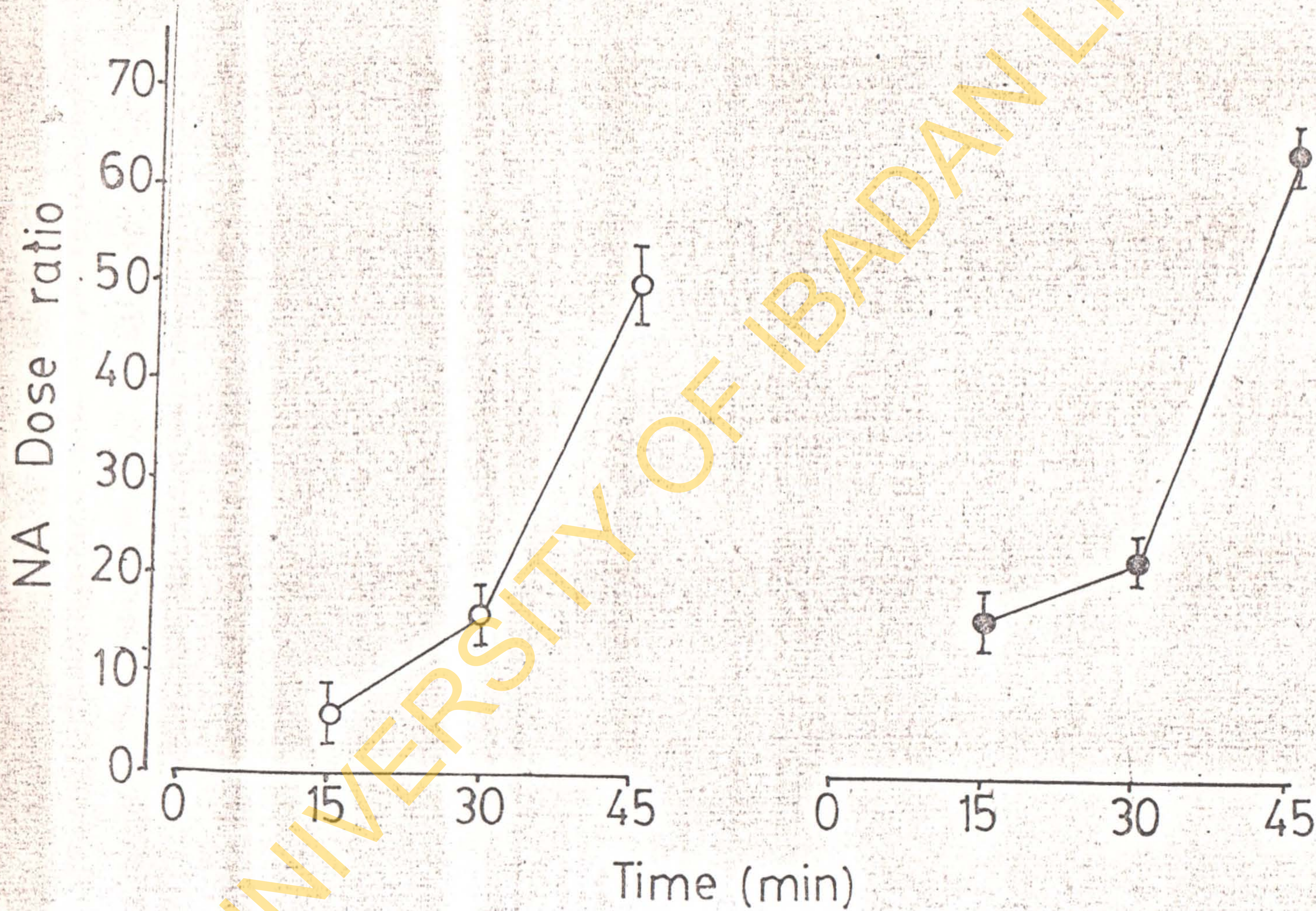

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FIGURE 18

Isolated rat mesenteric artery prěparation: effect of prazosin on the nature of NA Vasoconstrictor responses. Doses indicated by the dots are in ug. Responses to NA in the presence of indomethacin are similar.

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Fig. 18

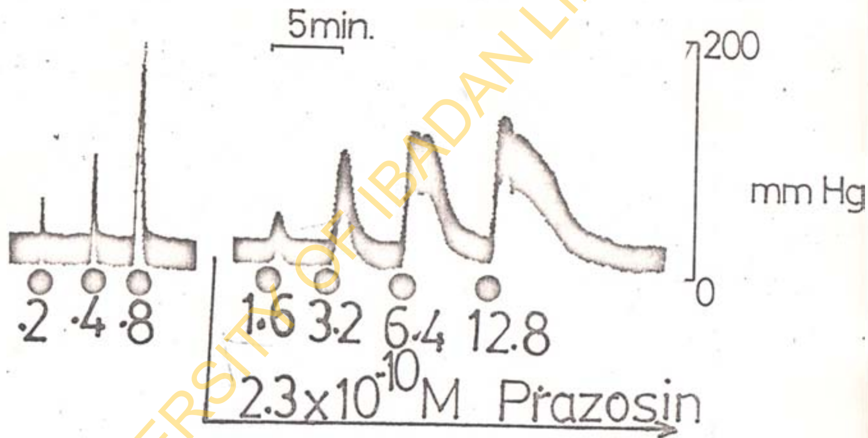

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FIGURE 19

The block of NA vasoconstriction by indomethacin and its reversal by PGE_2. The first panel are the control responses; the second are responses in the presence of 2.8×10^{-5}M indomethacin while the third panel represents responses in the presence of indomethacin and PGE_2 (10 ng/ml) combined. Doses of NA indicated by the dots are in ug.

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FIG 19

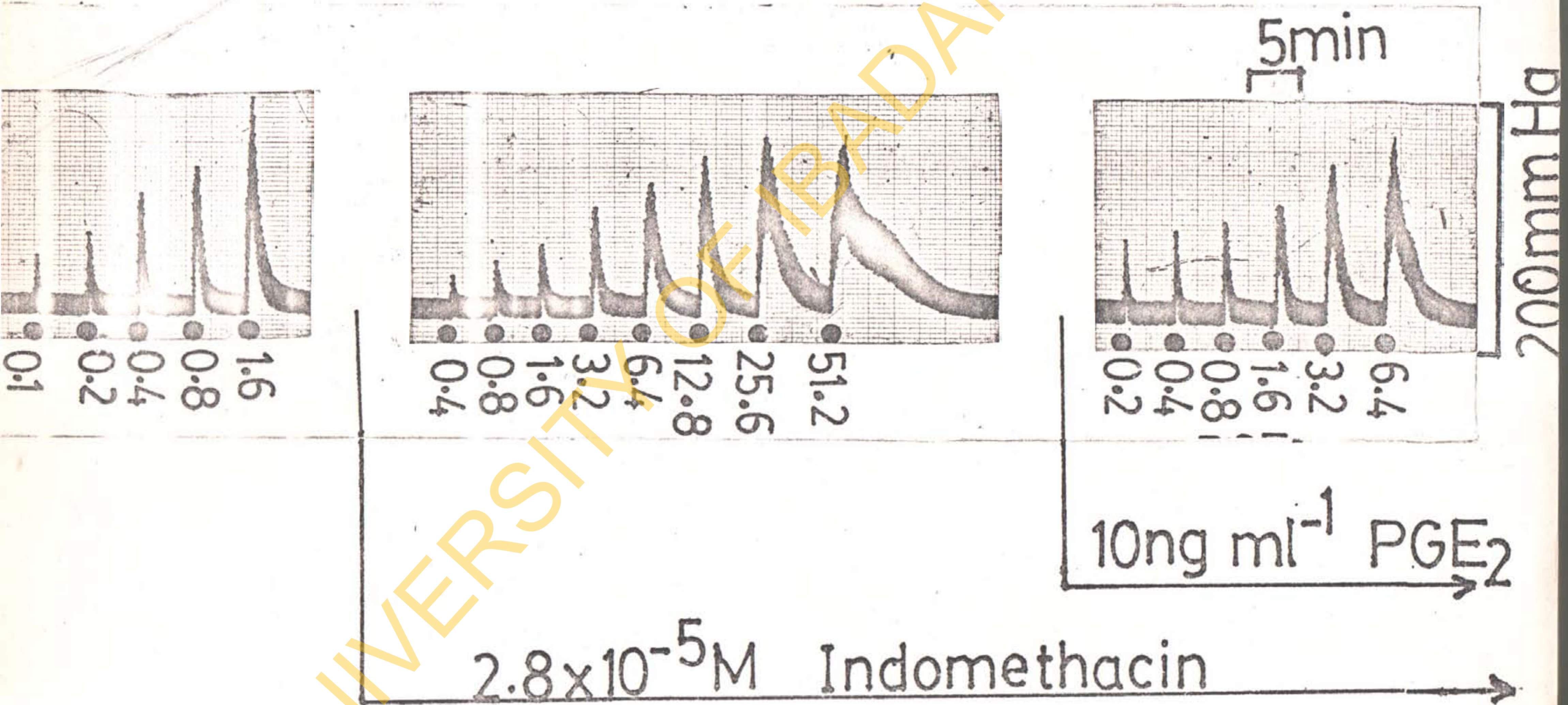

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FIGURE 20

Interaction of PGE_2 with indomethacin (A) and prazosin (B). (X) control responses to NA; (O) responses to NA in the presence of $2.8 - 10^{-8}$M indomethacin in A and 1.3×10^{-10}M prazosin in B; and (●) responses to NA in the presence of the antagonists and PGE_2 (2.8×10^{-8}M). Each point on the graph is a mean of 6 - 8 experiments. Vertical bars represent S.E.M.

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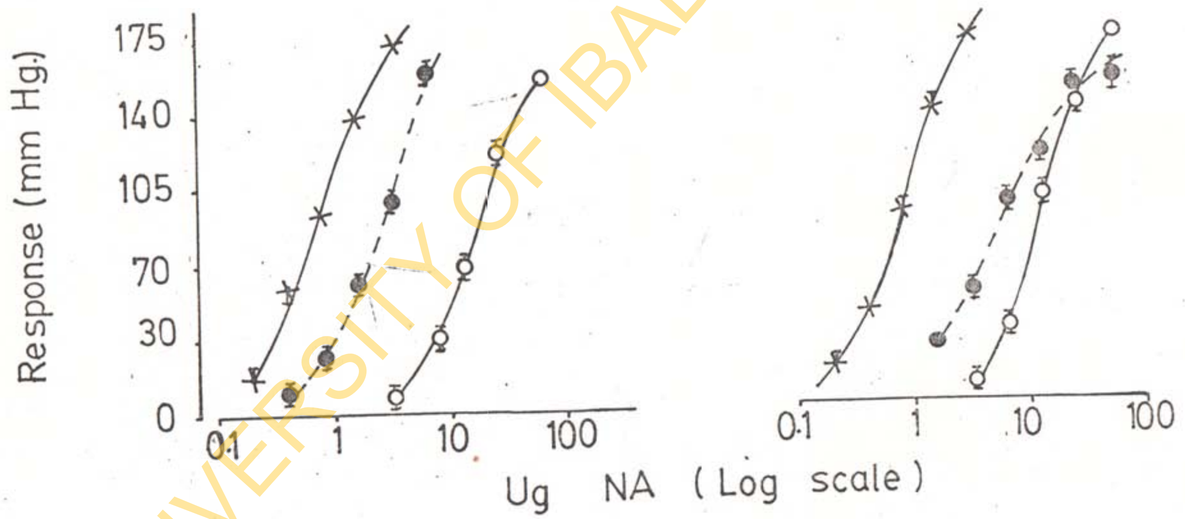
FIG 20



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(see chapter 2) were measured at any three concentrations at which maximum response to NA was not below 80%. When such pA_2 values were plotted against antagonist concentrations, the resultant line was not linear indicating that the block was non-competitive in nature (Fig. 21).

Effect of PGE_2 on NA block produced by cinnarizine and verapamil

Both cinnarizine and verapamil are potent antagonists of the excitation - contraction coupling system in the vascular smooth muscle (Godfraind & Kaba, 1972). Neither of these two substances has been shown to possess α - adrenoceptor blocking activity although it has been observed in the course of this study that both compounds are potent antagonists of NA -induced vasoconstrictor responses of the rat mesentery. NA block by these agents was always accompanied by severe depression of the maximum response i.e. the block was non-competitive in nature. Neither PGE_2 nor PGF_{2a} interferred with the blockade (Fig. 22).

Calcium ionophore A23187 influence on the basal perfusion pressure of the rat mesentery

In concentrations lower than 9.6×10^{-7}M A23187 did not alter the basal perfusion pressure of the rat mesenteric artery. But, all concentrations above 9.6×10^{-7}M consistently induced a characteristically slow contraction of the artery. The slow vasoconstrictor response was characteristic because it occured only on the first contact of the ionophore with the tissue. The

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FIGURE 21

Plot of pA_2 values against concentrations of indomethacin (left panel) and prazosin (right panel). Each point on the graph is derived from 6 - 8 experiments. Vertical bars are the S.e mean.

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FIG 21

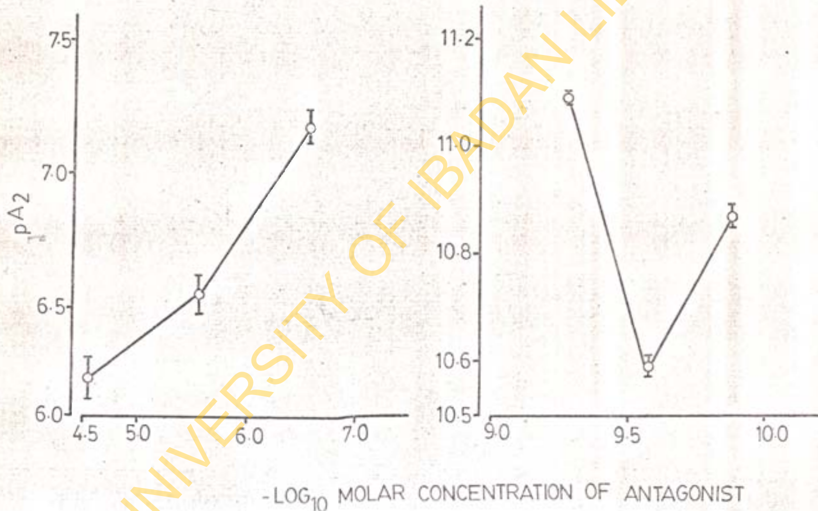

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FIGURE 22

Interaction of PGE_2 (10 ng/ml) with the block of NA by cinnarizine and verapamil. (●) represents control responses to NA; (O) responses in the presence of the stated doses of cinnarizine or verapamil, ●——● responses in the presence of cinnarizine or verapamil plus PGE_2 combined. Each point on the graph is derived from the mean of 8 - 12 experiments. Vertical bars represent S.E. mean.

