POST-JUNCTIONAL ALPHA ADRENOCEPTORS IN THE ANOCOCCYGEUS MUSCLE AND VAS DEFERENS: A COMPARATIVE STUDY IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

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Dedicated to

'Fola Tayo'

'Tunde Oriowo

Peter Akah

Sachia Khasar

Lecturers, Colleagues and dear

friends.

CERTIFICATION

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

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CONTENTS

CONTENTS

CONTENTS

ABSTRACT

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The characteristics of the post-junctional α-adrenoceptors in the isolated anococcygeus muscle and vas deferens were compared in spontaneously hypertensive rats (SHR) and normotensive rats (NCR).

Responses to α-adrenoceptor agonists were obtained in the absence and presence of cocaine and of antagonists. Noradrenaline (NA) and phenylephrine (PE) produced concentration related contractions of the preparations which were antagonised by phentolamine, prazosin and yohimbine in both rat strains, indicating α-adrenoceptor mediation. The effects of cocaine revealed the relative efficiency of the uptake mechanism in each preparation.

In the anococcygeus NA was equipotent in the NCR and SHR in the absence of cocaine whereas it was less potent in the SHR in the presence of cocaine. PE was less potent in the SHR in the absence and presence of cocaine.

Antagonism was assessed by pA_2 and K_{diss} determinations. Potencies were compared only when antagonism was competitive in both strains. In the anococcygeus low concentrations of prazosin (L-Praz) non-competitively antagonised NA but antagonised PE equally and competitively in both strains. Higher concentrations (H-Praz) competitively antagonised NA in both strains. Phentolamine was competitive against NA in NCR and against PE in both strains. However, it was non-competitive against NA in SHR. Low concentrations of yohimbine (L-YOH) competitively antagonised NA and PE in both the NCR and SHR but the K_{diss} values were significantly different. Higher

concentrations (H-YOH) was competitive against NA in the NCR and PE in both strains. In the vas L-Praz competitively antagonised both NA and PE in the NCR but gave non-competitive antagonism of both strains in the SHR. Phentolamine antagonised NA competitively in the NCR but non-competitively in the SHR. It was equipotent and competitive against PE in both strains. L-YOH non-competitively antagonised NA and PE in the NCR but in the SHR it was competitive. H-YOH antagonism was non-competitive against both NA and PE in both strains.

It is suggested that there might be both the α_1- and α_2-post-junctional adrenoceptor in the NCR anococcygeus muscle. Prazosin and yohimbine seem to be able to differentiate between the two receptor subtypes at low concentrations. It is suggested further that the α_2-subpopulation might not possess identical characteristics in the NCR and SHR anococcygeus. Also, there might be an alteration in NA uptake properties in the SHR. In the NCR vas deferens there seems to be a predominance of post-junctional α_1-adrenoceptors. In the SHR vas, there might be an increase in the post-junctional α-adrenoceptor population and/or sensitivity. Furthermore, it seems that the post-junctional α_2-adrenoceptor characteristics are somewhat different in the SHR. Uptake$_1$ is suggested to be less efficient in the SHR vas.

1.1

CHAPTER ONE

INTRODUCTION

INTRODUCTION.

1.1 GENERALITIES

"Hypertension" describes a pathological state thought to exist when the blood pressure exceeds the "normal" values. Values regarded as "normal" in themselves depend upon the age of the patient and the population or community wherein he lives. On the other hand, there occurs some exceptions wherein the blood pressure lies above or below the average values yet the individual is quite healthy. For such reasons as these the diagnosis of hypertension is based, alongside with the abnormal blood pressure, upon the findings of abnormalities in pressure-related organ or tissue characteristics like the renal function, urine and plasma composition, the electrocardiogram and the retinal blood vessel integrity. Primary, however, is the presence of a high blood pressure.

1.1.1 The blood pressure depends upon the cardiac output (volume of blood pumped by the heart per minute) and on the resistance to blood flow in the small vessels. This relationship may be expressed as follows:

$$B.P. = C.O. \times P.R.$$

where B.P. = the mean arterial blood pressure, C.O = cardiac output and P.R in the total peripheral resistance.

Thus, factors that will affect the cardiac output or the total peripheral resistance (hereafter referred to as peripheral resistance) will affect the mean arterial blood pressure. A brief consideration of some of these might be a good way to begin a retrocessive discourse on the aetiology of hypertension.

1.1.2 CARDIAC OUTPUT

The two basic variables upon which the cardiac output depends are

(i) the heart rate

(ii) the stroke volume or the extent to which the ventricles empty themselves during each contraction, both of which are in turn affected by some factors.

The magnitude of the venous filling (blood returned to the heart) plays an important role in the determination of the cardiac output, affecting mainly the stroke volume. Increased venous return causes a pressure rise in the atria. More blood is forced into the ventricles and the end diastolic stretch of the myocardial fibres is increased resulting in greater force of ventricular contraction - the Frank - Starling mechanism (Starling, 1918). Other factors include circulating substances that will alter the heart rate or force of ventricular contraction (chronotropic and inotropic responses (respectively) of the heart) which are mainly the circulating catecholamines (and the substances that sensitize the heart to their action) acting via stimulation of β-adrenergic receptors. Vagal factors will produce a lowering of the heart rate while the stimulation of the adrenergic innervation of the heart will increase the rate and force of cardiac contraction.

1.1.3 PERIPHERAL RESISTANCE

A lowering of the total peripheral resistance is usually the main focus of attention in lowering the blood pressure in almost all forms

of hypertension. This shows how crucial the peripheral resistance is in hypertension. The greater portion of the total resistance to blood flow is contributed by the small diameter vessels, which are normally maintained under a certain tone. Of these the thicker and more elastic arterioles provide the major share of this resistance. For this reason these arterioles are commonly referred to as the resistance vessels. The peripheral resistance is increased or decreased by a decrease (vasoconstriction) or an increase (vasodilatation), respectively, in the luminal diameter (that is, an inverse proportionality). From the relationship given above (B.P = C.O x P.R) any agent which changes the luminal diameter of these vessels will cause an alteration of the blood pressure, if the cardiac output remains constant. The extent to which changes in the blood vessel radius affects the blood pressure can be seen from the Poiseuille formula which deals with the flow of fluid through a cylindrical tube. It states that the magnitude of the forward flow (F) of a fluid is directly proportional to the product of the pressure gradient, ΔP, across the tube and the fourth power of the radius, r, of the tube and inversely proportional to the product of the length, l, of the tube and the viscosity, v, of the circulating fluid. It can be represented mathematically as

$$F = \frac{P r^4}{l.v} k$$

where k is a constant. Thus, due to this fourth power amplification, remarkable increases in the blood pressure values might be brought about by little excesses in the activities of agents which promote vasoconstriction or little insufficiencies in the activities of

vasodilators. However, the useful anti-hypertensive agents have been those that attenuate or disanul the excesses of the agents of vaso-constriction;; suggesting that excessive promotion of vasoconstriction is more important in hypertension than insufficient vasodilation. Factors which promote vasoconstriction include increased adrenergic neural activity leading to increased neurotransmitter (noradrenaline) release and excessive levels of circulating plasma catecholamines, all acting via the stimulation of α-adrenergic receptors on the vascular smooth muscle. Others include increased levels of other vasoconstrictors like angiotension II, vasopressin, 5-hydroxy-tryptamine; increased plasma concentrations of some cations (e.g. Calcium) and alkalosis. A variety of other vasoconstriction promoting conditions have been classed as 'passively acting' (Frohlich, 1977). These include oedema formation, intravascular thrombosis or embolism, haemoconcentration (e.g. in polycythemia) and contracted plasma volume leading to increased blood viscosity among others. Most of these "mediators" of hypertension are products of some tissue or organ malfunction which may themselves, be the remotest underlying cause(s) of the hypertension or the result of some unknown, primary disorder(s). Thus, hypertension is classified into two broad types, namely Primary and Secondary hypertension. Hypertension is termed primary (or Essential or Idiopathic) when the remote cause of the hypertension is unknown. It is said to account for up to 80% of the cases of hypertension (Bowman and Rand, 1980) and seems to be genetically determined. When the cause of the hypertension is known it is classed as secondary hypertension.