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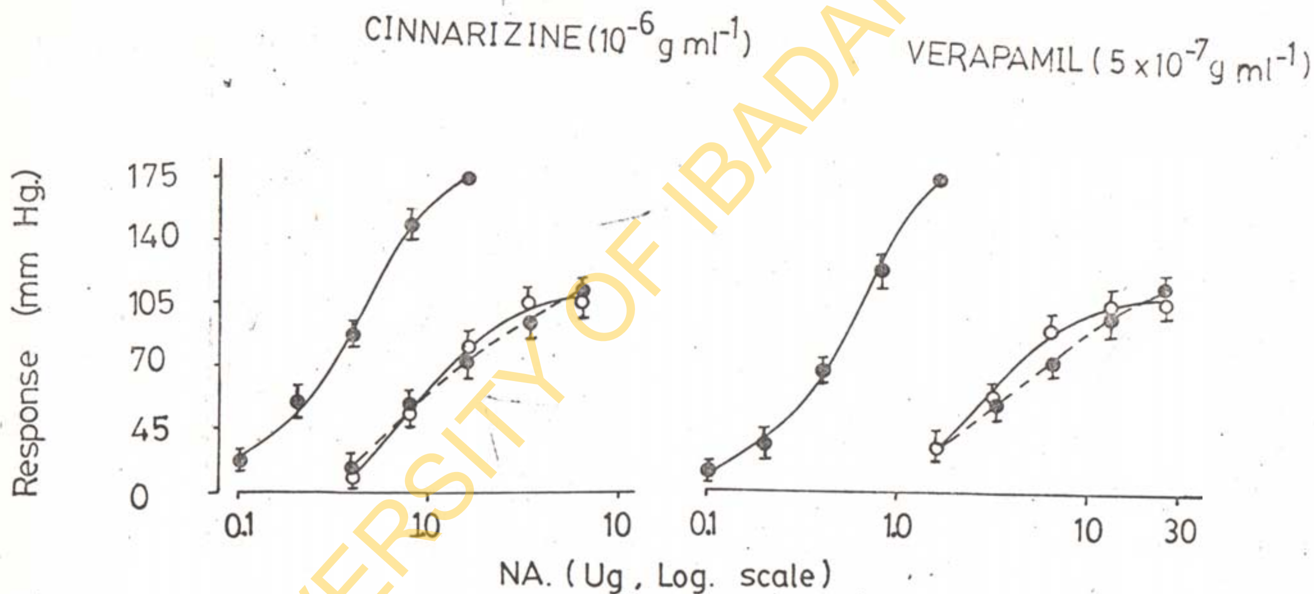
Fig. 22



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contraction faded away until base line pressure was again attained. Subsequent increase in concentration of ionophore in the perfusion medium did not induce vasoconstriction. However, using different tissue preparations for each concentration of ionophore in the perfusion medium, the transient vasoconstrictor effect seemed to increase with increasing dose of A23187 (Fig. 23).

Interaction of A23187 with NA and methoxamine

A23187, in concentrations ranging from 1.9×10^{-9}M - 9.6×10^{-7}M did not evoke any contractile response but greatly potentiated NA - and methoxamine - induced vasoconstrictor responses in the rat mesenteric artery. The magnitude of the potentiation faded with time (Fig. 24).

Influence of A23187 on α- adrenoceptor block by phentolamine, tolazoline and yohimbine

Like PGE_2, A23187 potentiated NA vasoconstriction and also reversed α-adrenoceptor blockade over a wide range of doses. 1.9×10^{-6}M A23187 potentiated NA nearly nine-fold. The effect of this concentration on α-adrenoceptor blockade by phentolamine, tolazoline and yohimbine was investigated since one possible way of overcoming antagonism might be by facilitation of excitation - contraction coupling. The results are shown in table 6.

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FIGURE 23

Vasoconstrictor action of increasing doses of A23187 in rat mesenteric artery preparation. The doses indicated by dots are in Molar (M). Each response is obtained from a separate artery preparation.

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FIG 23

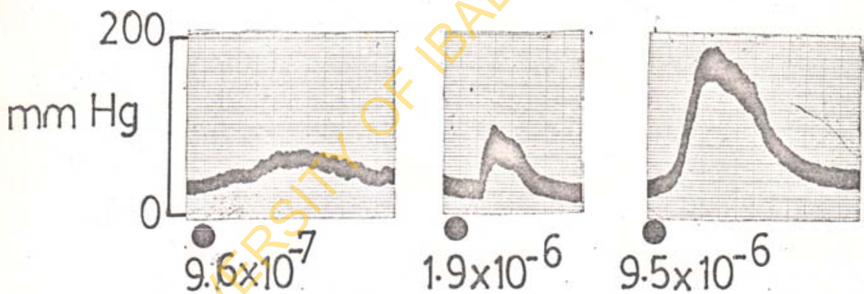

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FIGURE 24

The relationship of the potentiating effect of A23187 with time. Each point on the graph is a mean of observations derived from 8 experiments. Vertical bars represent s.e. mean.

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FIG. 24

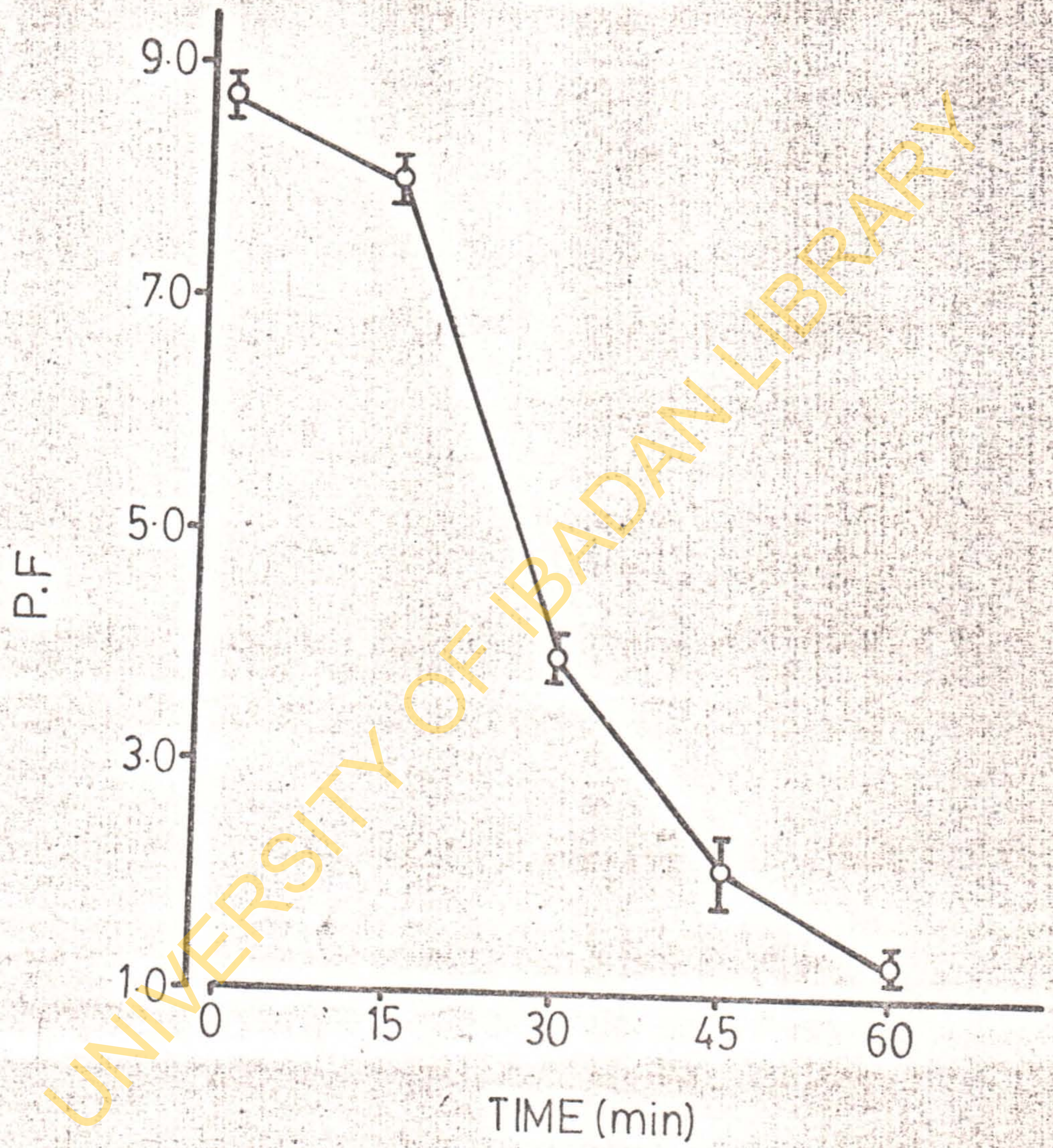

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TABLE 6

Effect of A23187 (1.6×10^{-6}M) on NA blockade caused by various α- adrenoceptor antagonists in isolated perfused rat mesenteric artery. Each mean value is derived from six experiments

ANTAGONIST	NA dose-ratio in the presence of antagonist alone (DR_A)	NA dose ratio in the presence of antagonist +A23187 $DR_{A+A23187}$	$DR_A - DR_{A+A23187}$ (Reversal factor)	NA dose-ratio in the presence of A23187 alone DR_P
PHENTOLAMINE (3.2×10^{-8}M)	16.1 ± 1.3	6.7 ± 0.6	9.4 ± 1.1	
TOLAZOLINE (4.1×10^{-5}M)	22.3 ± 1.0	14.3 ± 1.4	8.1 ± 0.9	8.7 ± 1.0
YOHIMBINE (1.28×10^{-6}M)	24.8 ± 0.4	17.0 ± 0.8	7.8 ± 0.5	

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It can be seen that A23187 reduced considerably the NA dose-ratios obtained in the presence of the three antagonists.

Interaction of A23187 with NA in reserpinized rats

In order to determine whether A23187 - induced potentiation was due to release of NA from the adrenergic nerve endings, 6 rats were injected intraperitoneally with reserpine (5 mg/kg) for two consecutive days. About 12 hrs after the second injection, the mesenteric arteries were isolated and set up as described under methods. Short-term reserpinization has been shown in an earlier part of this thesis not to sensitize mesenteric artery to NA. Reserpine pretreatment abolished the potentiating effect of A23187 (Fig. 25).

Effect of α- adrenoceptor antagonists, prostaglandin synthetase inhibitors (PGSIs), and Ca^{2+} antagonists on A23187 - induced contractions of the rat mesentery

The mechanism by which A23187 induced contraction of the mesenteric vascular bed was investigated. Three different classes of antagonists were tested. The first were the α- adrenoceptor antagonists phentolamine and yohimbine; the second were the PGSIs, indomethacin and sodium meclofenamate; and the third category were the Ca^{2+} antagonists, cinnarizine and verapamil. Since A23187-induced vasoconstriction was tachyphylactic, the control and the test arteries were from different rats. The height of contraction due to A23187 was measured in a set of arteries and compared with

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FIGURE 25

Interaction of A23187 (1.9×10^{-6} M) with NA in untreated (left) and reserpine pretreated (right) rats. (X) represents control responses in each of the panels while (O) represents the responses in the presence of A23187. Each point on the graph is a mean of 8 - 10 experiments.

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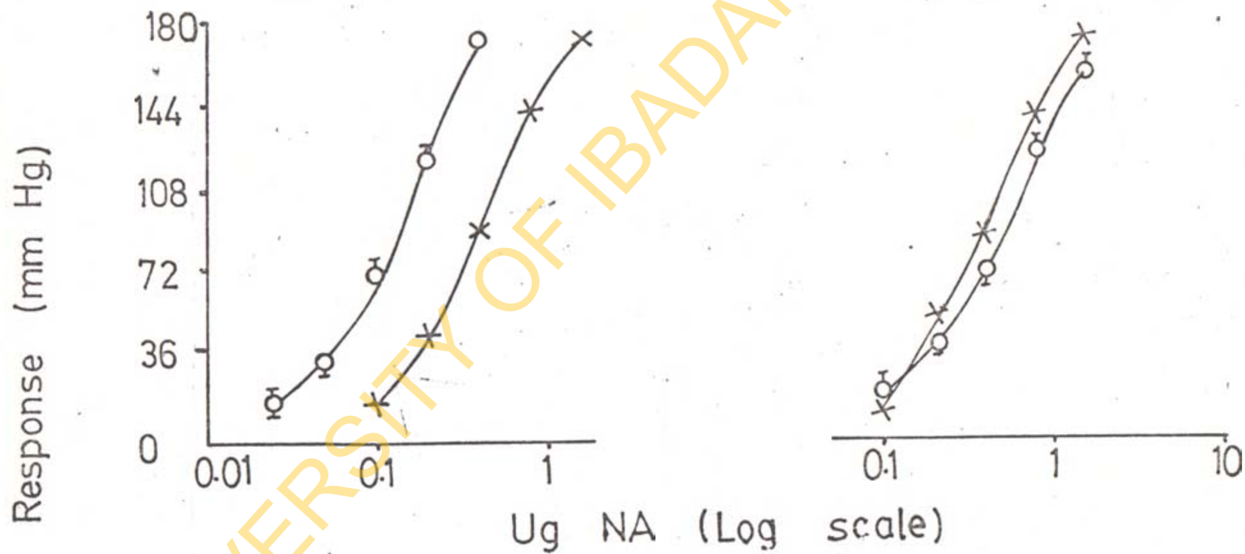
Fig. 25



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the height of contraction by the same dose of A23187 in another set of arteries perfused with Krebs solution containing a desired concentration of the test drug (Fig.26). The % inhibition was calculated from the relationship $\frac{H_A - H_{At}}{H_A} \times \frac{100}{1}$

where H_A = height of A23187 - induced vasoconstriction in Krebs solution,

H_{At} = height of A23187 - induced vasoconstriction during perfusion with Krebs solution containing the test drug.

The results are summarized in Table 7. It can be seen from the table that neither phentolamine nor yohimbine up to 1.6×10^{-5}M and 2.6×10^{-5}M respectively, inhibited A23187 - induced contraction appreciably. On the other hand, indomethacin, sodium meclofenamate, cinnarizine and verapamil inhibited and almost completely abolished A23187 - evoked contractions.

Vasoconstrictor responses of the rat mesentery to potassium chloride

High concentrations of potassium chloride (KCl) caused an increase in perfusion pressure - an indication of arterial vasoconstriction. The vasoconstrictor effect was clearly dose-dependent and rapidly reversible (see Fig. 5). Since K^+- induced contraction of this tissue is unaffected by short-term reserpinization of the experimental animals, the responses are not thought to be due to

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FIGURE 26

A23187 - induced vasoconstriction in rat mesenteric artery. I_1, I_2, and I_3 are responses to A23187 in the presence of increasing concentrations of indomethacin. B_t and V_t are responses in the presence of phentolamine and verapamil respectively.

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Fig 26

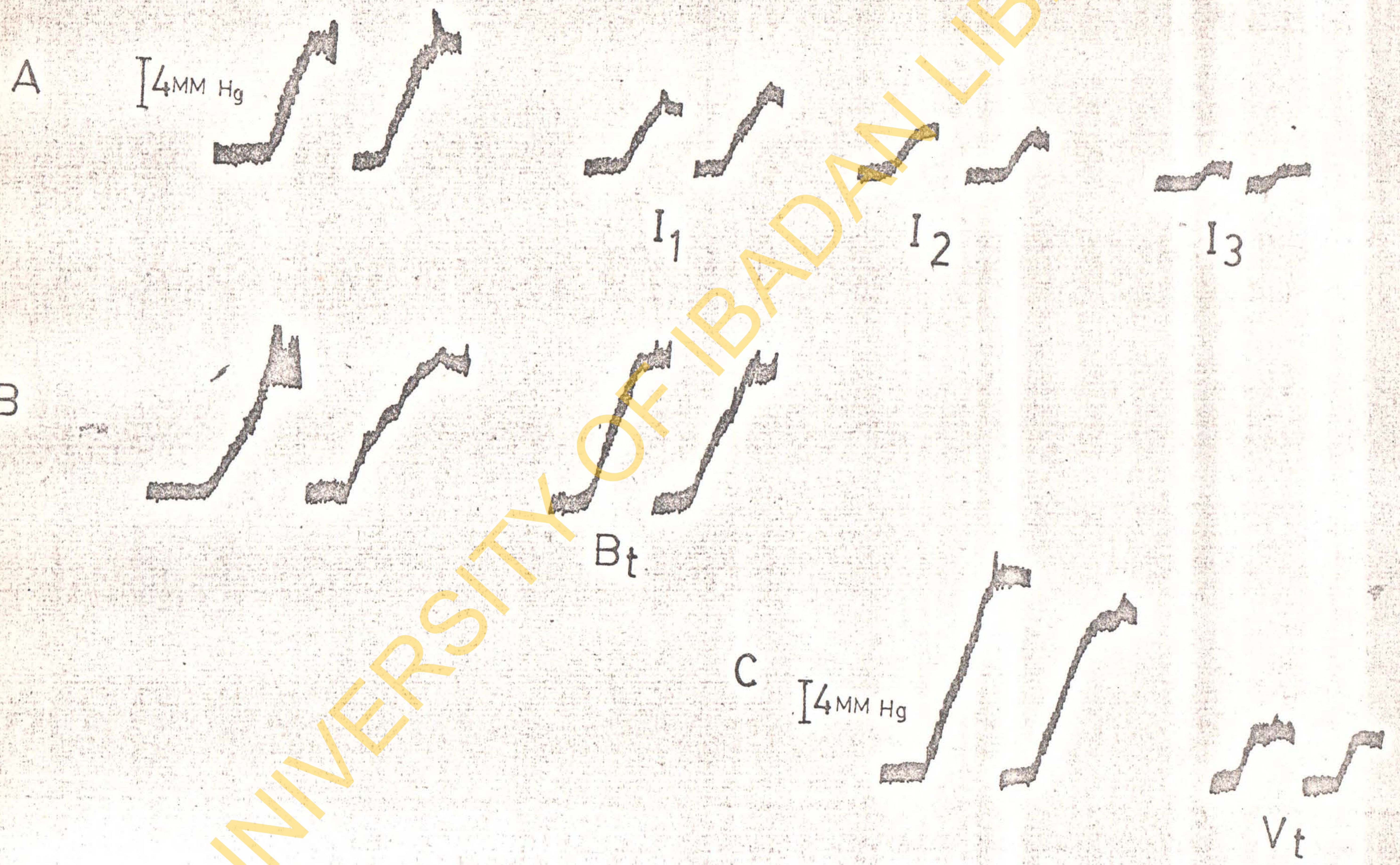

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TABLE 7

Antagonism of A23187-induced contractions of the rat mesentery by α- adrenoceptor antagonists (phentolamine and yohimbine); PGSIs (indomethacin and sodium meclofenamate) and Ca^{2+} antagonists (cinnarizine and verapamil)