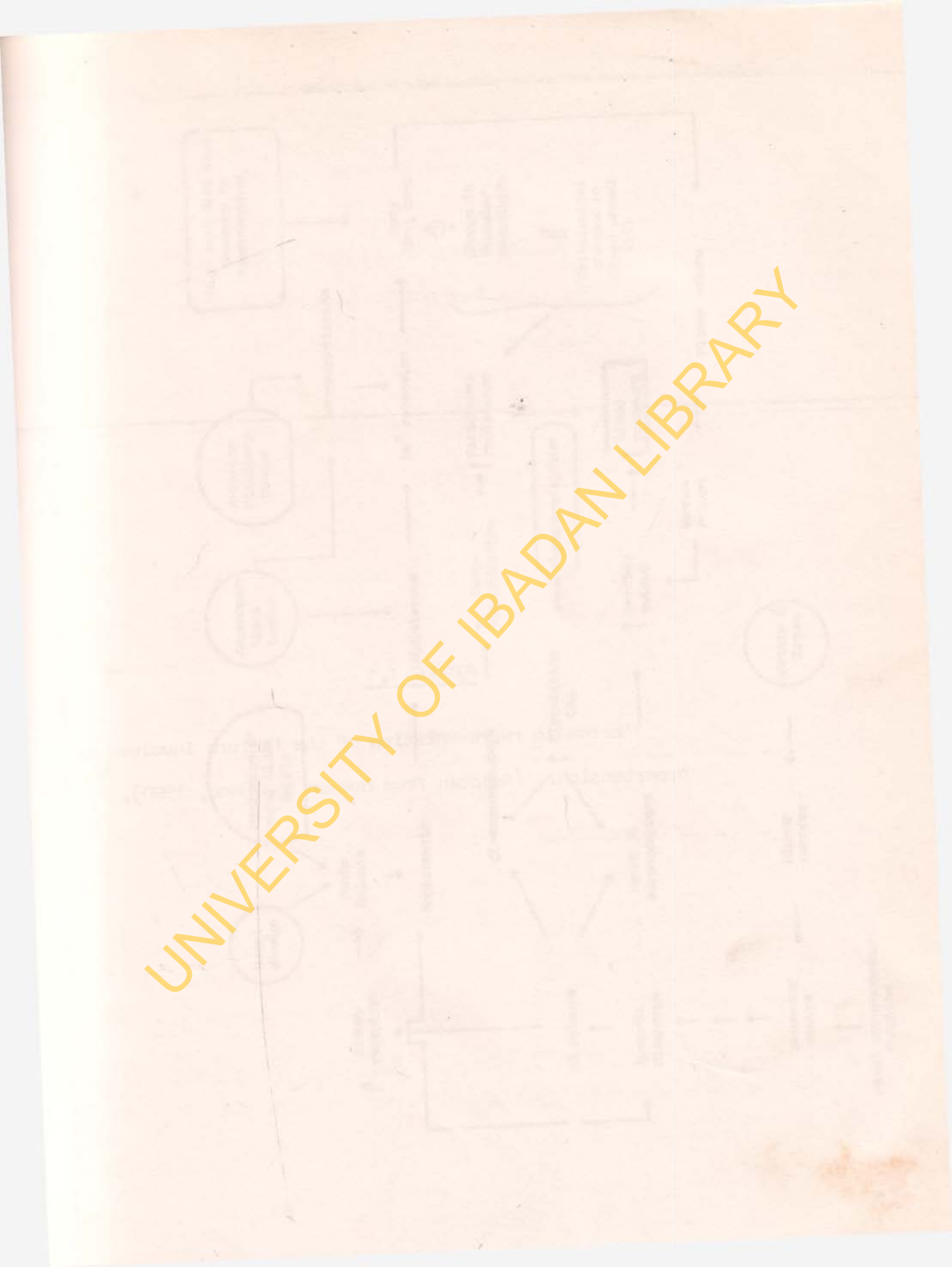


FIGURE 1.1

Schematic representation of the factors involved in hypertension. (Adapted from Bowman and Rand, 1980).

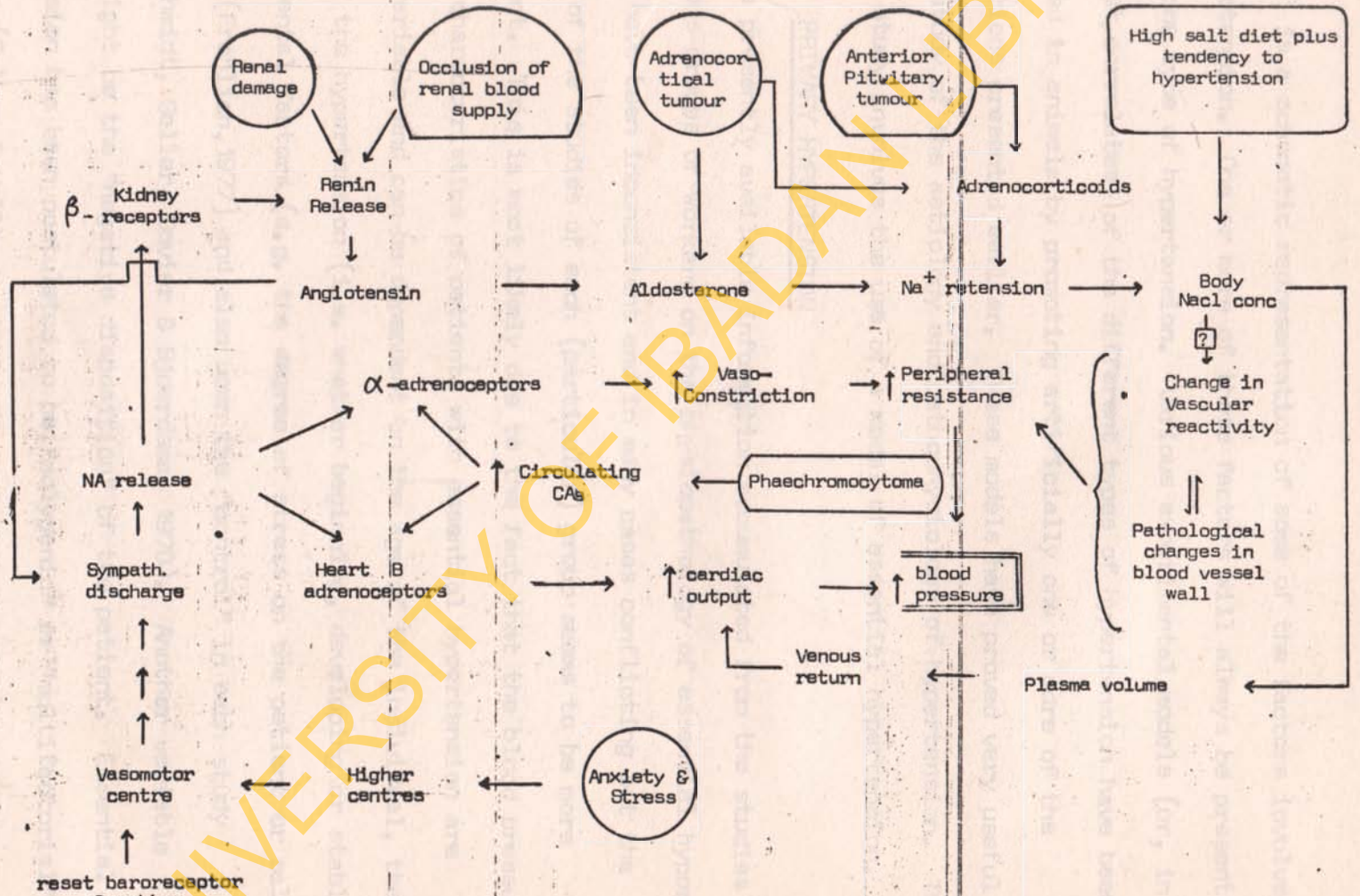


Figure 1 is a schematic representation of some of the factors involved in hypertension. One or more of these factors will always be present in any one type of hypertension. Various experimental models (or, in the least, correlates) of the different types of hypertension have been developed in animals by promoting artificially one or more of the dysfunctions presented earlier. These models have proved very useful in the study of the aetiology and pathophysiology of hypertension. The present study involves the use of a model of essential hypertension.

1.2 PRIMARY HYPERTENSION

The presently available information accumulated from the studies of various groups of workers on the physiopathology of essential hypertension have been inconsistent and in many cases conflicting but the results of the studies of each (particular) group seems to be more consistent. This is most likely due to the fact that the blood pressure related characteristics of patients with essential hypertension are highly variable and can be dependent on the age of the individual, the stage of the hypertension (i.e. whether beginning, developing or stable), environmental factors (e.g. the degree of stress on the patient or salt intake) (Frohlich, 1977) and also upon the "control" in each study (Clineschmidt, Geller, Govier & Sjoerdsma, 1970). Another variable factor might be the "negative disposition" of the patient. Essential hypertension has been postulated to be "polygenic" or "multifactorial" in origin (Folkow & Hallback, 1977; Beyer & Peuler, 1982); in other words a number of blood pressure related mechanisms exhibit a genetic deviation from the "normal". Thus, it can be inferred that if all the

key mechanisms contributing to blood pressure regulation possess a genetic (maybe latent) tendency to over-react, the particular one that precipitates the hypertensive response will be determined by the prevalent environmental stimulus (e.g. stressful conditions or salt intake). On the other hand, one or so of these mechanisms might exhibit a form of genetic dominance over the rest and is therefore more likely to generate a hypertensive state. However, it seems that when essential hypertension has attained a stable state it exhibits very similar physiopathology irrespective of the aetiology. It might be said therefore, that within the clinical population with essential hypertension, there may be sub-sets, each characterised by somewhat different aetiology. These sub-sets may be correlates of the presently known models of essential hypertension (Folkow & Hallback, 1977), each of which exhibits some degree of semblance to some variant of essential hypertension or another.

1.2.1 EXPERIMENTAL MODELS OF GENETIC (PRIMARY) HYPERTENSION

These have been developed by careful in-breeding of animals possessing higher blood pressure than the population mean which has resulted in the evolution of a naturally hypertensive strain of rabbits (Alexander, Hinshaw & Drury, 1954) and several such strains of rats. The rat models have been better developed and more extensively studied maybe because of their small size and relatively low cost. These rat strains include the spontaneously Hypertensive Rats (SHR) developed by Okamoto and Aoki (1963), who also have developed some more differentiated substrains; the Genetically Hypertensive Rats (GHR) developed in New Zealand by Smirk and Hall (1958); the Milan Hypertensive Strain (MHS)

developed by the Bianchi group (Bianchi, Fox, DiFrancesco, Bardi & Radice, 1973) and the Hypertension Sensitive Rats (HSR) developed by Dahl, Heine and Tassinari (1962). More recently is the Sabra strain developed in Israel by Zamir, Gutman and Ben-Ishay (1978) and the Lyon strain from France (Renaud, Fourniere, Denoroy, Vincent, Pujol & Sassard, 1978). The most extensively studied of these is the Japanese SHR, which was used in the present study. Thus, in this discourse the SHR constitutes the main focus of attention. Two other strains are briefly discussed in order to portray typical examples of the slight differences among the different strains in the pathophysiology of their hypertension. An integration of the information derived from all of these strains might produce a picture that is more representative of the pathophysiology of human essential hypertension than any one model.

1.2.2 NEW ZEALAND GENETICALLY HYPERTENSIVE RATS (GHR)

This was the first of genetically hypertensive rat strains to be developed and it was based upon systematic in-breeding among the Otago Stock Colony which itself originated from the Wistar strain. It took longer to obtain a pure hypertensive strain than in the breeding of SHR, suggesting that this strain is not a replica of the SHR. Within a few weeks significant difference in blood pressure could be observed between this strain and the normotensive control. The sympathetic nervous system seems to play an important role in the maintenance of hypertension in the GHR. Early immuno- or chemical sympathectomy normalises arterial blood pressure (Clark, 1971; Wood and Clark, 1974) and the increased heart

rate is blocked by propranolol (Lee & Simpson, 1973). Later, along with the development of hypertension sympathetic activity may become normalised but there are clear signs of ventricular hypertrophy and structural changes in the resistance vessels (Phelan, 1970).

Studies in young, prehypertensive and in mature GHR (Gresson, Bird and Simpson, 1973 a & b) reveal a lowered plasma renin level, a normal or slightly reduced plasma volume and a normal body sodium and water content throughout life. Plasma Sodium (Na^+) content is normal but the potassium (K^+) is slightly increased (in contrast to SHR where K^+ is slightly decreased). The renin-angiotensin - aldosterone system is normal or slightly suppressed. There is no good evidence of Na^+ retention or blood volume increase (Simpson, Phelan, Clark, Jones, Gresson, Lee & Bird, 1973) except at old age (Gresson & Simpson, 1974) in association with gradual cardiovascular failure, which are probably the result of the hypertension.

1.2.3 DAHL HYPERTENSION SENSITIVE (HSR) STRAIN

The development of this strain of hypertensive rats stems from the observation by Dahl and his collaborators that some of their Sprague-Dawley rats exhibited qualitative differences from some others in their response to salt (NaCl) loading (Dahl, Heine & Tassinari, 1962a & b). It seemed then that genetic factors were playing a great role. Thus, they initiated a selective and careful in-breeding that led to the development of two pure strains of rats, one developing hypertension in response to salt loading (Hypertension Sensitive Rats, HSR) and the other being resistant to salt loanding (hypertension Resistant

Rats, (HRR) (Dahl et al. 1962). They are often referred to as the "S" and "R" strains respectively.

Both the HSR and HRR are normotensive animals when on a normal NaCl diet although the cardiac output is slightly higher in the "S" strain (Tobian, 1977). However, HSR readily develop relatively very high systolic blood pressure than the control HRR. These two strains of rats also respond differently to artificial disturbances of some blood pressure related mechanisms. Thus, the HSR is more susceptible to pressure increases in response to desoxycorticosterone (DOCA) - NaCl, to renal artery clamping, to cortisone and to adrenal regeneration (Dahl et al., 1963, 1965). This model might therefore be a correlate of some variant of genetically hypertensives who do not exhibit any sign of hypertension but just remain "prone" until an environmental factor precipitates the hypertension.

The experiments of Dahl et al. point to the kidneys as the major site for triggering hypertension in the HSR. In cross-transplantations of kidneys between HSR and HRR, it was observed that HSR with HRR kidneys had lower pressures than HRR with HSR kidneys (Dahl et al., 1974). The participation of other extra-renal pre-disposing factors has not been excluded. Dahl et al. (1972) discussed the possibility of a change in ionic equilibrium in vascular smooth muscle membranes which, during salt loading precipitates an increased smooth muscle activity. Accordingly it has been reported that increased peripheral resistance accounts for almost half of the total rise in blood pressure observed in salt loaded HSR (Tobian, 1977). The sympathetic nervous system seems

to have a crucial role as well. The increased vasoconstriction during salt intake is said to possess a neurogenic component since it can be blocked by sympathetic denervation of the vascular bed under study (Takeshita & Mark, 1978). Also, after chemical sympathectomy with 6-hydroxydopamine the HSR do not develop the typical salt-induced hypertension (Takeshita, Mark & Brody, 1979). Whether this sympathetic component is due to differences in neuronal discharge rates or to α-adrenoceptor population is not yet fully elucidated. However, it has been reported that there are more α_1- and α_2- adrenoceptors in the kidneys of HSR than in the HRR (Pettinger, Sanshez, Saavedra, Haywood, Grandler & Rodes, 1982). This greater number of α-adrenoceptors is exaggerated in the HSR by high salt intake and is said to anticipate the rise in blood pressure (Pettinger et al., 1982).

1.2.4 THE JAPANESE SPONTANEOUSLY HYPERTENSIVE RAT (SHR)

1.2.4a PHYSICAL CHARACTERISTICS

The SHR do not exhibit any external physical differences from the Wistar-Kyoto rats (WKY) or the ordinary Wistar control rats (NCR) except that they are generally smaller in size i.e. when rats of the same age are compared. However, on opening up the animal, it can be observed that the left ventricle is bigger (Pfeffer, Pfeffer & Frohlich, 1977; Frohlich, 1977). We have observed (unpublished)that the SHR kidneys (to the naked eyes) look a lot more crimson than those of the NCR.

1.2.4b BLOOD PRESSURE DEVELOPMENT

The blood pressure in the SHR develops gradually as in the normotensive rats and as in man. But at about the age of five weeks the blood pressure values become significantly higher than in the NCR. The blood pressure continues to rise with age but does so at a faster rate in the SHR than the NCR (Okamoto, 1972; Trippodo, Walsh & Frohlich, 1978) until about 10 - 12 weeks of age when the blood pressure seems to maximise in the NCR but still increases, although at a much slower rate, in the SHR (Folkow & Hallback, 1977). Thus, animals of twelve weeks and above are taken to have attained relatively stable blood pressure levels and represent a model of "established hypertension", younger ones represent "labile" or developing hypertension and the very young (less than five weeks) are termed "pre-hypertensive" (Okamoto, 1972). Specific values of blood pressure recorded from SHR differ from laboratory to laboratory. It depends on the particular SHR colony studied, on environmental factors and on the method of measurement.