Antagonist	Concentration (M)	% inhibition of A23187-evoked contraction
Phentolamine	3.2×10^{-7}	0
	3.2×10^{-6}	8.0 ± 0.5
	1.6×10^{-5}	25.0 ± 3.0
Yohimbine	2.6×10^{-7}	0
	2.6×10^{-6}	5.0 ± 1.0
	2.6×10^{-5}	18.0 ± 2.5
Indomethacin	2.8×10^{-7}	15.0 ± 1.0
	2.8×10^{-6}	68.0 ± 3.5
	2.8×10^{-5}	82.0 ± 1.5
Sodium Meclofenamate	2.5×10^{-7}	20.0 ± 2.5
	2.5×10^{-6}	80.5 ± 3.5
	1.2×10^{-5}	90.0 ± 5.0
Cinnarizine	2.7×10^{-7}	19.0 ± 1.8
	2.7×10^{-6}	50.5 ± 5.0
	1.4×10^{-5}	95.0 ± 3.0
Verapamil	2×10^{-7}	20.0 ± 2.0
	2×10^{-6}	65.0 ± 4.0
	1×10^{-5}	98.0 ± 4.5

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catecholamine release (Fig. 27), rather they could be due to massive Ca^{2+} influx consequent upon the membrane depolarizing action of potassium.

Effect of E - C coupling antagonists on K^+ - induced vasoconstriction

The α- adrenoceptor antagonist, phentolamine, up to the concentration of 3.2×10^{-5}M, did not inhibit K^+- induced vasoconstriction of the rat mesentery. Also, prazosin, a very potent NA antagonist, did not antagonize responses to KCl even when its concentration in the perfusion medium was as high as 2.6×10^{-4}M. Similarly, indomethacin, up to 2.8×10^{-5}M did not affect K^+- induced vasoconstriction. On the other hand, PBZ in doses that have been shown to block NA non-competitively (i.e. above 2.9 $\times 10^{-10}$M) and low concentrations of cinnarizine and verapamil were potent antagonists of the K^+- induced vasoconstriction. The order of potency being cinnarizine = verapamil > PBZ. The results are summarized in (Fig. 28). Another figure, (Fig. 29) compares these antagonists against NA and KCl. It can be observed from the latter figure that the potent antagonists of NA-induced vasoconstriction, prazosin, phentolamine and indomethacin in that order were inactive against K^+- induced vasoconstriction.

Effect of PGE_2 on K^+- induced vasoconstriction

It was of interest to determine the effect of PGE_2 on K^+-induced vasoconstriction. Doses of PGE_2 which have been shown to potentiate NA were tested against K^+- induced vasoconstriction. The results

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FIGURE 27

Isolated rat mesenteric artery preparation : Dose - response curves to potassium chloride in normal (X) and reserpinized (O) rats. Each point on the graph represents measurements from 6 - 8 separate artery preparations. Vertical bars represent s.e. mean.

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Fig 27

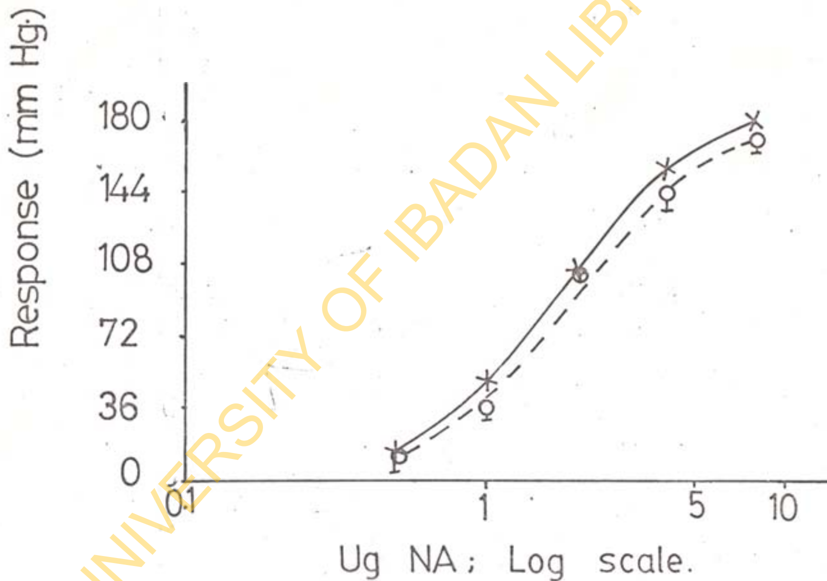

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FIGURE 28

Antagonism of potassium chloride vasoconstrictor responses by cinnarizine (△); verapamil (◉); phenoxybenzamine (X) and indomethacin (□) in the isolated rat mesenteric artery preparation. Each point on the graph is a mean of measurements from 8 - 10 separate artery preparations. S.E mean are small and cannot be represented due to large symbols used on the graph.

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FIG 28

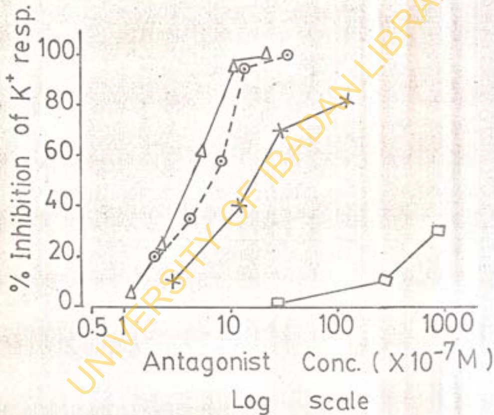

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FIGURE 29

Comparative effect of α- receptor antagonists, Ca^{2+} antagonists; and indomethacin on NA - (A) and Kcl - (B) evoked vasoconstrictor responses in rat mesenteric artery preparation. Responses in the presence of prazosin (O); phenoxybenzamine (X); phentolamine (●); cinnarizine (△); verapamil (⊗) and indomethacin (□) are shown on the graphs. Prazosin and phentolamine did not antagonize Kcl - evoked contractions at all concentrations used. Each point on the graphs is a mean of 6 - 9 experiments. S.E mean are small and cannot be represented.

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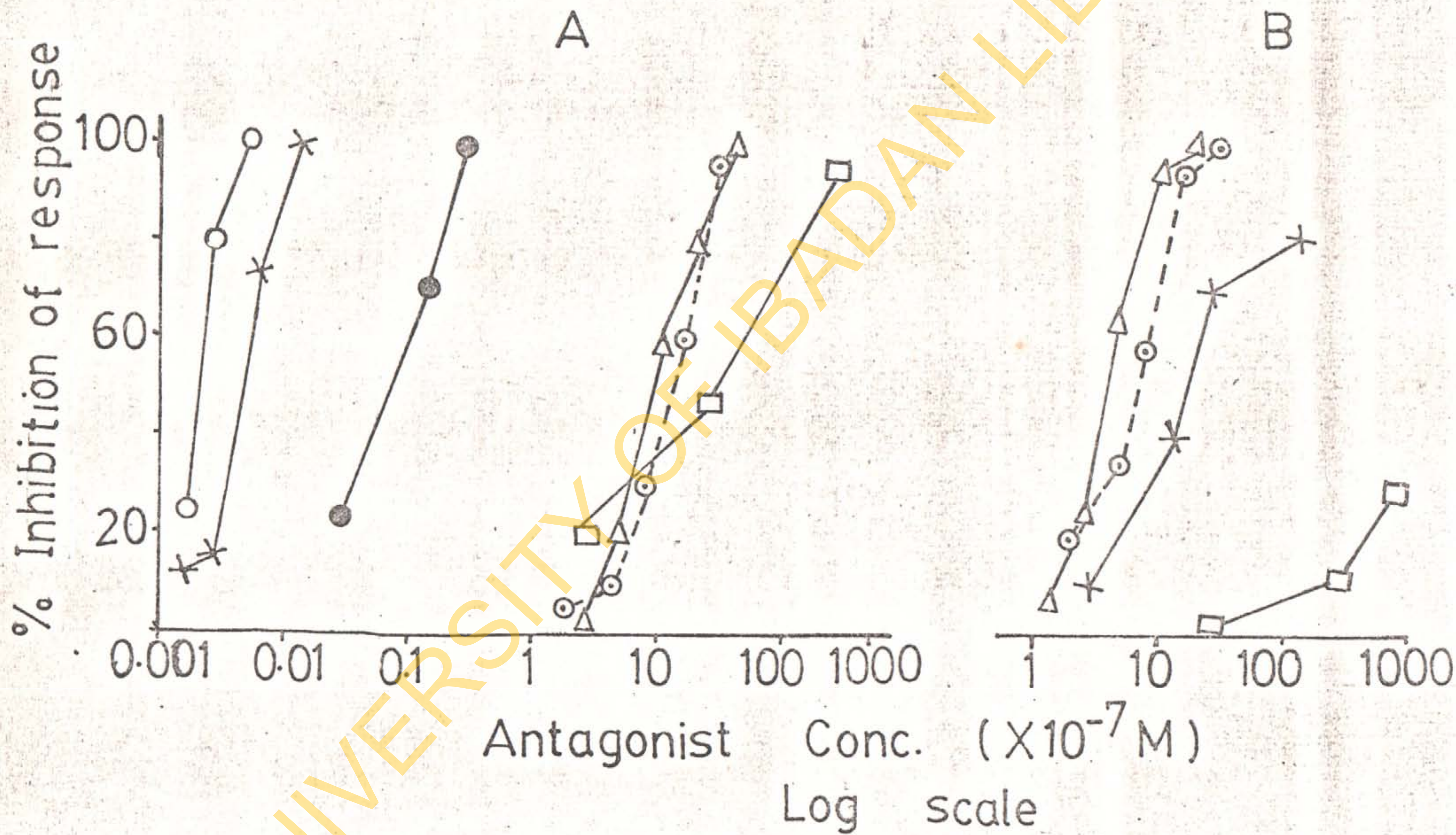
FIG 29



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are presented in Fig.30. It can be observed from the figure that PGE_2 did not potentiate K^+- induced vasoconstrictor effect in the rat mesentery.

EFFECT OF EXTERNAL Ca^{2+}

It is known that NA and K^+ utilize different sources of activator Ca^{2+} for contraction. Therefore, it was important to know the influence of external Ca^{2+}:

(a) on the vasoconstriction induced by these agonists (i.e. NA and K^+). Towards this end, experiments were carried out to study the effect of omitting Ca^{2+} from the perfusing Krebs solution on NA - and KCl - induced vasoconstrictor effects. After allowing the preparation to equilibrate for 30 min. in Ca^{2+} - free Krebs, 0.8 ug NA or 16 mg KCl was injected at 5 min. interval over a period of 1 hr. It was observed that NA action was substantially sustained over this period of time while responses to kcl were drastically reduced within the first 15 min. (Figs 31a and 31b). It was thus evident that external Ca^{2+} played a greater role in K^+- induced contraction than it did in NA - induced contraction.

(b) on the potentiation of NA by PGE_2, PGF_{2a} and Ca^{2+} ionophore, A23187

A possible involvement of Ca^{2+} on the potentiation of NA induced by prostaglandins or A23187 was investigated by eliminating Ca^{2+} ions from the krebs solution perfusing the artery. First, the effect of omitting Ca^{2+} on dose response curves to NA was studied.

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FIGURE 30

Effect of prostaglandin E_2 (PGE_2, 10 ng/ml) on vasoconstrictor responses of rat mesenteric artery to potassium chloride: (O) control responses; ● responses in the presence of PGE_2. Each point is a mean of measurements from at least 6 separate preparations. Vertical bars represent s.e. mean.

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FIG 30

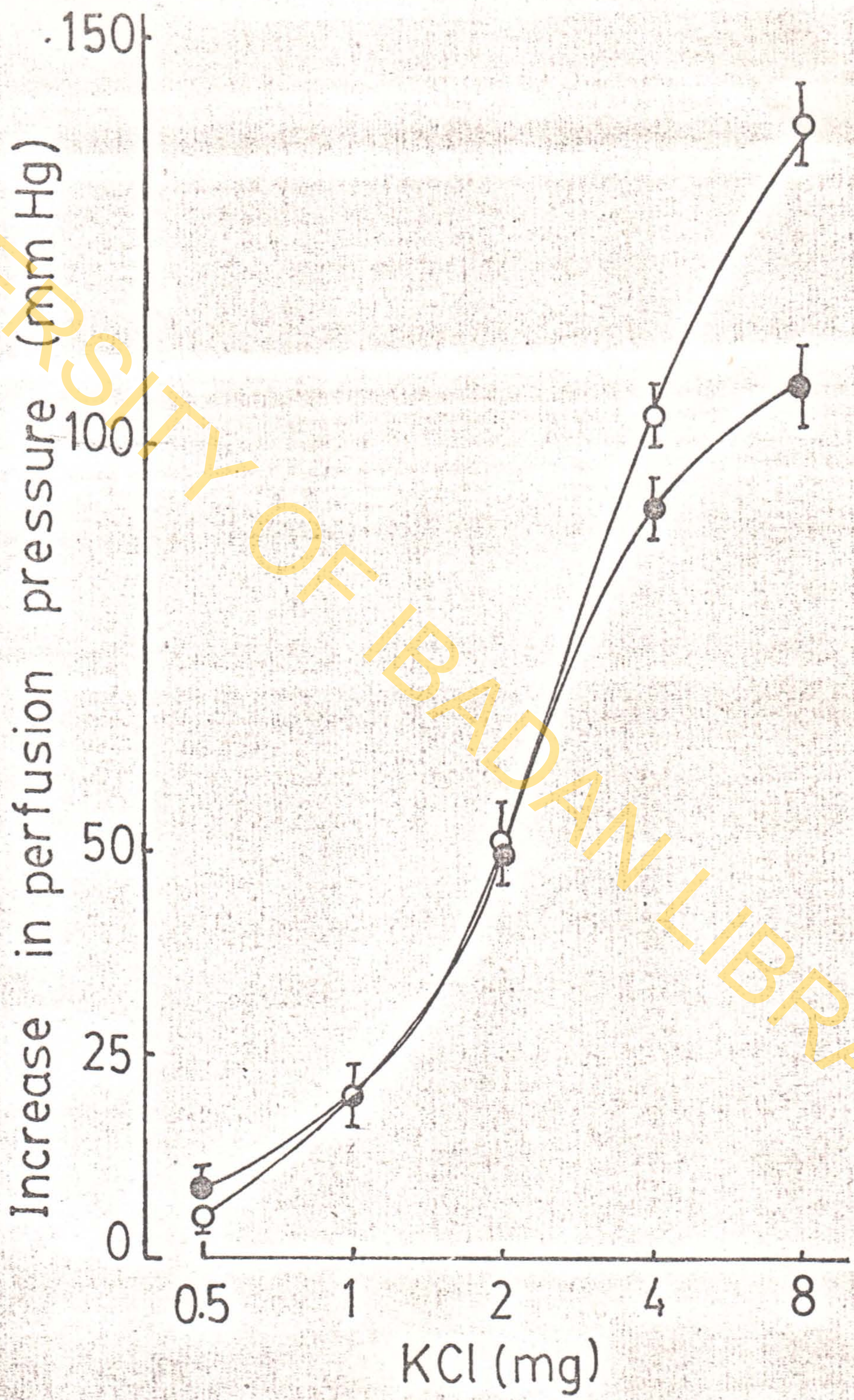

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FIGURE 31a

Rat mesenteric artery preparation: Effect of Ca^{2+} omission from the Krebs solution perfusing the mesentery on NA vasoconstrictor responses. The dots indicate responses to 8 ug NA injected at 5 min. intervals over a period of 1 hr.