1.2.4c HAEMODYNAMICS

In the adult SHR the cardiac output is normal but there is an increased total peripheral resistance (Pfeffer & Frohlich, 1973; Smith & Hutchins, 1979). However, in the young (less than 12 weeks old) rats the results have been inconsistent. Frohlich & Pfeffer (1975) ascribed this to the use of different strains as control. They observed that the cardiac output in the young SHR is significantly greater than in the NCR but not different with respect to WKY (Pfeffer & Frohlich, 1973; Frohlich & Pfeffer, 1975; Frohlich, 1977). Nevertheless, even if increased, the

cardiac output does not seem to contribute to the high blood pressure nor is the raised peripheral resistance a cardiovascular response to the cardiac output (Pfeffer et al., 1974; Frohlich & Pfeffer, 1975). There is a higher heart rate, however, in the SHR throughout life (although it seems to decrease at old age) and the left ventricle undergoes hypertrophy (Frohlich, 1977). The blood volume may either be normal or slightly reduced both in the young (Trippodo et al., 1978) and adult (Sen, Hoffman, Stowe, Smeby & Bumpus, 1972; Ripper, Lundin & Folkow, 1978).

1.2.4d. RENAL AND HUMORAL FACTORS

The kidney might have some role to play in the development of hypertension in the SHR. A delay in the onset of hypertension has been observed (Liard, 1977, Kline, Kelton & Mercer, 1978) after renal denervation. Renal blood flow has been reported normal with a normal glomerular filtration rate but with increased filtration (Beierwaltes & Arendshorst, 1978; Steele & Underwood, 1978, Arendshorst & Beierwaltes, 1979). Thus, renal vascular resistance is elevated but it seems like it results from a generally overactive nervous system (Arendshorst & Beierwaltes, 1979).

Renal handling of water and electrolytes does not appear to be different (Norman, Enobakhare, De Clue, Douglas & Guyton, 1978) and both extracellular fluid volume and plasma sodium levels seem normal in the SHR (Trippodo et al., 1978; Willis & Bauer, 1978). Koletsky, Shook & Rivera Valez (1972) reported a normal or slightly lower plasma renin level. However, Sen et al. (1972), reported a slightly increased plasma

renin activity in the young SHR. Other workers have suggested that the initial increase in plasma renin levels may be as a result of a general overactivity in the adrenergic system since renin release is also modulated neurogenically via β-adrenoceptor mechanisms (Folkow & Hallback, 1977). By this explanation there should be a slight increase in the angiotensin - aldosterone levels too but it does not seem the adrenal cortex and mineralocorticoids play an important role in the genesis of SHR hypertension. SHR hypertension is not eliminated by adrenalectomy provided a sodium chloride supplement is given (Folkow and Hallback, 1977).

1.2.4e BARORECEPTOR FUNCTION

The persistently faster heart rate and normal cardiac output in the presence of an increased peripheral resistance suggest some malfunction in the baroreceptor reflex. Nosaka & Okamoto (1970) and Nosaka & Wang (1972) demonstrated the occurrence of altered baroreceptor reflex characteristics in the SHR. Pfeffer & Frohlich (1973) also showed that the baroceptor reflex was not totally absent but rather set at higher levels of arterial pressure. More recently it has been shown that baroreceptor sensitivity was essentially the same in very young (4 - 6 weeks old) SHR and WKY rats. However, that same level of sensitivity remain in the SHR while it almost trippled in the WKY by the time the animals were 20 weeks old (Struyker-Boudier, Evenwel, Smits & Van Essen, 1982). This suggests that the differences in the baroreceptor functions are genetic.

1.2.4f NEURAL FACTORS

Differences have been observed right from the level of the higher centres of the brain through to the peripheral network and even as regards events at the nerve terminal. The hyperfunction of the higher centres expresses itself in greater aggressiveness and alertness to environmental stimuli than in NCR (Okamoto, 1972; Rifkin, Silverman, Chawez & Frankl, 1974) and an exaggerated increase in blood pressure to stress (Yamori, Matsumoto, Yamobe & Okamoto, 1969; Hallback & Folkow, 1974). It was recently reported that simple changing from single unit housing to several SHR in a cage is enough to provoke a sharp rise in blood pressure such as was not observed with WKY rats (Lloyd & Boyd, 1981). Other studies have indicated the involvement of lower centres of the brain. Posterior hypothalamic spontaneous activity has been shown to be greater in the young (9 weeks) SHR (Bunag & Takeda, 1979). Hyperactivity in these brain areas express themselves also in the peripheral autonomic system and Folkow & Hallback (1977) reported that they are more intense in the 'prehypertension' or 'labile hypertension' age than in the older SHR. This suggests that the hyperactivity precedes the hypertension rather than being a consequence thereof. Furthermore, they remarked that the central hyperactivity in SHR does not seem to express itself in all autonomic efferent links but rather in the sympathetic outflow alone -- based on some of their earlier observations (Hallback, Magunsson & Weiss, 1974). This suggests that there seems to exist some genetically determined peculiarities in the sympathetic nervous system of the SHR that affects its function and

relation to other blood pressure related functions of the body. It will be recalled that the sympathetic system was mentioned earlier on as very crucial in the induction of hypertension by NaCl in Dahl HSR, in which there is no impressive evidence of any central nervous system hyperactivity (Dahl et al., 1968). Hyperactivity in the sympathetic system of the SHR has been reported from experiments involving direct recordings of nerve activity (Okamoto, Nosaka, Yamori & Matsumoto, 1967; Judy, Watanabe, Henry, Beseh, Murphy & Hockel, 1976; Schramm & Barton, 1979). Less direct evidence has been reported from experimental studies in the SHR where surgical or pharmacological reduction or abolition of sympathetic influence resulted in greater reduction of arterial blood pressure in the SHR than in the NCR (Folkow, Hallback, Lundgreen & Weiss, 1972; Numao & Iriuchijima, 1974; and Yamori, 1976). As in its maintenance, the sympathetic nervous system seems to be very crucial in the development of SHR hypertension as shown by experimental studies in which immunosympathectomy (Cutilletta, Erinoff, Heller, Low & Opril, 1977) or chemical sympathectomy (Provoost & De Jong, 1978) prevented the development of hypertension. At the level of the nerve ending, the prejunctionally located α_2-adrenoceptors have also been implicated in SHR/NCR comparative studies. Galloway & Westfall (1982) reported that the prejunctional α_2-adrenoceptor population in the adult SHR kidney in less sensitive than normal.

1.2.4g. VASCULAR REACTIVITY

The increased total peripheral resistance and the exaggerated rise in blood pressure observed in response to experimental stimuli suggests that there is a difference in the reactivity of the SHR vascular smooth muscle to vasoactive substances. Folkow and his co-workers have maintained over the years that the altered blood vessel design (a hypertrophy of the vessel wall which increases the wall-to-luman ratio) in the least, contributes to resistance to blood flow and also to the hyper-responsiveness of the resistance vessels to constrictor agents (Folkow, Hallback, Lundgreen, Silverton & Weiss, 1972, 1975; Collis and Vanhoutte, 1977; Mulvany, Hansen & Aakjaer, 1978; Folkow, 1982). Others who have reported increased reactivity felt it was due mainly to a functional (rather than structural) alterations in the vascular smooth muscle (Haeusler & Finch, 1972, Holloway & Bohr, 1973, Finch & Haeusler, 1974; Bohr, 1974; Lais & Brody, 1975; Hermsmeyer, 1976).

Experiments in which the effect of the increased wall-to-lumen ratio of the vessels will be of no consequence became important. A strip made from some isolated blood vessel should not exhibit any hyperactivity, if it is mainly due to geometric alteration. Hallback, Lundgreen & Weiss (1971) working on such strips could not obtain any differences in reactivity whereas Shibata, Kurahashi & Kuchii (1973) obtained qualitative differences in the response of SHR arteries to some non-physiologic cations. This kind of conflicting reports have been the case in vascular

strips (Friedman, 1977). This may be due to variety in the control strain of animal or possibly due to variations in the particular blood vessel employed in the study. Some have attempted to relate the hyper-responsiveness to altered membrane ion (Na^+) fluxes and enzymes (Na^+, K^+ - ATPase) (Jones, 1973; Friedman 1977, 1979; Pamani, Clough & Haddy, 1979) and this is increasingly becoming an area of great interest.

1.2.5 NON-VASCULAR TISSUES

There is presently very little literature on pharmacological studies on non-vascular tissues of the SHR. Altman, Da Ponte & Worcel (1976, 1977) studied the reactivity of the SHR stomach fundus strip to a series of cations (Sr^{2+}, La^{3+}, Ca^{2+}, Mn^{2+} & Ba^{2+}). They observed that male SHR fundus strips were more reactive to Ba^{2+} and Sr^{2+} than the normotensive control strips. The contractile action of Ca^{2+} in depolarised preparations were enhanced in both male and female SHR strips and the effect of diazoxide (a calcium antagonist) was more marked in SHR than in the control rats. Corbett, Goldberg, Swamy, Triggle & Triggle (1980) compared between normotensive and SHR, the sensitivity of vasa deferentia to noradrenaline (NA) K^+, Ba^{2+}, La^{3+}, H^+ and Ca^{2+}. Furthermore, Nghiem, Swamy & Triggle (1982) studied the effect of a calcium antagonist on NA and KCl induced contractions of vasa deferentia from normotensive and SHR. Results from these two studies suggested that there may be alterations in Ca^{2+} handling in the SHR. Also, Caufield, Paterson & Wayyes (1977) reported altered response to NA of vasa deferentia from genetically hypertensive rats.

In all these studies the aim was to determine if the altered reactivity observed in vascular tissue was generalised (in smooth muscles) in which case, it might suggest that the cellular modifications responsible for the altered reactivity in the SHR is not an adaptive reaction to high blood pressure. Their results suggest that there might be a generalised smooth muscle abnormality with a genetic background. Thus, it is possible that there might be some alteration in the functions of some visceral organs of the body. Further studies in this area might bring to light some disorders which might accompany hypertension but which have hitherto been taken to be present "only incidentally".

1.3 THE PRESENT STUDY

The present work attempts to study the reactivity of two extra-vascular smooth muscle preparations (the anococcygeus muscle and the vas deferens) in response to α-adrenoceptor agonists and antagonists. Like in the studies cited in the preceeding section the presence of alterations will suggest that there might be a general smooth muscle abnormality that is primary in the SHR.

In this study, attention is focused on the post-junctional α-adrenoceptor populations of the smooth muscle preparations studied. The α-adrenoceptors mediate vasoconstriction and the use of some α-adrenoceptor blockers (prazosin, labetalol and indoramin) in the treatment of hypertension shows their importance in the disorder. Thus, it was considered expedient, in studying smooth muscle reactivity, to look at the α-adrenoceptor population. It was also decided that it might be wise to choose tissues which (like blood vessel strips) elicit

contractile responses to α-adrenoceptor stimulation. Thus, the rat anococcygeus muscle and the vas deferens were chosen.

1.4 SMOOTH MUSCLE α-ADRENOCEPTORS: PRESENT STATE OF THE CONCEPT

In 1948, Ahlquist classified adrenoceptors into α- and β- types based on his observation of two different orders of potency among six sympathomimetic amines all acting on a number of sympathetic functions which were subsequently divided into two groups. In support of this classification was the inhibition of the α-adrenoceptor mediated functions by the then known α-adrenoceptor antagonists without any effect on the β-mediated functions. Selective inhibition of the β-mediated sympathetic functions was first demonstrated by Powell & Slater in 1958 using dichloro-isoprenaline (DCI). Other specific agonists and antagonists of the α- and β-receptors have since been developed. The β-adrenoceptors were later subdivided into two on the basis of a series of studies similar to that of Ahlquist and carried out by Lands and his colleagues (1964 - 1967). They found that the order of potency among some sympathomimetics acting on β-adrenoceptors like in lipolysis and cardiac function were similar and statistically correlated on one hand while the relative potencies on others like bronchodilation and vasodepression were similar and correlated on the other hand but there was neither similarity nor correlation between the former and the latter pairs of functions. This subclassification was verified by the description (Levy, 1964, 1966) of a selective antagonist for the group of β-effectors typified by the rat uterus or peripheral blood vessels

(subsequently named β_2-adrenoceptors) and the description by Dunlop and Shanks (1968) of ICI 50172 (practolol) which selectively blocks the other types of β-receptor typified by cardiac function (β_1-adrenoceptor). Thus, two phenomena have been used in adrenoceptor classification:

(i) order of potency among drugs and

(ii) drug selectivity.

Up to as late as 1972, it could be concluded that the α-adrenoceptors were of a single type since the agonists and antagonists used did not exhibit any appreciable discrepancies in the two phenomena described above, among the different preparations used (Furchgott, 1972). Soon afterwards the existence became evident of α-adrenoceptors that mediate, by a negative feedback mechanism, the regulation of noradrenaline release from sympathetic nerve terminals. They are suggested to be located somewhere on the nerve endings i.e. "pre-junctionally" (Langer, Alder, Enero & Stefano, 1971; Starke, 1972, Rand, McCulloch & Storey, 1973; Langer, 1973). Langer, 1974 then designated the post-junctional α-adrenoceptor as "α_1-" and the pre-junctional as "α_2" to distinguish between the two receptor subtypes. This classification was plainly on the basis of the anatomical locations of the receptors until pharmacological differences between the two receptor populations was demonstrated - using drug selectivity, order of potency and potency ratios (i.e. between the pre- and post-junctional receptors). It was shown in various preparations that α-adrenoceptor agonists like

clonidine, xylazine, guanabenz, B-HT 920 and antagonists like yohimbine and rauwolscine are relatively more potent on the α_2-subtype while agonists like phenylephrine and methoxamine and antagonists like prazosin, clozapine and azapetine exhibit preference for the α_1-subtype. Noradrenaline and phentolamine typify respectively agonists and antagonists that do not discriminate between the two adrenoceptor subtypes (see reviews by Starke, 1977, Langer, 1974, 1977). Thus at present, the terminologies " α_1" and α_2" are used independently of the anatomical location or physiological function of the α-adrenoceptors but rather according to the relative affinity for agonists and antagonists. Furthermore, α-adrenoceptor agonists and antagonists might as well be taken to have more or less been classified according to their preference for the receptor subtypes.

More recently the existence of more than one type of post-junctional α-adrenoceptor have been reported. These have come from blood pressure studies in various pithed animal preparations on the interaction with the post-junctional α-adrenoceptor of agonist and antagonists whose preference for the α-adrenoceptor subtypes had been established. It has been shown that along with the conventional α_1-adrenoceptor there seems to exist, post-junctionally, another α-adrenoceptor with very similar pharmacological characteristics to the α_2-adrenoceptors in the vascular smooth muscle (Bentley, Drew & Whiting, 1977; Drew & Whiting, 1979; Timmermans & Van Zwieten, 1980, 1981, Docherty and McGrath, 1980; Yamaguchi & Kopin, 1980; Van Meel, De Jonge, Timmermans & Van Zwieten, 1981), and the anococcygeus muscle (Docherty, MacDonald

and McGrath, 1979; Docherty & McGrath, 1980) of the pithed rat, the vascular smooth muscle of the anaesthesized cat (Drew & Whiting, 1979), the vascular smooth muscle of the pithed dog (Constantine, Gunnel & Weeks, 1980) and the vascular smooth muscle of the conscious rabbit (Hamilton & Reid, 1980). Others include the isolated rat aorta (Ruffolo, Yaden & Waddell, 1980) and human palma digital artery Jauernig, Moulds & Shaw, 1978). Other studies have indicated the possible existence of some other variants of the α-adrenoceptor. MacDonald & McGrath (1980) presented evidences that suggest that the post-junctional α_1-adrenoceptor population in the isolated rat vas deferens may not be the same as in the anococcygeus muscle and yet does not possess α_2-adrenoceptor characteristics. Ruffolo et al.,(1980) reported the possible existence in the rat spleen, portal vein and bladder of a post-junctional α-adrenoceptor subpopulation with characteristics that fall somewhat midway between the α_1- and the α_2. Hirst and Neild (1980,a,b) have suggested that some excitatory effects of noradrenaline in arterioles that they found to be resistant to phentolamine (α_1- and α_2-adrenoceptor antagonist) might be mediated by what they termed (gamma) γ-adrenoceptors. However, it seems some more detailed studies need to be done to ascertain the existence of these α-adrenoceptor variants. Thus, McGrath (1982) in a commentary on the subclassification of post-junctional α-adrenoceptors suggested that great caution be exercised in subclassifying these receptors. The question then arises: 'Is there also a dual existence of post-junctional α-adrenoceptor in the extra vascular smooth muscle

of the SHR?