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FIG 31a

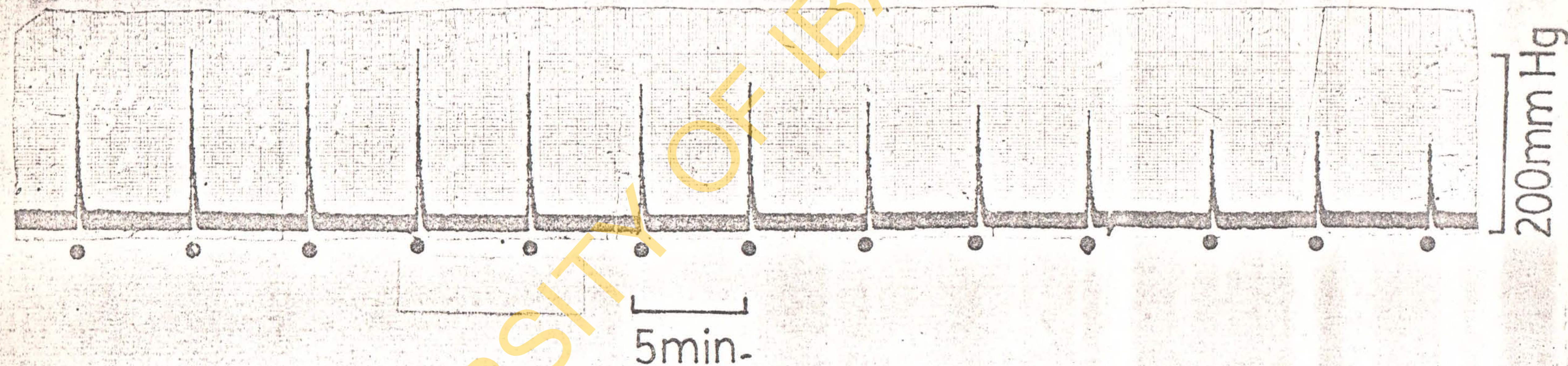

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FIGURE 31b

Response(s) of the perfused rat mesenteric artery preparation to potassium chloride (16 mg) in normal Krebs solution (A). The responses in B, were induced to Kcl during Ca^{2+} omission, at 5 min. intervals after allowing the artery to equilibrate for 30 min. in Ca^{2+} - free Krebs.

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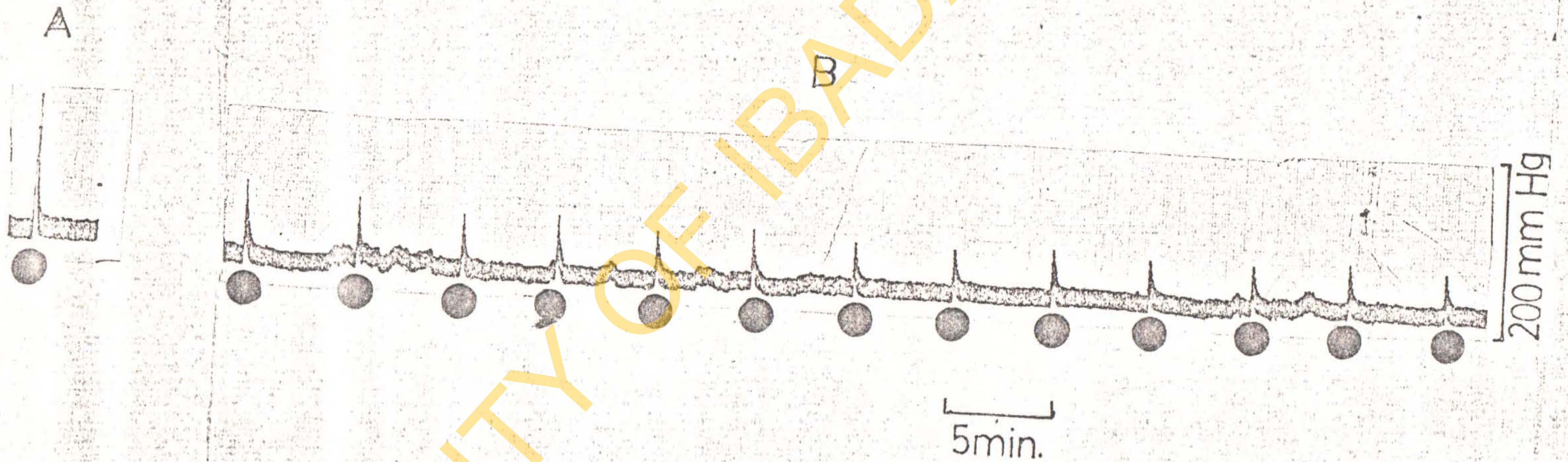
FIG 31b



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Control dose-response curves were first obtained in normal Krebs, then the tissues were exposed to Ca^{2+}-free Krebs for 30 min. Dose - response curves to NA constructed within 60 min. of Ca^{2+} removal were not significantly different from control curves constructed in Krebs containing 2.5 mM Ca^{2+}- (Fig. 32). The effects of prostaglandins or A23187 on NA vasoconstriction in Ca^{2+} free Krebs were studied by constructing dose-response curves to NA in the absence and presence of prostaglandins or A23187. The results are shown in Figs 33 and 34 for PGE_2 and PGF_{2a} respectively and Fig. 35 for A23187. From the graphs, it can be seen that prostaglandin - or A23187 - induced potentiation of NA was completely abolished in Ca^{2+}-free Krebs solution.

In another set of experiments, the relationship between prostaglandin - or A23187 - induced potentiation and concentration of Ca^{2+} in the perfusion medium was examined. This was done by obtaining PF values in Krebs solution containing different concentrations of Ca^{2+} ions. A separate preparation was used at each Ca^{2+} ion concentration. It can be seen from Fig. 36 that the PF values increased with increasing Ca^{2+} concentration up to (2.5mM for PGE_2; 1.25mM for A23187) and decreased on further increasing the Ca^{2+} ions in Krebs solution. This results showed that external Ca^{2+} was crucial for the potentiating effect of PGE_2 and A23187.

(c) on NA- blockade by α- adrenoceptor antagonists and on reversal by PGE_2

NA caused vasoconstriction by stimulating α- adrenoceptors

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FIGURE 32

Vasoconstrictor responses to NA in normal Krebs (X) and in Ca^{2+} - free Krebs solution (O). Responses in calcium - free Krebs were obtained within 45 min. of Ca^{2+} - free perfusion of the artery. Each point on the graph is a mean of observations from 6 - 8 separate artery preparations. Vertical bars represent s.e. mean.

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FIG 32

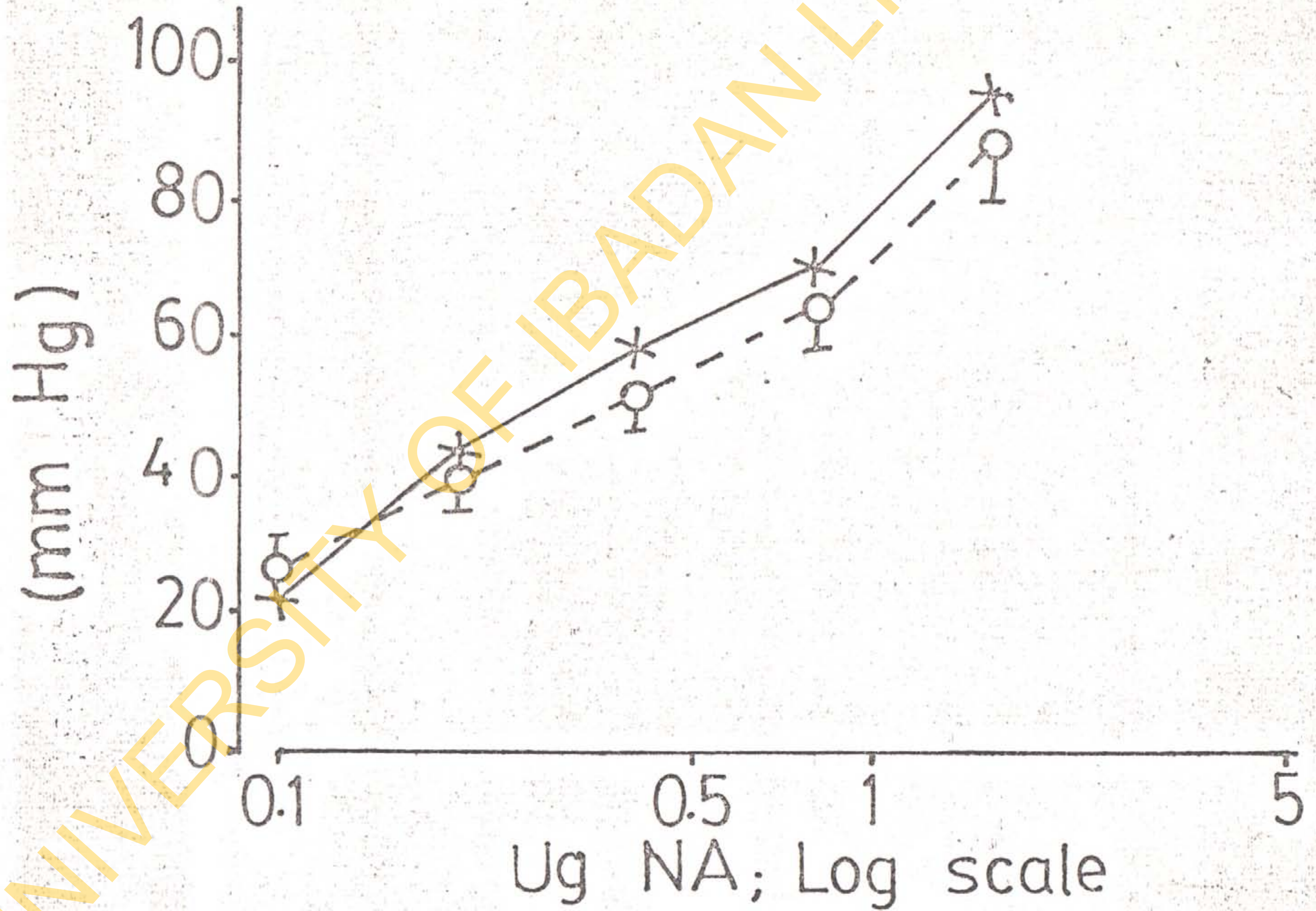

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FIGURE 33

Effect of PGE_2 on NA vasoconstrictor responses in rat mesenteric artery perfused with normal (left panel) and Ca^{2+} - free (right panel) Krebs solution. The curves represent dose response to NA (X) alone, and (O) in the presence of 10 ng/ml PGE_2. Each point on the graph represents the mean $\pm$ S.E. of 6 - 8 experiments.

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FIG 33

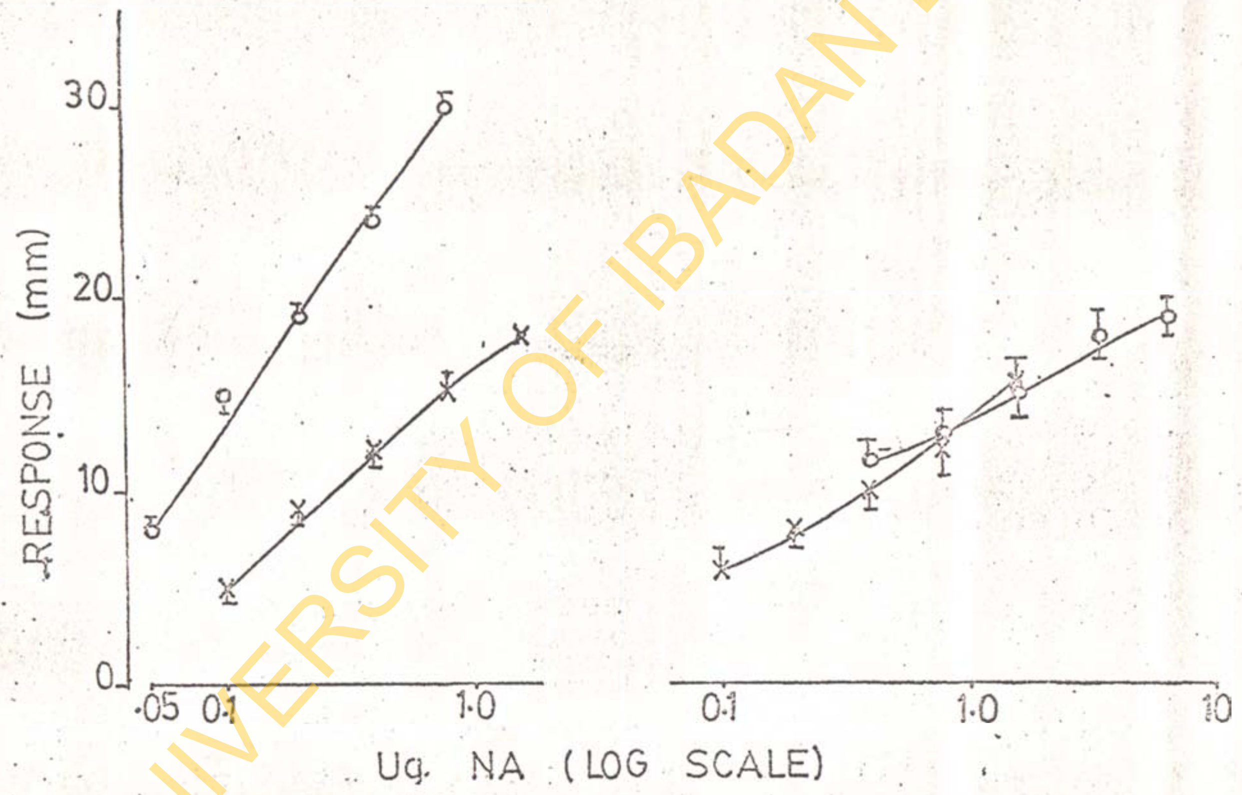

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FIGURE 34

Effect of PGF_{2a} on NA vasoconstrictor responses in rat mesenteric artery perfused with normal (left panel) and Ca^{2+}-free (right panel) Krebs solution. The curves represent dose responses to NA (X) alone, and (O) in the presence of 10 ng/ml PGF_{2a}. Each point on the graph represents the mean $\pm$ S.E of 6 experiments.