1.5.1 THE RAT VAS DEFERENS

The isolated rat vas deferens preparation has been in experimental use for quite long (see Waddell, 1916; Match, 1917; Martin & Valle, 1939). It is very densely innervated with a dominant proportion of the nerves containing catecholamines (Lane & Rodin 1964; Norberg & Hamberger, 1964; Sjostrand, 1965; Swedin, 1971). Sjostrand (1965) reported that the innervation is very similar to that of the guinea pig except that the innervation seems denser but more delicate. The guinea pig vas deferens was said to possess a very dense nerve plexus within both the circular and longitudinal muscle layers. The nerve terminals run along the muscle cells chiefly following their direction. Thus, in the middle layer, the major part of the varicose terminals run circularly and in the inner and outer layers run longitudinally. In the peripheral parts and just outside the vas deferens nerve bundles are found running parallel to the vas deferens and branching into small fascicles which penetrated the wall to ramify the organ (Sjostrand, 1965). Contractions of the vas deferens can be elicited either by electrical stimulation of the pre-ganglionic nerve fibre or by transmural stimulation of the post-ganglionic nerves (Swedin, 1971). These contractions seem to be due to the stimulation of noradrenergic nerves and their subsequent release of noradrenaline (NA) since they are abolished by adrenergic neurone blocking drugs and pre-treatment with 6-hydroxydopamine (Gillespie & McGrath, 1975). There are however, misgivings about the mediator(s) of

motor transmission in this preparation. These arose because the twitch response to nerve stimulation is resistant to blockade by prazosin (Docherty, Mac Donald & McGrath, 1979) and some other α-adrenoceptor antagonists (Swedin, 1971) or by reserpine treatment (Swedin, 1971; Ambache & Aboo Zar, 1971). A number of hypotheses have been put forward in an attempt to account for these discrepant observations but more information seems necessary for greater precision in explaining them. Thus, the use of the neuronally evoked contractions of the isolated rat vas deferens in limited to studies on the pre-junctional feedback mechanism which seem somewhat generally accepted to be mediated by NA and α_2-adrenoceptors (see review by Doxey and Roach, 1980).

Exogenously applied NA and other sympathomimetic amines produce contractions of the rat vas deferens (Barnett, Symchowicz & Taber, 1968; Pennefather, 1973, Jurkiewicz & Jurkiewicz, 1976; Simon & Van Maanen, 1976; Tayo, 1979a, 1980, Mac Donald & McGrath, 1980) which can be antagonised by α-adrenoceptor antagonists like yohimbine, piperoxan, phentolamine, tolazoline, prazosin & WB 4101 (Jurkiewicz, Jurkiewicz & Valle, 1971; Kapur & Mottram, 1978, McGrath, 1978; Mac Donald and McGrath, 1980). This indicates post-junctional α-adrenoceptor mechanism. Thus, post-junctional α-adrenoceptors can be studied using drug induced contractions of the vas deferens.

Post junctional β-adrenoceptors have also been shown to be present in the rat vas deferens. These are inhibitory and can be stimulcted or blocked by β-adrenoceptor agonists and antagonists respectively (Ganguly & Battacharya, 1970, Mac Donald & McGrath, 1980).

1.5.2 THE RAT ANOCOCCYGEUS MUSCLE

The anococcygeus muscle preparation was first described about a decade ago (Gillespie, 1971, 1972). It occurs in the body in a pair. The two muscles arise in a tendon from the upper coccygeal vertebrae very close to one another in the midline of the pelvic cavity. They lie dorsal to and slightly to each side of the colon, their end portions joining together to form a bar ventral to and beyond the colon, less than one centimeter (1 cm.) from the anus. The bar thus lies caudal to the point of origin (Gillespie, 1972).

The rat anococcygeus muscle consists entirely of parallel bundles of smooth muscle cells forming a thin sheet. It has a dense autonomic innervation, the nerves running between the muscle fibres. The nerves ramify throughout the muscle presenting something similar to the innervation of the inctitating membrane (Gillespie & Maxwell, 1971). A good proportion of the nerves is noradrenergic as shown by many workers through various methods (Gillespie & Maxwell, 1971; Gibson & Gillespie, 1973; Gillespie & Lullmann-Rauch, 1974; McKirdy & Muir, 1978) although other types of varicosities seem present (Gibbins & Haller, 1979). The extrinsic nerve supply is from two main groups of nerve fibres. One originates from the upper lumbar outflow and the other from the sacral cord between L5 and S3 (Gillespie & McGrath, 1973). Stimulation of any of the two extrinsic nerves produces motor or inhibitory responses depending on the experimental conditions (Gillespie, 1972; Creed, Gillespie & Muir, 1975; Gillespie & Tilmisany, 1976; McKirdy & Muir, 1976, 1978).

Motor responses produced by field or extrinsic nerve stimulation are reduced or abolished by guanethidine (adrenergic neurone blocker) (Gillespie, 1972) or by α-adrenoceptor antagonists like phentolamine, phenoxybenzamine and yohimbine (Doxey, Smith and Walker, 1977) suggesting that they are mediated via the release of noradrenaline acting on α-adrenoceptors. These (post-junctional) α-adrenoceptors have been suggested to be a mixed population consisting of the α_1-adrenoceptor subtype and an α_2- like subpopulation, both mediating contractions (Docherty, Mac Donald & McGrath, 1979; Docherty & McGrath, 1980, McGrath, 1982). Atropine has no effect on these nerve evoked motor responses (Gillespie, 1972) suggesting that there is no muscarinic contribution to the motor responses. However, exogenously applied acetylcholine and carbachol produce contractions antagonised by atropine suggesting the presence of muscarinic receptors. It also contracts to α-adrenoceptor agonists and to 5-hydroxytryptamine (5-HT), KCl and $BaCl_2$ (see review by Gillespie, 1980). Isoprenaline produces no visible effect except in very high doses which produce propranolol resistant contractions. This suggests the absence of post-junctional β-adrenoceptors (Gillespie, 1972).

The inhibitory response to nerve stimulation is normally masked and is visible only when the tone of the muscle is sufficiently raised by an agonist like 5-HT or the excitatory response abolished by guanethidine or by a combination of the two experimental conditions (Gillespie, 1972). These responses are nerve mediated since they are

abolished by low concentrations of tetrodotoxin (Gillespie, 1972). However, the transmitter mediating the inhibitory responses and the receptor on which it acts are still unknown (see Gillespie, 1980).

CHAPTER TWO

MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 ANIMALS

Male albino rats of above 16 weeks of age were used in this study. The control (250-350 g) rats (NCR) were of the Wistar strain bred locally in the Animal House of the University of Ibadan. The spontaneously Hypertensive rats (SHR) (200-300 g) originated as a hypertensive mutant of a Wistar strain in Japan (Okamoto and Aoki, 1963), brother - sister mating being continued in this department. The rats were fed on standard livestock cubes (Pfizer Nigeria Limited) and were given water to take at will.

2.2 MEASUREMENT OF BLOOD PRESSURE

The animals were anaesthetized with 60 mg/kg Sodium Pentobarbitone given intraperitoneally 30 minutes before the cannulation. The animal was placed on its back on a small operating board and its limbs fastened. The neck was opened about the mid-line and the trachea exposed. The trachea was not cannulated in most cases since the experiment was short. The common carotid lies deep in the neck between the trachea and a bundle of muscle. The carotid artery was exposed and carefully separated from the nerve and vein that lie beside it. Three thread segments were placed around it. The most anterior one was then ligated. The most posterior one was used to occlude temporarily the carotid artery by tightening it around the artery in a reversible way. An incision was made (posterior to the first ligature) and the artery was cannulated. The third thread was used to tie the cannula in place. The cannula had been connected to a Gould Statham P 231D pressure transducer and the whole unit filled with

0.9% w/v NaCl solution containing heparin (4 units/ml). It was assured that air bubbles were expelled from the unit and a little saline flushed in. Blood pressure was recorded using a Grass Polygraph (Model 7D) that had previously been calibrated. Pulses were recorded and the blood pressure values were estimated from the latter part of the recording, after the attainment of a steady state which usually took about 10 minutes.