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FIG 34

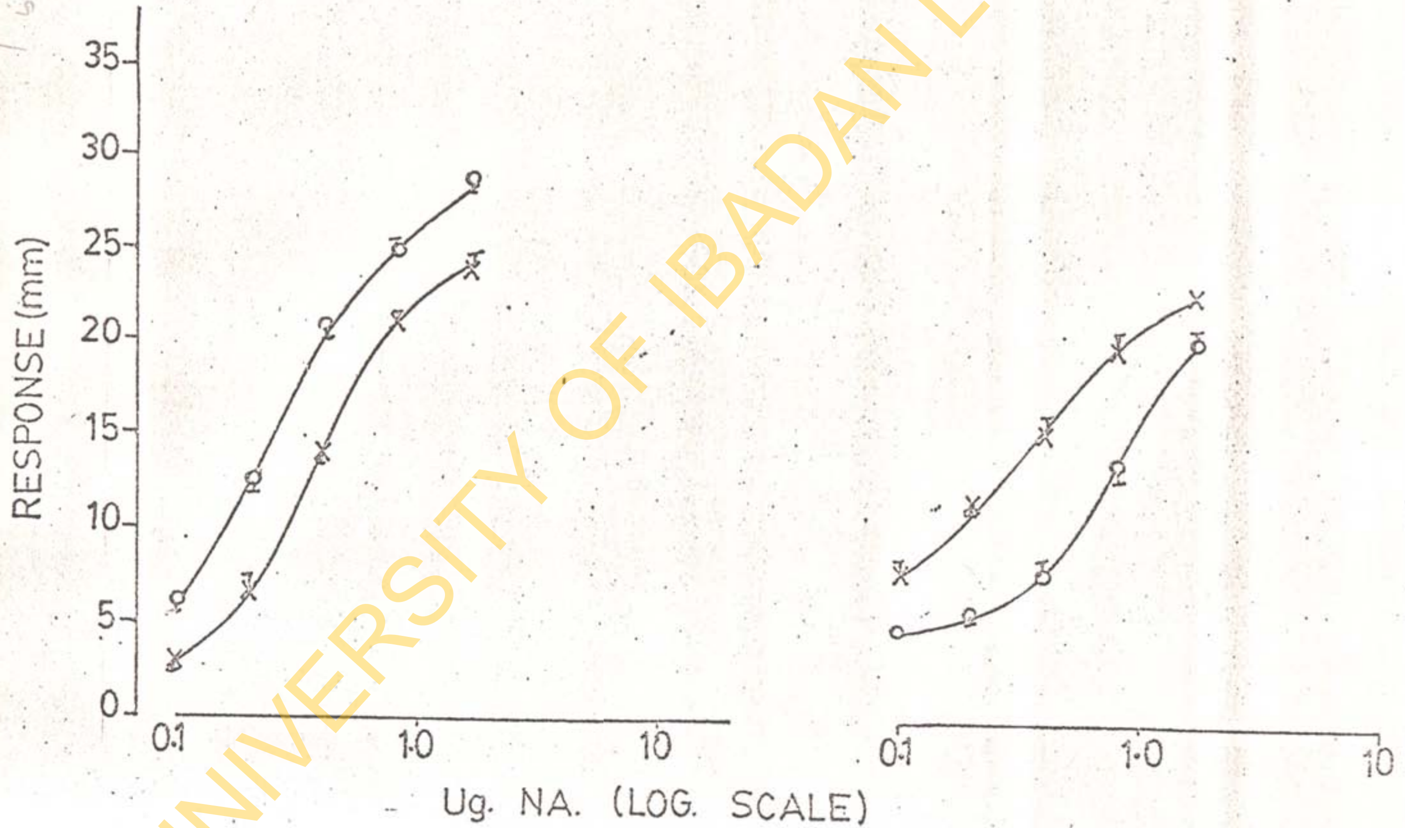

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FIGURE 35

Effect of A23187 (1.9×10^{-6}M) on NA vasoconstrictor responses in rat mesenteric artery perfused with normal (left panel) and Ca^{2+}- free (right panel) Krebs solution. The curves represent dose responses to NA (X) alone, and (o) in the presence of 1.9×10^{-6}M A23187. Each point on the graph represents the mean $\pm$ S.E of 6 experiments.

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Fig. 35

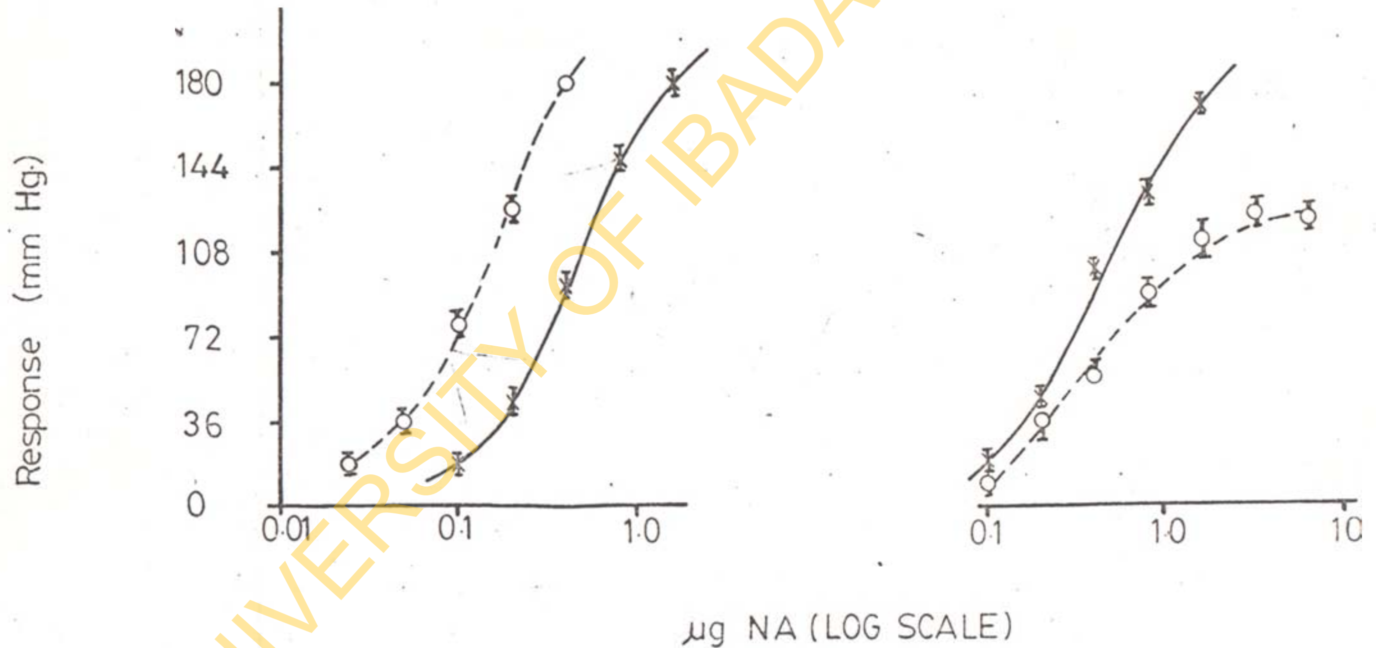

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FIGURE 36

Effect of increasing calcium chloride concentration on noradrenaline vasoconstrictor potentiation (PF) caused by 2.8×10^{-8}M prostaglandin E_2 (left panel) and 1.9×10^{-6}M A23187 (right panel) in rat mesenteric artery. Each point is a mean of measurements made in 7 - 8 preparations. Vertical bars represent S.e. mean.

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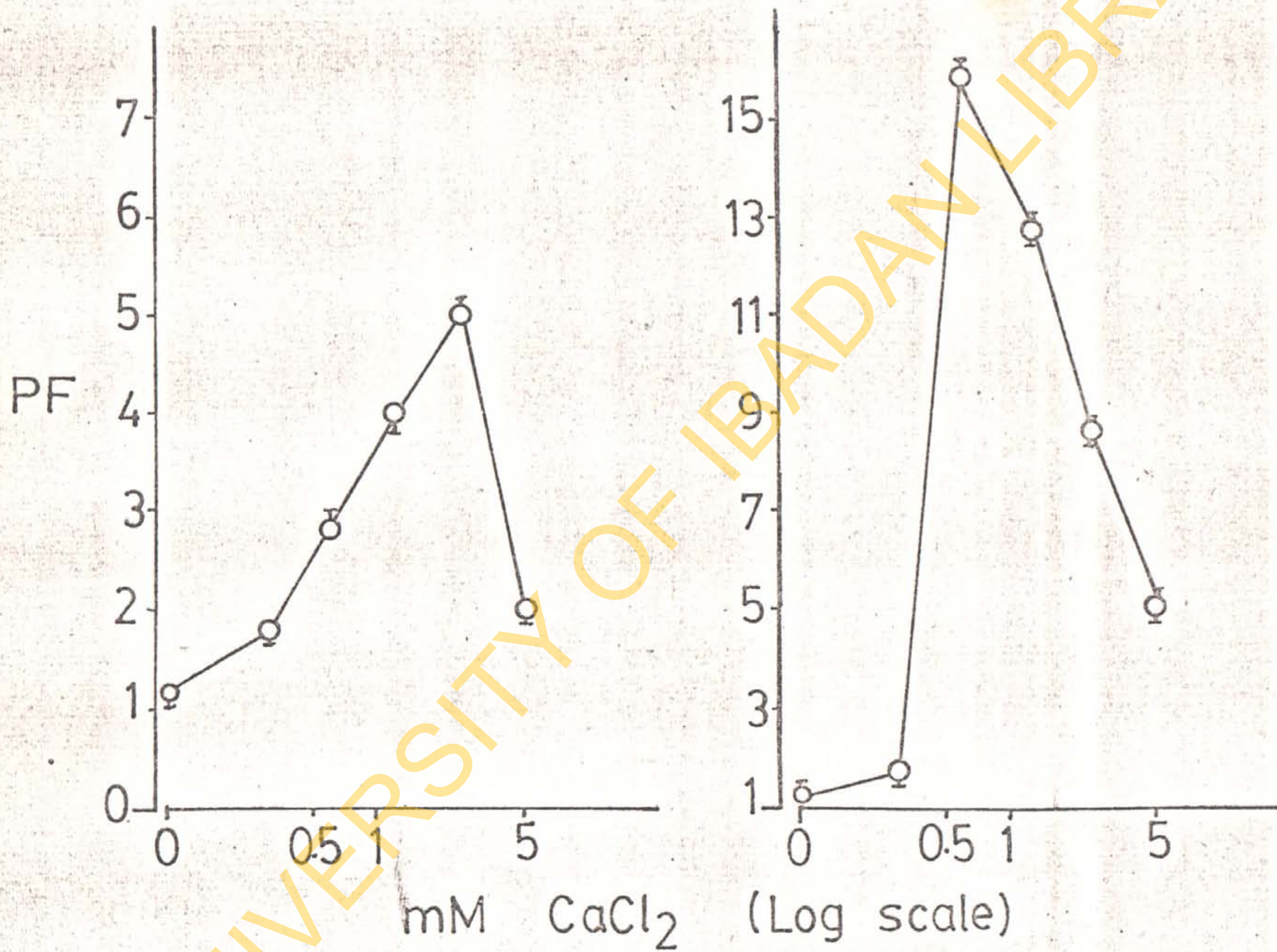
FIG 36



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of the mesenteric artery. The responses to NA were potentiated by prostaglandins in normal Krebs but not when Ca^{2+} was omitted from the perfusion medium. NA vasoconstriction was blocked by α- adrenoceptor antagonists phentolamine, tolazoline and yohimbine in normal Krebs and the blockade can be reversed by low concentrations of PGE_2. It was important to know (i) the effect of Ca^{2+} omission on the α- adrenoceptor block by these antagonists, and (ii) how prostaglandins (if at all) interact with the blockade during Ca^{2+} omission. The aim of this experiment was to determine whether the mechanism by which PGE_2 potentiated NA vasoconstriction was the same as that by which it reversed α- adrenoceptor blockade. The α- adrenoceptor block induced by phentolamine, tolazoline and yohimbine was unaffected by Ca^{2+} omission although PGE_2 (2.8×10^{-8}M) which did not potentiate NA in Ca^{2+} - free Krebs, reversed the α-adrenoceptor blockade due to each of the antagonists during Ca^{2+} omission to about the same extent as in normal Krebs.

Effect of variation in external Ca^{2+} on antagonism by indomethacin and prazosin

If an inhibitor of constriction prevented the entry of Ca^{2+} ions into the muscle cell, the concentration of Ca^{2+} ions in the perfusing fluid might be expected to influence the action of the inhibitor. Indomethacin and prazosin were tested against NA in normal Krebs solution. Cinnarizine and verapamil were not tested since both agents have been conclusively shown to antagonize Ca^{2+} influx across cell membranes (Godfraind & Kaba, 1972). The results summarized in Table 9 show that variation of the concentration of $CaCl_2$ in the external medium did not appear to affect antagonistic effect of indomethacin and prazosin in the rat mesentery.

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TABLE 8

Isolated Rat mesenteric artery: effect of PGE_2 (2.8×10^{-9}M on NA blockade caused by various α- adrenoceptor antagonists in Ca^{2+}- free krebs. Each mean value is calculated from measurements made in six experiments

ANTAGONIST	NA dose ratio in the presence of antagonist alone	NA dose ratio in the presence of antagonist+PGE_2
PHENTOLAMINE (3.2×10^{-8}M)	17.6 ± 0.9	5.3 ± 0.6
TOLAZOLINE (4.07×10^{-5}M)	27.2 ± 2.7	8.6 ± 0.8
YOHIMBINE (12.8×10^{-7}M)	20.2 ± 1.0	1.9 ± 0.2

TABLE 9

The effect of variation in external Ca^{2+} on NA antagonism by indomethacin and prazosin in the isolated rat mesentery Figures in this table represent the mean values from 6 - 8 experiments

Concentration of $CaCl_2$ in external medium mM	Dose ratios to NA	
	5.3×10^{-10}M Prazosin	2.8×10^{-5}M Indomethacin
0	60.0 ± 5.2	58.5 ± 3.5
0.3125	Not done	54.0 ± 3.0
0.625	67.5 ± 6.5	55.8 ± 4.8
1.25	65.0 ± 3.4	56.8 ± 5.0
2.50	66.2 ± 1.7	56.2 ± 7.9
5.0	64.8 ± 2.5	12.8 ± 3.1

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The magnitude of antagonism by either of the antagonists on NA vasoconstriction was similar in either normal or Ca^{2+}- free Krebs solution. On the other hand, doubling the concentration of $Cacl_2$ in the perfusion medium to 5.0 mM reduced the dose ratios to indomethacin, but not to prazosin. This result suggested that external Ca^{2+} antagonized the inhibitory effect of indomethacin.

Effect of cinnarizine and verapamil on PGE_2 - induced potentiation of NA

Cinnarizine and verapamil are both very potent antagonists of K^+- induced vasoconstriction of rat mesentery. Since the vasoconstrictor action has been shown to be exclusively due to influx of external Ca^{2+}, it follows that both cinnarizine and verapamil are antagonists of Ca^{2+} influx. Thus, if PGE_2 potentiates NA by causing Ca^{2+} influx, low concentrations of both antagonists will be expected to inhibit the potentiation. Indeed, concentrations of cinnarizine and verapamil too low to inhibit NA vasoconstriction, completely abolished the potentiating effect of PGE_2(Fig. 37). This further strengthens the view that external Ca^{2+} is essential for the prostaglandin - induced potentiation of NA.

Blockade by indomethacin, of Ca^{2+}- induced vasoconstriction in the mesenteric artery perfused with Ca^{2+}- free depolarizing krebs solution

When the mesenteric artery was perfused with Ca^{2+}- free depolarizing solution (see chapter 2 for the composition), bolus injection of $Cacl_2$ caused a rise in perfusion pressure. The rise

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FIGURE 37

Antagonism of PGE_2 - induced potentiation of NA by concentrations of cinnarizine (A) and verapamil (B) in rat mesenteric artery. Dose of NA used in all panels is 0.2 ug. The first set of responses in each panel are control responses (0.2 ug NA). In the second panel are the responses in the presence of 10 ng/ml PGE_2 while the third panel represents responses to 0.2 ug NA in the presence of PGE_2 and 2.7×10^{-8}M cinnarizine (A) combined; and PGE_2 plus 2.0×10^{-8}M verapamil combined (B).