2.3 ORGAN PREPARATIONS

The rats were made unconscious either by a sharp blow to the head or by anaesthesia and subsequently sacrificed by exsanguination. The abdomen was cut open in the mid-line and the tissues were isolated as follows:

2.3.1 VAS DEFERENS

The urethra was cut and the testes were gently pushed out of the scrotum thereby exposing the vasa deferentia. The connective tissue around the vasa were removed and each vas deferens was desheathed as well as possible. The organ was then isolated by making two ligatures spaced at a distance of approximately 3.5 cm. from each other and about the central segment. Thus, it was ensured that the preparation had about equal portions of the epididymal and prostatic "halves" of the organ without necessarily using the whole vas length. The organ was then cut beyond the ligatures and suspended (with the prostatic end being fixed to tissue holder) under 0.5 g tension in a 15 ml. organ bath containing physiological salt solution. The physiological salt solution was maintained at 36°C (using a thermostat) and gased with air.

2.3.2 ANOCOCCYGEUS MUSCLE

The testes and the bladder were then removed. The pelvic bone was split in the midline and the underlying muscles cleared until the rectum became well exposed. The colon was then cut at the proximal end and the rest of the intestines cleared aside. The pelvic portion of the colon was pulled upwards and the delicate connective tissues behind it cleared until the anococcygeus muscles came into view. The connective tissues immediately around and between the two muscles were cleared and the two muscles isolated. The isolation was done by making ligatures at the two ends of each muscle. The muscle was then carefully cut beyond the ligatures. The length of the muscle was 1 - 1.5 cm. It was suspended (with the coccygeal end fixed to the tissue holder) under 1.0 g tension in a 10 ml. organ bath filled with physiological salt solution maintained at 36°C and gased with air.

2.4 PHYSIOLOGICAL SALT SOLUTION

The physiological salt solution used in this study was Tyrode solution of the following composition (mmol/litre): NaCl, 136.9; KCl, 2.7; $CaCl_2$ 1.8; $MgCl_2$, 0.9; NaH_2PO_4, 0.3; $NaHCO_3$, 11.9 and D-Glucose, 5.6.

2.5 EXPERIMENTAL PROCEDURE

The preparations were allowed an equilibration time of 60 minutes or - in cases where the animals were anaesthetised before sacrifice - 120 minutes. Individual contractions to increasing concentrations of the agonist were recorded - in the absence and presence of increasing concentrations of an antagonist - on smoked paper through an isotonic lever with

a tenfold magnification in the case of the anococcygeus muscle and an eightfold magnification in the case of the vas deferens. Each concentration of the agonist introduced into the bath was allowed a contact time of 90 seconds with the anococcygeus and 60 seconds with the vas deferens. Each antagonist concentration was allowed an initial 15 minutes to equilibrate with the muscle before a new concentration-response curve was constructed in the presence of the antagonist.

2.5.1 COCAINE EXPERIMENTS

In these experiments concentration-response curves were obtained in the absence and presence of cocaine in order to investigate the effect of cocaine on the responses. Cocaine was added into the bath 2 minutes before the introduction of each dose of the agonist. The main experiment (described above) was then done in the presence of cocaine.

2.6 ASSESSMENT OF RECEPTOR CHARACTERISTICS

2.6.1 RELATIVE AGONIST POTENCY

The relative potencies of the agonists and antagonists were used to characterise the receptor types or subtypes present in the preparation. Agonist potency was expressed as the pD_2 value according to Ariens & Van Rossum (1957). pD_2 is the negative logarithm of the molar concentration (EC_{50}) of the agonist that produces 50% of the maximum response possible.

2.6.2a. ANTAGONIST POTENCY (pA_2)

Antagonists were assessed by pA_2 values according to the method of Arunlakshana & Schild (1959). The pA_2 value is the negative logarithm of the molar concentration of the antagonist that renders a certain median

response to an agonist reproducible only after the agonist concentration has been doubled (Schild, 1947). It is an indirect estimation of the dissociation constant since they are mathematically related (see Appendix I). It is usually estimated by a graphical method which may at times produce a value that is not a very accurate estimate of the dissociation constant (See Appendix I). Thus, the antagonism was also assessed by the values of the dissociation constant K_{diss} obtained by direct calculation.

2.6.2b ANTAGONIST POTENCY (DISSOCIATION CONSTANT K_{diss})

K_{diss} was calculated from the relationship

$$K_{diss} = \frac{B}{x - 1}$$

where B is the molar concentration of the antagonist and "x" is the dose ratio. The dose ratio is the ratio of the agonist concentration producing a certain magnitude of response in the presence of an antagonist (of a certain concentration) to the agonist concentration producing the same response in the absence of the antagonist (Schild, 1947). This is more explicitly discussed in Appendix I. K_{diss} was calculated at each of the three concentrations of the antagonist employed in each of "n" observations. The mean K_{diss} value $\pm$ the standard error at each antagonist concentration was obtained and the "grand mean" K_{diss} for the antagonist was the mean $\pm$ standard error of all "N" K_{diss} values obtained at all three antagonist concentrations, where N is the sum of n, the number of observations at each antagonist concentration. K_{diss} values are expressed in molar.

2.6.3 NATURE OF ANTAGONISM

The nature of the antagonism (with respect to the competitive nature or otherwise) was also used to study receptor characteristics.

2.6.3a ARUNLAKSHANA & SCHILD (A - S) PLOT SLOPE

An antagonism is competitive when the slope of the Arunlakshana and Schild (A-S) regression line is not significantly different from unity and the pA_2 is obtained from the graph as the absolute value of the intercept on the abscissa (Arunlakshana & Schild, 1959). In cases where the slope of the line is different from unity it implies that the pA_2 value will change with the concentration of antagonist used and that is contrary to the properties of competitive antagonism (Schild, 1947).

2.6.3b MACKAY PLOT

Another method used to test whether the pA_2 was changing with the antagonist dose was by the regression of pA_2 values on the logarithm molar concentration of the antagonist (Arunlakshana & Schild, 1959; Mackay, 1978). The plot should exhibit no significant regression if the pA_2 is constant. pA_2 values obtained from plots in which the A-S slope is significantly different from one or the Mackay plot shows significant regression are referred to in this report as the "apparent pA_2". A more explicit description of these parameters as well as the method of determination is given in Appendix I.

2.6.3c K_{diss} ANALYSIS OF VARIANCE

Like the pA_2, the mean K_{diss} values at the three antagonist doses should not be significantly different if the antagonism is competitive

(Schild, 1947). The values were statistically treated in order to test for a difference and in cases where all the values are not the same, the antagonism was regarded as non-competitive. The grand mean K_{diss} in such cases are denoted in this report as grand mean K'_{diss} Grand mean K_{diss} values were compared between the NCR and SHR using unpaired t-test.

2.7 STATISTICAL ANALYSES

Log. molar concentration vs. percentage maximal response plots were made for the agonist induced contractions and each point represents a mean of "n" number of observations. Results were expressed as means ± standard error of the mean. In the determination of pA_2 values the line of best fit was drawn by regression analysis (See Appendix II) and its slope ± standard error thus determined. The value of the slope was then subjected to significance test.

Antagonist - Agonist pairs with values not significantly different from unity were regarded as having a constant pA_2 irrespective of the antagonist concentration in accordance with the theory of competitive antagonism. With the pA_2 vs log. (antagonist) plots, referred to in this report as "Mackay plot" a regression co-efficient significantly different from zero was taken to mean that the pA_2 varied with the antagonist concentration. The mean K_{diss} values at each antagonist concentration were compared by Analysis of Variance (ANOVA). The α-level was 0.05 and antagonisms having K_{diss} values not all the same were regarded as non-competitive. Comparison was also made between pD_2 values. The significance of differences was calculated using Student's t-tests.

The accepted level of significance was $p < 0.05$. See Appendix II for more details on the statistical analyses of results.

2.8 DRUGS USED

L-Noradrenaline (British Drug Houses) was prepared by adding an equivalent amount of sodium metabisulphite and dissolved in distilled water. Cocaine hydrochloride (Krakowski Zargad, Poland), yohimbine hydrochloride (Sigma) and phentolamine methane sulphonate (Ciba-Geigy) were dissolved in distilled water. Phenylephrine hydrochloride (Sigma) was obtained as the commercially available eye drops or as the powder (Winthrop) and dissolved in distilled water. Prazosin hydrochloride (Pfizer) stock solution was prepared by dissolving the salt in 25% methanol and subsequently diluting appropriately with distilled water. Pentobarbitone Sodium (Sagatal®; May & Baker) and Heparin (Evans Medical).