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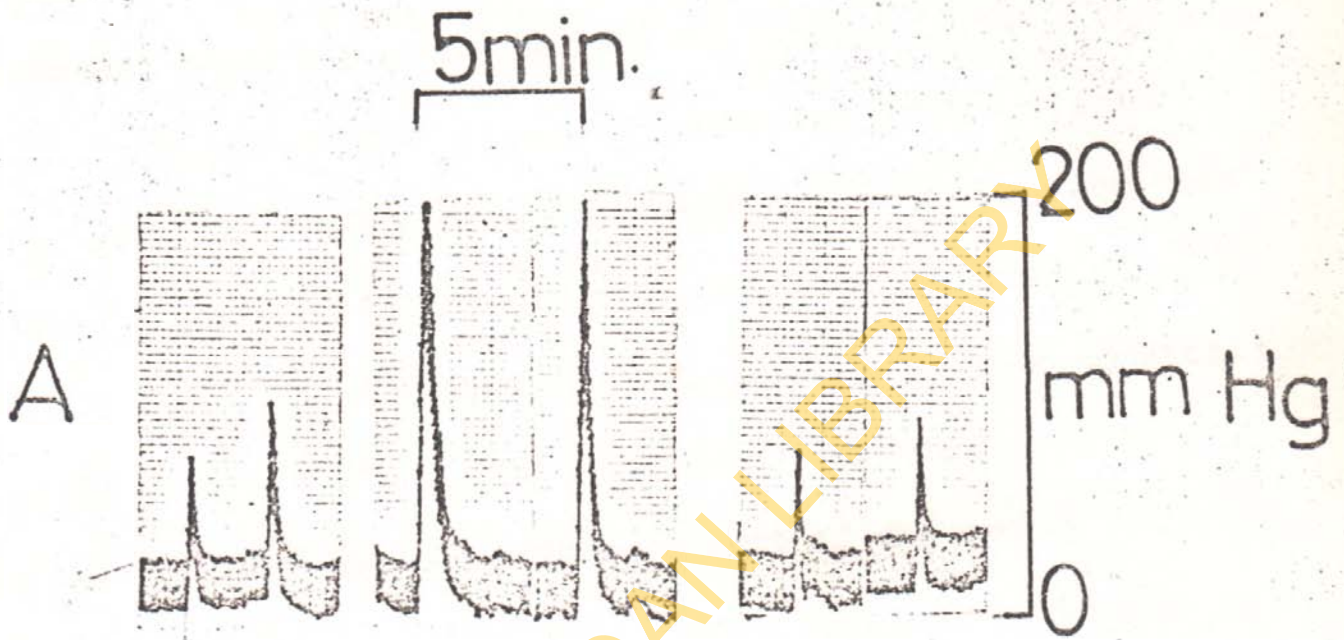
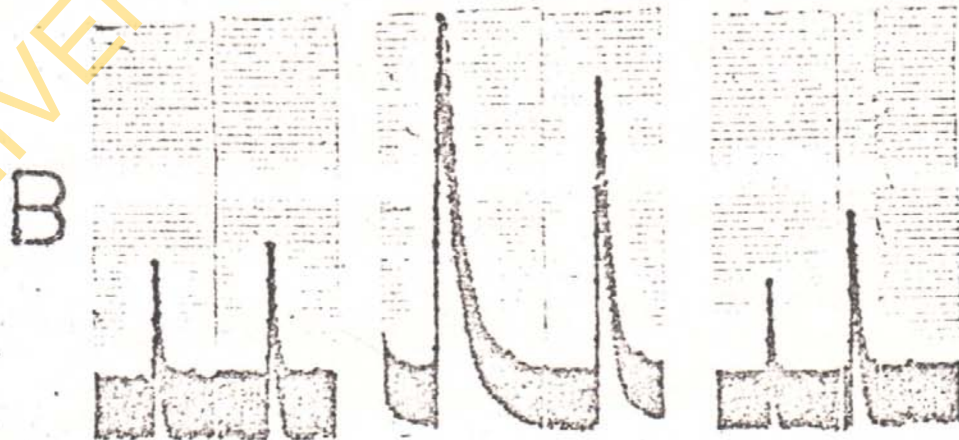
FIG 37





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was immediate, quite brief and measurable. The threshold concentration of $CaCl_2$ necessary to cause detectable constriction was about 25 mg, higher concentration producing a greater effect but not strictly graded. Vasoconstrictor responses were obtained at 5 min. intervals. It is noteworthy that there was no change in the base line of the artery when it was continuously perfused with Ca^{2+}- free depolarizing solution. Indomethacin (2.8×10^{-5}M) completely abolished Ca^{2+} - induced constriction of the artery (Fig. 38). PGE_2 on the other hand, neither potentiated nor inhibited Ca^{2+} induced vasoconstrictor effect of the artery (Fig.39). This result thus showed clearly that this concentration of indomethacin acted beyond cell membrane level and points to different sites of action for PGE_2 and indomethacin in NA - induced vasoconstriction.

Effect of Ca^{2+} on the mesenteric artery perfused with Ca^{2+} - containing depolarizing solution

Continuous perfusion of rat mesenteric artery with Ca^{2+}- containing (0.625mM) depolarizing solution caused a persistent elevation of the perfusion pressure. The artery slowly relaxed back to base line when perfusing solution was changed to one not containing Ca^{2+}. Addition of indomethacin (2.8×10^{-5}M) to Ca^{2+} - containing depolarizing solution caused a rapid and complete relaxation of the vessel (Fig. 40). Since the completion of this experiment, it has been realised that the Krebs depolarising solution was probably hypertonic. Therefore, the above results and their interpretations should be read with caution. To establish this point, future experiments would need to utilize isotonic Krebs solution.

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FIGURE 38

Blockade by indomethacin, of Ca^{2+} - induced vasoconstriction in the mesenteric artery perfused with Ca^{2+} - free depolarizing Krebs solution. The doses of Ca^{2+} indicated by dots are in mg. The arrow indicates when indomethacin was introduced to the perfusion medium.

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Fig. 38

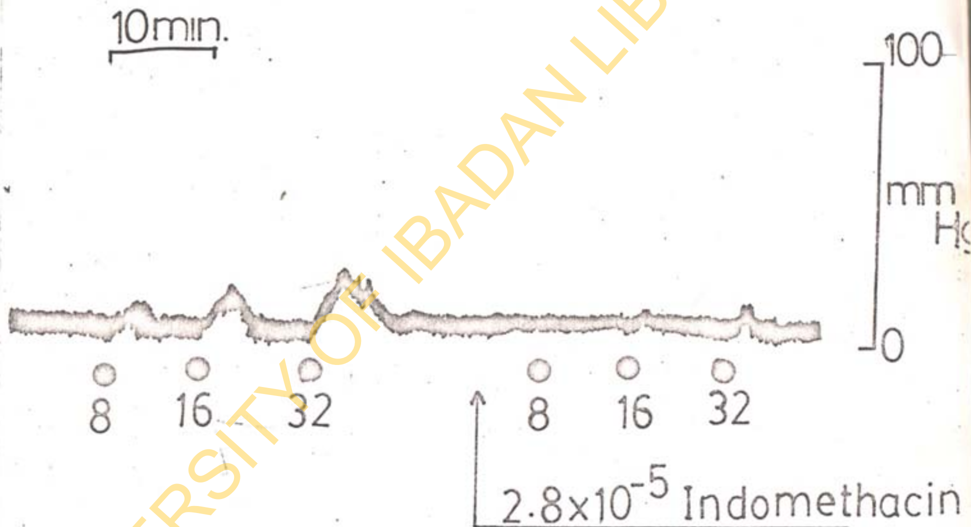

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FIGURE 39

Interaction of PGE_2 with calcium chloride in mesenteric artery perfused with Ca^{2+} - free depolarizing Krebs solution. (O) represents the control; while (●) represents responses in the presence of PGE_2 (10 ng/ml). Each point on the graph represents mean ± S.E of six experiments.

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FIG 39

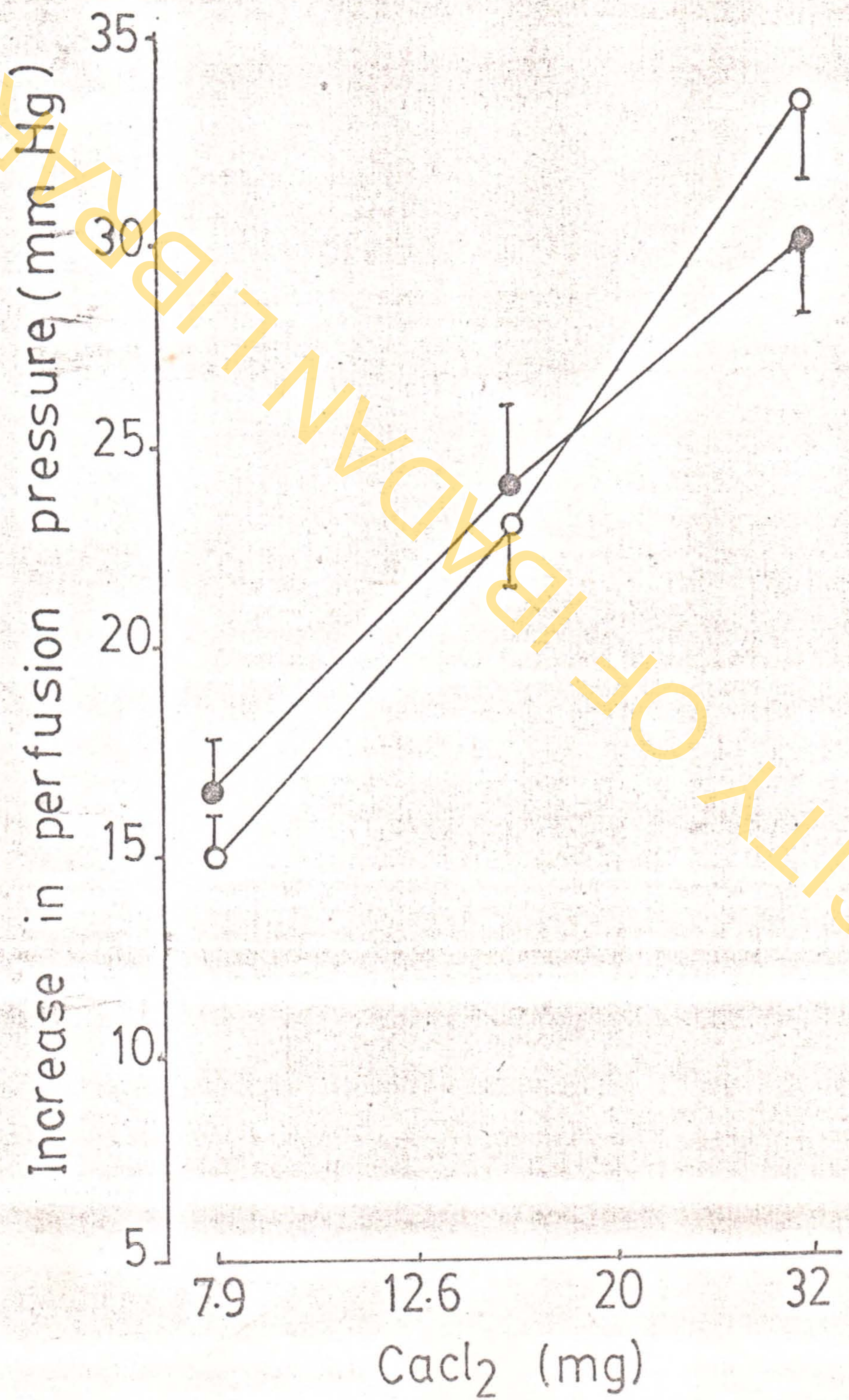

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FIGURE 40

Inhibitory effect of indomethacin on the persistent vasoconstriction caused by change of Ca^{2+} - free to Ca^{2+} - containing depolarizing perfusion medium. (A): perfusion with Ca^{2+} - free depolarizing solution, (B): perfusion with Ca^{2+} - containing depolarizing Krebs solution and (C) shows perfusion with Ca^{2+} - containing depolarizing Krebs solution to which 2.8×10^{-5}M indomethacin has been added.

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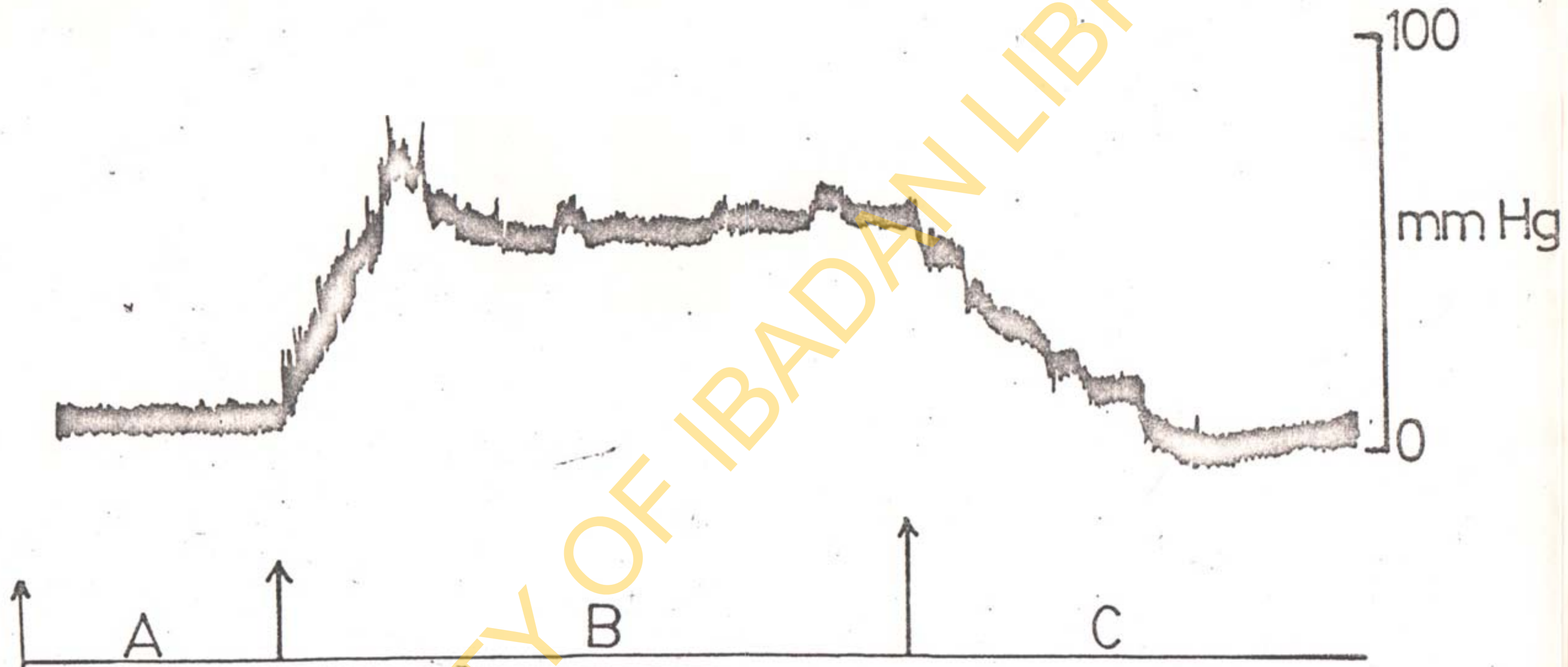
Fig. 40



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CHAPTER FOUR

DISCUSSION

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DISCUSSION

It is generally accepted that the contraction of vascular smooth muscle is initiated by a rise in the concentration of intracellular free or activator Ca^{2+} and that the resting membrane is impermeable to Ca^{2+}. Two types of excitation - contraction coupling (corresponding to the two pathways by which Ca^{2+} leaks into the cell) have been described, electromechanical and pharmacomechanical (Somlyo & Somlyo, 1968; Rasmussen & Goodman, 1977). These two pathways have been elaborately described in Chapter 1. The low doses of NA used in this work are comparable with those used by Casteels & Droogmans (1976) and have been shown not to cause depolarization of the vascular smooth muscle cell and will thus serve as a suitable example for pharmacomechanical pathway of mobilizing Ca^{2+} for contraction. Bolus injections of high doses of potassium chloride on the other hand, have been shown to cause vasoconstriction by a mechanism which utilizes extracellular Ca^{2+} (Bevan, Osher & Su, 1963; Hudgin & Weiss, 1968). KCl does not act on any specific receptor site but causes depolarization of the cell membrane thus rendering the latter permeable to extracellular Ca^{2+} whose influx causes the contraction. Vascular contraction induced this way represents an "electromechanical" pathway for Ca^{2+}.

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Mechanism of prostaglandin - induced potentiation of NA.

Exogenously administered prostaglandins had no action on its own in the isolated mesenteric artery preparation, but greatly potentiated the vasoconstrictor action of the neurohumoral trasmitter substance, noradrenaline, (NA) used as an agonist in this study. For PGE_2, the potentiation is dose - related at least up to 10^{-7} gml^{-1} whereas PGF_{2a} and PGI_2, there was no clear cut dose - relationship (Table 1). This result is in agreement with results obtained in other vascular smooth muscle preparations such as rabbit aorta and mesenteric artery in vitro (Strong & Chandler, 1972; Tobian & Viet, 1970); rabbit mesenteric artery (Malik et al, 1972); canine saphenous veins and hindpaw resistance vessels (Kadowitz, Sweet & Broody, 1971; Kadowitz, et al, 1971; Brody & Kadowitz, 1974) but different from those of Coupar & McLennan, (1978). The latter authors had observed that PGA and PGE_1 but not PGE_2 potentiated NA in rat mesenteric artery preparation. The failure of these authors to observe potentiation with PGE_2 may be due to the high doses of NA and also of PGE_2 used in their studies. In our experiments, the PGE_2 - dose potentiation curve was bell shaped (See Fig. 7). Prostaglandin - induced supersensitivity was probably due to the perfused prostaglandins since according to the results expressed in Table 2, only about

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20% of the PGE_2 and PGF_{2a} infused into the mesenteric artery was inactivated. Prostaglandin metabolites possess a variety of biological activities (Anggard, 1966; Pike, Kupiecki & Weeks, 1967; Crutchley & Piper, 1975) on a variety of smooth muscle preparations. It is furthermore, clear that products of PGE_2 or PGF_{2a} metabolism did not contribute to enhancement of NA vasoconstriction since the perfusate of the prostaglandin after passage through the guinea-pig lung pulmonary artery did not display any NA - potentiating effect. PGE_2 and PGF_{2a} undergo more than 90% inactivation on single passage through the guinea-pig pulmonary vasculature (Piper, Vane & Wyllie, 1970; Crutchley & Piper, 1975).

There is evidence that the innervation of the mesenteric artery is adrenergic in nature. The abolition of responses to stimulation by bretylium and by guanethidine, and also the reduction or abolition of responses to nerve stimulation by pretreatment with reserpine, together with the lack of effect of atropine and physostigmine, are all evidence supporting the fact that vasoconstriction caused by stimulation was due to excitation of adrenergic fibres (McGregor, 1965). Further support is derived from the observation of Furness & Marshall (1974) that the principal and small arteries and terminal arterioles of the mesenteric vascular bed are all innervated by a network of adrenergic fibres and all constrict in response to stimulation

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of paravascular nerves and exogenous NA. Prostaglandin - induced supersensitivity may therefore be pre - or post - synaptically mediated. Presynaptically, it could be due to release or facilitation of release of transmitter substance from the nerve endings since there is evidence that PGE_2 and PGF_{2a} facilitates NA release in vascular tissue. For example, in the hindlimb of the dog, PGF_{2a} causes venoconstriction which is abolished after denervation (Ducharme, Weeks & Montgomery, 1968). Also, both PGE_2 and PGF_{2a} have been shown to selectively facilitate the responses to nerve stimulation in the resistance vessels of the dog hindpaw (Kadowitz et al, 1971; Brody & Kadowitz, 1974). Our results show that both PGE_2 and PGF_{2a} still potentiated NA even after the adrenegic neurones had been depleted of NA by reserpine treatment or blocked with bretylium. The prostaglandin - induced supersensitivity could not therefore be due to release of NA from adrenergic nerve endings. Another interesting result was the observed increase in sensitivity of the mesenteric artery to NA following 3 - 4 days consecutive pretreatment with reserpine. In such arteries, PGE_2 - induced potentiation of NA responses was significantly greater than in control arteries. Bretylium on the other hand, reduced the degree of potentiation. The mechanism by which 'prolonged' reserpine pretreatment and bretylium quantitatively affect prostaglandin enhancement of NA vasoconstriction

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is not yet clear. But synergism with reserpine would be expected if PGE_2 was acting in a similar way to reserpine. It is noteworthy that the supersensitivity due to reserpine has been related to an enhanced ability of the tissue to retain and utilize Ca^{2+} (Carrier et al, 1970; Carrier & Jurevics, 1973; Carrier & Hester, 1976). Prostaglandin enhancement of NA action too, seems related to Ca^{2+} fluxes as will be shown later in this thesis.

Prostaglandin enhancement of NA vasoconstriction does not appear to involve inhibition of NA uptake. Firstly, in the presence of maximal potentiating concentration of cocaine, prostaglandins still caused further enhancement of NA vasoconstriction (Fig. 10). Secondly, in the present experiments, methoxamine vasoconstrictor responses were potentiated by PGE_2 and PGF_{2a} (See Fig. 11). Methoxamine is a predominantly post-synaptic α-adrenoceptor agonist which because of the hydroxyl group on the B - carbon of the side chain and more importantly because of the methoxyl substituents in the ring, is almost completely devoid of affinity for the uptake site. Thus, methoxamine is not a substrate for the uptake mechanism (Iversen, 1970) and its action was not potentiated by cocaine (Trendelenburg et al, 1970). That PGE_2 does not inhibit NA uptake is further supported by the finding of Hedqvist (1970) that the prostaglandin did not alter the removal of tritium - labelled exogenous NA infused into the cat spleen.

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The results of Coupar & McLennan (1978) are also compatible with those presented here.

The Role of External Ca^{2+}

The present studies show that elimination of Ca^{2+} ions from solution perfusing the mesentery completely abolished the prostaglandin - induced potentiation of NA vasoconstriction. This result suggests that the potentiation depends on external Ca^{2+}. NA vasoconstriction induced every 5 min. was maintained for up to 60 min. after an initial equilibration in Ca^{2+} - free krebs medium for 30 min. (Fig.31a). This result demonstrates that, in the rat mesenteric artery preparation, NA can induce vasoconstriction in the absence of external Ca^{2+}. Since it is known that vascular smooth muscle contraction requires Ca^{2+} (Burks, Whitacre & Long, 1967; Bohr, 1973) it would appear that in this preparation, the Ca^{2+} required for contraction originated from an intracellular source. Hinke (1965) also attributed the persistence of NA vasoconstrictor effect in rat tail artery after Ca^{2+} removal from the perfusion medium to utilization of sequestered Ca^{2+}, a suggestion that was supported by Hudgins & Weiss (1968). However, this sequestered Ca^{2+} (or bound Ca^{2+}) has been shown to be in equilibrium with the extracellular Ca^{2+} (Daniel, Sehder & Robinson, 1962; Edman & Schild, 1962). Omission

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of Ca^{2+} from the external medium was observed in the short run, not to affect the NA dose - response curve. This will suggest that Ca^{2+} influx induced by NA does not directly influence the magnitude of the response. PGE_2 did not appear to affect the release of Ca^{2+} from intracellular stores when NA induced vasoconstriction in Ca^{2+} free media, since potentiation was completely abolished during perfusion with Ca^{2+} free krebs. Moreover, our finding that the prostaglandin potentiation of NA was minimum in Ca^{2+} - free krebs, but increased in proportion to the concentration of Ca^{2+} ions in the external medium suggests strongly that the prostaglandin potentiation was due to increased availability of Ca^{2+} from the external source probably by facilitating Ca^{2+} - influx. This may be so, if the prostaglandins were acting as a lipid soluble chelating agent which complex with free Ca^{2+} ions as suggested by Eagling, Lovell & Pickles (1972). Under this condition, utilizable Ca^{2+} is made more abundant to the intracellular stores and could thus be released to the contractile system far more easily and in sufficiently large quantity. In Ca^{2+} - free Krebs solution, "PG - Ca^{2+}" complex is not formed therefore keeping the agonist action at control level. Alternatively, prostaglandin - induced potentiation of NA may be due to a non-specific increase in membrane permeability since prostaglandins have been shown to produce vascular permeability

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changes in guinea-pigs (Horton, 1973) and rats (Arora, Lahiri & Sanyal, 1970; Kaley & Weiner, 1971; Crunkhorn & Willis, 1971; Thomas & West, 1973, 1974).

Effect of high K^+

High doses of KCl induced vasoconstriction of the rat mesenteric artery in a graded manner. Responses to KCl induced at 5 min. intervals rapidly faded off in Ca^{2+} - free Krebs (Fig.31b). This suggests and also confirms earlier observations by Waugh (1962); Bevan, Osher & Su, (1963) and Hudgins & Weiss (1968) that KCl causes vasoconstriction by utilizing exclusively, the extracellular Ca^{2+}. If prostaglandins were potentiating NA by a mechanism which involved promotion of Ca^{2+} influx across the cell membrane exclusively, then, K^+ - induced vasoconstriction should also be greatly potentiated. Interestingly but surprisingly too, neither PGE_2 nor PGF_{2a} potentiated K^+ - induced vasoconstriction of the rat mesentery. This would suggest that facilitation of external Ca^{2+} influx was not the primary event bringing about the potentiating effect. In addition, in the mesenteric artery perfused with Ca^{2+} - free high potassium depolarizing solution, bolus injection of large doses of Ca^{2+} caused vasoconstriction. This, according to Bohr, (1973) is due to free passage of Ca^{2+} through the membrane which has been rendered highly permeable by the K^+ - induced depolarization.

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In the present studies, prostaglandins had no effect on the responses of such a depolarized preparation to Ca^{2+}. This suggests that the prostaglandins have no effect on the contractile machinery itself nor do they have any effect on Ca^{2+} permeability or on the subsequent suquestration or extrusion of Ca^{2+} in at least a depolarized artery. It thus appears that the integrity of the vascular smooth muscle membrane is crucial to the potentiating action of the prostaglandins. The potentiating effect can thus be explained on the grounds that prostaglandins lower the otherwise stable membrane potential of highly rectified cell membrane of the smooth muscle in the isolated artery. As a result of the lowered membrane potential, NA which normally activates the pharmacomechanical pathways, now causes depolarization or may even produce action potentials. Activator Ca^{2+} is thus provided by the electrical as well as the non-electrical pathway and therefore the potentiated contractile responses.

Agonist potentiation by PGE_1, PGE_2 and PGF_{2a} have been reported by Khairallah, Page & Turker (1967); Paton & Daniel (1967); Clegg, Hall & Pickles, (1966); Eagling, Lovell & Pickles, (1972) and Greenberg, Kadowitz, Diecke & Long (1974). The mechanism suggested by Khairallah et al (1967) that prostaglandins cause localized areas of depolarization in smooth muscles thereby increasing its responsiveness to other spasmogens, has been contradicted by the sucrose gap recording of Clegg et al (1966) which gave no

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evidence for such decrease in resting membrane potential of polarized myometrium. Greenberg _et al_ (1974) observed that PGF_{2a} increased Ca^{2+} uptake and also enhanced NA - induced Ca^{2+} uptake by strips of dog saphenous veins and therefore suggested that PGF_{2a} acted at smooth muscle membranes to enhance permeability to Ca^{2+} ions. In the rat mesenteric artery or any other vascular muscle preparation, no information on the effect of prostaglandins on membrane potential is available. However, since PGE_2 and PGF_{2a} are potent spasmogens in the visceral smooth muscle, it seems likely that the prostaglandins can also cause depolarization. It is very well known that membrane permeability to Ca^{2+} is increased as the membrane potential is decreased (Glover, 1978); therefore, it is possible that the prostaglandins depolarize the cell membrane to an extent that may or may not be sufficient to produce a contraction, but which in either case enhances an increase in Ca^{2+} permeability brought about by the pharmacomechanical action of NA.

In conclusion therefore, the prostaglandins seem to have an effect on the membrane of the vascular smooth muscle which appears to mediate the Ca^{2+} - dependent potentiating effect on NA. Whether or not they act by enabling NA to cause depolarization thereby facilitating Ca^{2+} influx, or by altering the resting membrane potential requires an electrophysiological investigation.

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Mode of action of the Ca^{2+} ionophore A23187

In the course of this study, compound A23187 has been chosen as a valuable tool for probing the location of the site of action of prostaglandins and other agents studied. Although, this compound has been extensively studied in many isolated smooth muscle preparations, none of such studies has been carried out on the isolated mesenteric arteries.

This investigation has revealed two distinct actions of the compound A23187. These are (i) agonist potentiating (or indirect), and (ii) contractile (or direct) actions. A23187 in the dose range 1.9×10^{-9}M-9.6×10^{-7}M caused a rapidly fading potentiating effect of NA and methoxamine. Inhibition of uptake is not a probable mechanism of the potentiation since methoxamine was also potentiated. A23187 did not potentiate NA in reserpine pretreated rats suggesting that the mechanism of the potentiation involves release of catecholamines from the adrenergic neuronal endings. This suggestion agrees with the conclusions of Thoa et al (1975) and Fairhurst et al (1975) that A23187 releases catecholamines from peripheral adrenergic neurones, and rat brain synaptosomes respectively. The techyphylactic nature of the potentiating effect is also consistent with this view. The observation that there was no potentiation when Ca^{2+} was omitted from the perfusing Krebs solution is important and would suggest that catecholamine release

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by A23187 is dependent upon calcium. Calcium dependence, according to Wooten, Thoa, Kopin & Axelrod, (1973) is an index of release by exocytosis.

However, concentrations of A23187 above $9.6 \times 10^{-7}M$ induced a characteristically slow and prolonged contractile responses. The contractions were only slightly reduced by high concentrations of phentolamine and yohimbine suggesting that the responses were not largely mediated by stimulation of α- adrenoceptors. Well known Ca^{2+} antagonists, cinnarizine (Godfraind& Kaba, 1968, 1972) and verapamil (Rasmussen & Goodman, 1977) reduced the ionophore - induced contractions substantially even though the contractions were sustained during Ca^{2+} omission in the perfusing Krebs solution. Also, small doses of indomethacin and sodium meclofenamate nearly abolished the A23187 - induced contractions. These latter agents have also been shown to antagonize vasoconstrictors by preventing them from utilizing Ca^{2+} (Northover, 1968, 1972, 1977). The results with cinnarizine and verapamil suggest that the action of these agents involved factors other than mere blockade of Ca^{2+} influx. They could be acting further by blocking the mobilization of sequestered Ca^{2+} or by preventing the utilization of mobilized Ca^{2+} by the contractile proteins. From these results therefore, it is concievable that the mesenteric vascular muscle contraction induced by compound A23187 resulted from massive release of Ca^{2+} ions from bound intra cellular sites (probably the

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sarcoplasmic reticulum and the mitochondria) and the consequent rise in free Ca^{2+} in the vicinity of the contractile proteins. This is supported by the findings of Publicover, Duncan & Smith (1968) that A23187 release Ca^{2+} from the sarcoplasmic reticulum in the mouse diaphragm and that of Statham, Duncan & Smith (1976) that A23187 causes an increase in resting tension and development of contraction in the cutaneous pectoris muscle of the frog. The divalent cation ionophore A23187 will therefore be a very useful tool in the studies of antagonists acting beyond receptor or membrane levels (as it is made use of in this investigations).

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Types and mechanisms of blockade of vasoconstriction in rat mesentery

It is an established fact that the measured response to NA results from its combination with specific receptors on the tissue membrane. Consequent upon this primary combination, a chain of events of undetermined length and nature, which culminates in the response is triggered off. Antagonists which interfere with the first step (i.e. the combination of the agonist with specific receptors) appear to be in mass - action equilibrium with the receptor and the blockade produced is a measure of competition between the agonist and the antagonist for receptor occupancy. Such agents are termed "classical competitive antagonists" (Nickerson, 1959). In their own submissions, Arunlakshana &

Schild (1959) introduced conditions which an agent must satisfy before its antagnoism of another could be classified as "competitive". These conditions include (i) that the dose - response curve in the presence of the agent must be shifted rightwards parallel to the control curve; (ii) nearly constant magnitude for the maximal responses in the control and in the presence of antagonist; and (iii) that the slopes of "A - S plots" (described in Chapter 2) should not be significantly different from unity. A forth condition which infact is a further explanation on (iii) above, is that pA2 values (see Chapter 2) of any competitive antagonist should be constant regardless of the concentration of the antagonist (Mackay, 1978). Based on these grounds, phentolamine,tolazoline and yohimbine were all observed in the present studies to block the action of NA by competing with it for the α- adrenoceptors in the rat mesentery. Low concentrations of phenoxybenzamine acted by a mechanism similar to that of the three antagonists.

Indomethacin(2.8×10^{-7}M - 2.8×10^{-5}M) inhibited the vasoconstrictor action of NA. There was a characteristic difference in the nature of the block produced by indomethacin e.g. the duration of action of each dose of NA administered in the presence of indomethacin was prolonged. However, indomethacin block of NA was overcome by increasing agonist concentration. In contrast to conventional competitive receptor antagonism, indomethacin caused

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severe depression of the maximal agonist responses. Also, "pA_2" values for this agent decreased with increasing concentration of the antagonist in the perfusion medium. This, according to Mackay, (1978) shows the antagonism is not of the competitive type. That indomethacin does not block NA via α- adrenoceptors is supported by the observation that its antagonism is unspecific in nature. Apart from NA, it blocks histamine and acetylcholine in guinea-pig ileal muscle (Bennett, Eley & Stockley, 1975; Sokunbi, 1979); angiotensin, vasopressin, barium chloride and serotonin in rat mesenteric arteries, (Northover, 1968; Horrobin et al, 1976). Indomethacin block of NA has been ascribed to its inhibitory effect on the endogenous prostaglandin synthesis. This conclusion is based on the finding that low concentrations of prostaglandins especially of the E series, cause complete reversal of the blockade (Horrobin et al, 1974; Couper & McLennan, 1978; Couper, 1980). Although, near complete reversal of indomethacin block was observed in about 70% of the experiments during these studies, it seems the blocking action cannot solely be explained in terms of prevention of endogenous prostaglandin generation. The evidence for this view is as follows:- (a) the block develops within 10 - 15 min. of tissue contact with indomethacin. This period is short for antagonism of an enzyme system; prostaglandin synthetase inhibition usually requires up to 30 min (Flower, 1974). (b) the block is completely overcome

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by washing the tissue with plain Krebs solutions for 15 - 20 min; whereas indomethacin binding to cyclooxygenase is usually considered irreversible (Flower, 1974); (c) reversal of the block by PGE_2 is only about 70% consistent in the rat mesentery (present studies).

Alternatively, indomethacin may act by blocking Ca^{2+} influx. However, the magnitude of indomethacin block was not affected by alterations in external Ca^{2+} concentration up to 2.5 mM Ca^{2+}. This will suggest that indomethacin was not acting by preventing Ca^{2+} influx. This is buttressed by the observation that indomethacin up to 2.8×10^{-5}M did not block vasoconstrictor responses induced by KCl. Responses to $CaCl_2$ in Ca^{2+} - free depolarizing Krebs solution were blocked by indomethacin suggesting that the site of indomethacin action was beyond membrane level probably blocking Ca^{2+} mobilization from sequestration sites or preventing utilization of free Ca^{2+} by the contractile proteins. But in the presence of excess Ca^{2+} in the perfusion medium (5.0 mM Ca^{2+}), indomethacin block of NA was drastically reduced. This situation will be expected if the membrane stabilizing action of high doses of indomethacin (Northover, 1971, 1972; Flower, 1974; Famaey, Fontaine & Reusse, 1977) was prevented or reduced by excess Ca^{2+}. The vasoconstrictor response to A23187, shown earlier in this thesis to be due to massive release of Ca^{2+} from bound intracellular sites was almost completely abolished by

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2.8×10^{-5} M indomethacin (Table 7). This observation can be taken to mean that the site of indomethacin action is intracellular preventing release from the mitochondria or the sarcoplasmic reticulum. This suggestion is compatible with that of Northover,(1977) who reported that indomethacin did not block responses of glycerinated smooth muscle strips made to contract by the addition of a mixture of adenosine triphosphate (ATP), Ca^{2+} ions and Mg^{2+} ions.

<u>Action of Prazosin</u>

Prazosin (1.3×10^{-10} M — 5.3×10^{-10} M) potently inhibited NA vasoconstriction in the rat mesentery. Like indomethacin, there was prolongation of the duration of the agonist action and severe depression of the maximal responses to the agonist by high concentrations of the antagonist. Also, its blockade of NA can be overcome by increasing agonist concentration. However, prazosin block of NA in rat mesentery is non-competitive in nature since the values of "pA_2" obtained with prazosin vary with varying concentrations of the antagonist. This conclusion contradicts earlier reports by Cavero (1976), Cavero <u>et al</u> (1978) that prazosin is a competitive post-synaptic α- adrenoceptor blocking agent in vascular smooth muscles. The latter conclusion has been based on receptor characterization experiments in anaesthesized animals

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(rats and dogs). The magnitude of prazosin block was not affected by changes in external Ca^{2+} concentration up to 2.5 mM Ca^{2+}. Doubling the external Ca^{2+} concentration did not affect the block. These put together will suggest that prazosin was not preventing Ca^{2+} influx across cell membrane. In contrast to indomethacin, prazosin block was if anything only partially reversed by PGE_2 (Fig. 20). Prazosin block appears very selective to NA action. At the concentration of 2.6×10^{-10} M in the perfusion medium, NA dose ratio was 11.2 ± 0.4, whereas up to a concentration of 1.3×10^{-4} M, (i.e. one million fold increase in concentration), prazosin did not show any antagonistic effect on vasoconstriction induced by KCl. This interesting observation suggests that prazosin demonstrates some specificity for the pharmacomechanical pathway of vasoconstriction. However, since prazosin is not a competitive α- adrenoceptor antagonist (present work) its inhibitory action is probably on the events following receptor stimulation (i.e. block Ca^{2+} channels mobilized specifically by pharmacomechanical coupling). This is consistent with the conclusions of Constantine et al (1973) that prazosin caused functional blockade of vasoconstriction at a point distal to the α- adrenoceptors.

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Cinnarizine and Verapamil

Cinnarizine and verapamil antagonised both NA - and KCl - induced vasoconstriction in rat mesentery. Both agents were clearly more potent against KCl - induced vasoconstriction than NA vasoconstriction (Fig. 29). Both cinnarizine and verapamil blocked NA in a non-competitive manner with the magnitude of the maximal responses to NA always suppressed. This is expected since both compounds have been shown to prevent Ca^{2+} influx across biological membranes (Godfraind & Kaba, 1969, 1972). This conclusion is supported by our observation that concentrations of these agent, lower than required to block NA vasoconstriction, completely abolished PGE_2 - induced potentiation of NA (Fig. 37) shown earlier in the thesis to be dependent on external Ca^{2+}. Furthermore, cinnarizine and verapamil were potent antagonists of A23187 - induced vasoconstriction of the rat mesentery; suggesting that the site of action of these two compounds may extend beyond the membrane level. They could be preventing the mobilization of Ca^{2+} from bound sites or preventing utilization of free Ca^{2+} by the contractile machine. Godfraind & Kaba (1969) had ruled unlikely the probability that cinnarizine was acting directly on the contractile proteins based on the observation that the compound did not modify the contraction of isolated rabbit, mesenteric artery evoked by adrenaline in Ca^{2+} - free depolarizing solution. It can therefore be suggested by exclusion, that

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cinnarizine prevents the mobilization of sequestered Ca^{2+} required for NA action. Verapamil probably acts by the same mechanism. This conclusion is in addition to the fact that both compounds prevents Ca^{2+} influx, a fact vindicated in this study by the observed inhibition of K^{+} - induced vasoconstriction.

Mechanism of prostaglandin reversal of NA - antagonism

The possibility that prostaglandins may act by interfering with the pharmacological receptors was considered. In a study of the interaction of PGE_2 with phentolamine, tolazoline, yohimbine and phenoxybenzamine, it was observed that the blockade by the latter was consistently and dose - dependently reversed by PGE_2. The responses were reversed almost to control levels when the block was of the competitive type. PGF_{2a}, similarly reversed the block by phentolamine, tolazoline and yohimbine. The block by cinnarizine, verapamil and prazosin were unaffected by doses of PGE_2 as high as 2.8×10^{-6} M. These results are similar to those of Maxwell, Plummer, Povalski Schneider & Coombs (1959) who showed that cocaine reversed the blocking action of surmountable antagonists of NA but did not affect the blocking action of an unsurmountable antagonist. They suggested that cocaine caused supersensitivity by producing a change in the configuration of the receptor (i.e. "deforming" the receptor). If this were so, a different binding characteristics for the receptor antagonists might be

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expected since according to Green & Fleming, (1967); Taylor & Green (1971), any changes in the nature of the α- adrenoceptor might be expected to lead to changes in the binding characteristics of the receptor with its specific antagonist. In the present experiments, PGE_2 could not be said to reverse α- adrenoceptor blockade by altering the receptor in such a way as to reduce its affinity for the antagonist, since pA_2 values determined in the presence of PGE_2 were, if anything, increased. The reason for this apparent increase in the affinity of the antagonist for the receptor in the presence of exogenous PGE_2 is not clear. A similar apparent increase in the effectiveness of phentolamine blockade at α- adrenoceptors has been observed in rabbit ear artery made supersensitive by prior reserpine treatment (Okpako, personal communication).

As reported earlier in this thesis, (Fig. 36 .), PGE_2 potentiates NA vasoconstriction in the rat mesenteric artery preparation by a mechanism which involved utilization of external Ca^{2+}. Part of the evidence was that potentiation was absent in Ca^{2+} - free Krebs and increased in proportion to Ca^{2+} in the external medium. Reveral of antagonism described here appears to be more complex than can be accounted for in terms of enhancement of NA vasoconstriction caused by PGE_2. In the first place, NA potentiation is not observed in Ca^{2+} - free Krebs

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(Adeagbo & Okpako, 1980 and Fig. 33), but NA antagonism by phentolamine, tolazoline and yohimbine in Ca^{2+} - free Krebs was reversed by PGE_2 to about the same extent as in normal Krebs. This suggests that external Ca^{2+} may not be involved in blockade reversal by PGE_2. Secondly, if enhancement of NA vasoconstriction by PGE_2 would account for the reversal, then the following relationship should hold for a given dose of PGE_2:

$$DR_p \text{ (Potentiation factor)} = DR_A - DR_{APG} \text{ (Reveral factor)}$$

where DR_p = NA dose - ratio in the presence of PGE_2

DR_A = NA dose - ratio in the presence of antagonist.

DR_{APG} = NA dose - ratio in the presence of antagonist + PGE_2.

For all the antagonists (Table 4) the reversal factors were significantly higher ($p < 0.005$) than the potentiation factor obtained for NA with 2.8×10^{-8} M PGE_2. This would suggest that the reversal of antagonism by PGE_2 cannot be accounted for in terms of simple enhancement of NA vasoconstriction.

In contrast, the partial reversal by A23187 of NA antagonism appeared to be due to its ability to enhance NA vasoconstriction, since A23187 reversed NA blockade by the same order of magnitude as that by which it potentiated NA (Table 6). This compound facilitates Ca^{2+} influx(Reed, 1968; Reed & Lardy, 1972; Pressman, 1976) which would account for potentiation of NA vasoconstriction.

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The "selectivity" of PGE_2 in its mode of blockade reversal is noteworthy and interesting. NA antagonistic actions of cinnarizine, PBZ (above 2.9 x 10^{-10} M) and verapamil were unaffected by PGE_2. All these agents have been shown to block excitation - contraction (E - C) coupling. For instance, verapamil has been shown to block E - C coupling in the heart - an action which has been attributed to Ca^{2+} antagonism (Fleckenstein, Tritthart, Fleckenstein, Herbst & Grun, 1969) and/or specific blocking effect of potential dependent Ca^{2+} permeability channels (Rasmussen & Goodman, 1977). Similarly, cinnarizine (Godfrain & Kaba, 1968, 1972) and PBZ in high doses (Bevan, Osher & Su, 1963; Shibata & Carrier, 1967) have been shown to be Ca^{2+} antagonists. In contrast, non of the so - called competitive antagonists at the α- adrenoceptor site have been shown to be a Ca^{2+} antagonist. Catecholamine - α - receptor interaction has been shown to initiate the mobilization of a directly proportional number of Ca^{2+} ions to generate a proportionate response (Moran, Swammy & Triggle, 1970). Competitive α - adrenoceptor blockers therefore reduce the amount of bound Ca^{2+} mobilized by the agonist. Consequently, the response is reduced. It thus seems, that PGE_2 probably augments the mobilization of internal Ca^{2+} to induce blockade reversal. PGE_2 would therefore not be expected to reverse the NA antagonistic actions of cinnarizine,

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PBZ (in high doses) and verapamil since these agents are blockers of the mobilizable Ca^{2+} ions. PGE_2 is thus a useful tool for distinguishing between types of α - adrenoceptor blockade.

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CHAPTER FIVE

SUMMARY AND CONCLUSION

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SUMMARY AND CONCLUSION

1. PGE_2 causes potentiation of NA vasoconstriction by a mechanism which involves utilization of external Ca^{2+}. The evidence for this includes:-

   (a) potentiation is directly related to the concentration of Ca^{2+} in the external medium and it is absent when no Ca^{2+} is present in external medium;

   (b) effects of PGE_2 is additive with cocaine and reserpine, two agents reported to cause potentiation by their effects on calcium movement (Summers & Tillman, 1979; Carrier & Hester, 1976 for cocaine and reserpine respectively);

   (c) potentiation was blocked by bretylium, cinnarizine, chlorpromazine and verapamil in doses at which these substances did not affect the responses to NA. These substances are known to block Ca^{2+} influx.

2. High K^+ vasoconstriction shown in this study to depend solely on external Ca^{2+} was not potentiated by PGE_2. Similarly, PGE_2 did not potentiate NA when mesenteric artery was perfused with Ca^{2+} - free depolarizing krebs solution.

3. Ca^{2+} ionophore, A23187, enhanced NA vasoconstric NA vasoconstriction by a mechanism which involves Ca^{2+} - dependent neurotransmitter release from adrenergic nerve endings in the isolated

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rat mesentery. This is evident because A23187 induced enhancement of NA was absent in reserpine pretreated rats, and also during perfusion of the artery with Ca^{2+} - free krebs solution.

4. High doses of A23187 caused vasoconstriction which was not blocked by α- adrenoceptor antagonists but was almost completely abolished by putative Ca^{2+} antagonists. The vasoconstriction was sustained in Ca^{2+} - free krebs solution.

5. Blockade of NA vasoconstriction caused by "competitive" α- adrenoceptor agents such as phentolamine, tolazoline and yohimbine was reversed by low doses of PGE_2. The reversal can be demonstrated in Ca^{2+}- free krebs where NA potentiation is absent, and the extent of reversal ("reversal factor") of the blockade is greater than could be expected from simple potentiation.

6. The block produced by the so-called non-competitive antagonists such as high doses of phenoxybenzamine, cinnarizine, and verapamil was not reversed by PGE_2. The block caused by these agents was not of the competitive type and may have involved Ca^{2+} antagonism.

7. Prazosin block of NA was also non-competitive and not reversed by PGE_2.

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8. The results showed PGE_2 to be a useful tool for differentiating between types of α- adrenoceptor blockade. Furthermore, the finding that blockade caused by phentolamine, tolazoline and yohimbine which are not known to be prostaglandin synthetase inhibitor can be reversed by low doses of PGE_2 shows that this prostaglandin can reverse blockade even if the mechanism of inhibition did not involve prostaglandin inhibition in the first place.

The following mechanism of PGE_2- induced potentiation of NA have therefore been proposed based on the fact that NA causes vasoconstriction by activating the pharmacomechanical pathway (Somlyo & Somlyo, 1968) - in the dose range used in this study. The contraction following this pathway does not require external Ca^{2+} - a view supported by the finding that NA dose - response curves with or without external Ca^{2+} were superimposable (present study). Thus, presence of PGE_2 in perfusion medium enables NA to cause depolarization of the vascular smooth muscle, thereby activating the electromechanical pathway in addition to the pharmacomechanical pathway. On the other hand, the mechanism by which PGE_2 reversed "competitive" α- adrenoceptor block is not fully clear but appears to be due to enhanced mobilization of sequestered Ca^{2+} by the prostaglandin.

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