CHAPTER THREE

RESULTS

R E S U L T S

3.1. Blood Pressure

The mean systolic blood pressure values for the SHR were 180 ± 2 mm Hg (n = 10) and for the NCR, 144 ± 9 mm Hg (n = 4). The values were significantly different ($p < 0.05$).

3.2. Agonist Studies

3.2.1. Anococcygeus: The two agonists used in this study were noradrenaline (NA) and phenylephrine (PE). Both of them produced concentration dependent contractions of the anococcygeus muscle in the normotensive control rats (NCR) and the spontaneously hypertensive rats (SHR) (plate 1). NA ($7.3 \times 10^{-7} - 9.5 \times 10^{-5}$M) gave pD_2 values of 5.03 ± 0.05 and 5.17 ± 0.07 in the NCR and SHR respectively. Statistically, these values were not significantly different ($p > 0.05$) (Table 1). PE ($1.01 \times 10^{-7} - 1.29 \times 10^{-5}$) had significantly different ($p < 0.05$) pD_2 values of 6.14 ± 0.08 and 5.80 ± 0.01 in the NCR and SHR respectively (Table 1).

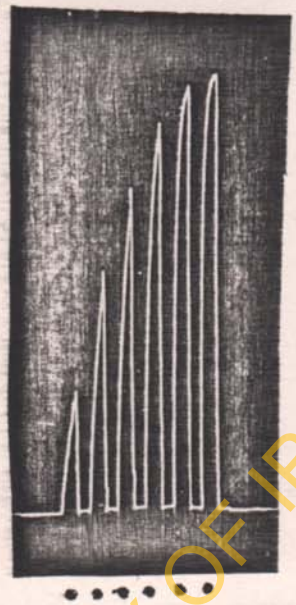
3.2.2. Vas:

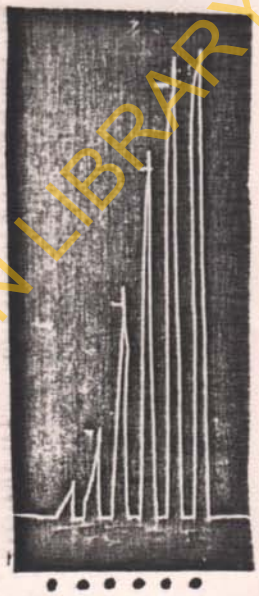
NA and PE produced concentration dependent contractions of the vas deferens in both rat strains (plate 2). NA ($5.9 \times 10^{-7} - 1.5 \times 10^{-5}$M) gave pD_2 values of 5.16 ± 0.13 and 5.46 ± 0.04 while PE ($4.03 \times 10^{-7} - 2.58 \times 10^{-5}$M) gave pD_2 values of 5.47 ± 0.05 and 5.32 ± 0.06 in NCR and SHR respectively. The NA values were signifi-

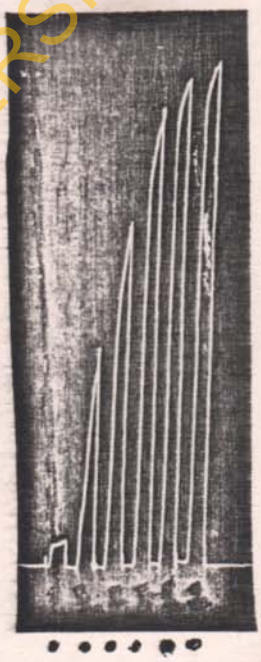
PLATE 1

A. Noradrenaline evoked contractions of the anococcygeus muscle of the NCR and SHR.

B. Phenylephrine evoked contractions of the anococcygeus muscle of the NCR and SHR.







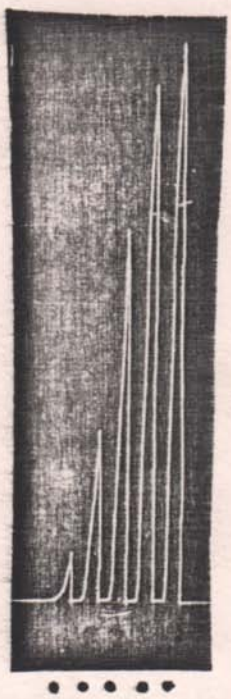


PLATE 2

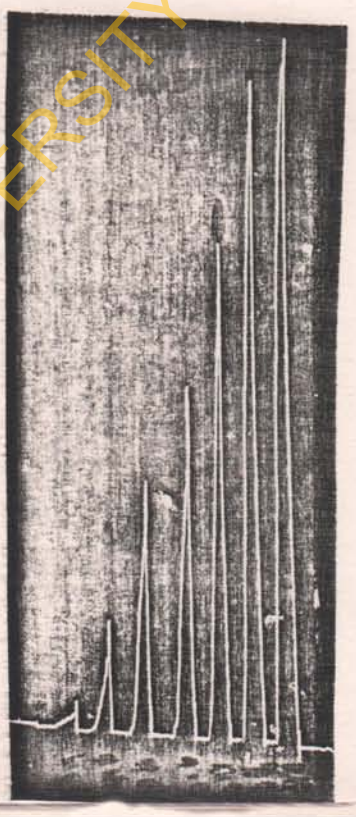
A. Noradrenaline evoked contractions of the vas deferens of the NCR and SHR.

B. Phenlephrine evoked contractions of the vas deferens of the NCR and SHR.

NCR

SHR

NCR



SHR

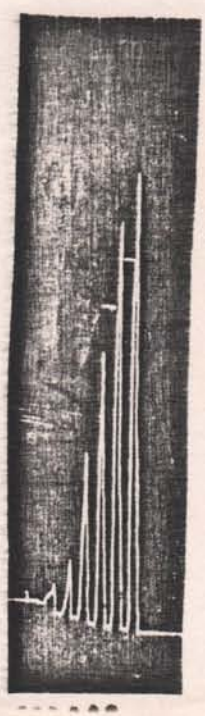


TABLE 1

A. Agonist potencies on the anococcygeus muscle in the absence and presence of cocaine and the cocaine potentiation ratios.

B. Agonist potencies on the vas deferens in the absence and presence of cocaine and the cocaine potentiation ratios.

Values represent the mean of n observations ± S.E. The figures in the parentheses are n, number of observations.

nd = not significantly different and

sd = significantly different between the upper and the lower values.

D-R = dose ratio after 5.24×10^{-7} M cocaine.

pD_2 Values

ANOC.

	NA	NA + COCA.	D-R	PE	PE + COCA.	D-R
NCR	5.03 ± 0.05(9)	5.83 ± 0.06(18)	6.31 ± 0.08	6.14 ± 0.08(2)	6.68 ± 0.04(12)	3.47 ± 0.09
	nd	sd	sd	sd	sd	nd
SHR	5.17 ± 0.07(4)	5.61 ± 0.06(18)	2.75 ± 0.09	5.80 ± 0.01(7)	6.37 ± 0.07(13)	3.72 ± 0.12

VAS

	NA	NA + COCA.	D-R	PE	PE + COCA.	D-R
NCR	5.16 ± 0.13(4)	5.42 ± 0.03(20)	1.82 ± 0.13	5.47 ± 0.05(8)	5.72 ± 0.04(16)	1.78 ± 0.06
	sd	sd	sd	nd	nd	sd
SHR	5.46 ± 0.04(5)	5.86 ± 0.05(19)	2.51 ± 0.06	5.32 ± 0.06(8)	5.80 ± 0.05(14)	3.02 ± 0.08

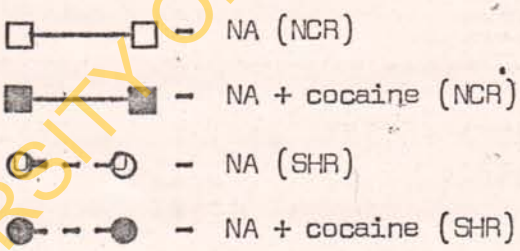
cantly different ($p < 0.05$) whilst the PE values were not ($p > 0.05$) (Table 1).

3.3. Cocaine Experiments

Cocaine (5.24×10^{-7}M) shifted the log-concentration - response curves to the left. (It was found that concentrations higher than this would raise the tone of the anococcygeus muscle but yet could not increase the potentiation in the vas deferens). The maximal responses were not altered by cocaine in the anococcygeus muscle. However, in the vas deferens the maximal responses were found to be increased in some experiments. Increase in maximal contraction to PE was observed in 60% each of the NCR and SHR vasa preparations but with NA, 40% exhibited such an increase in the SHR. No increase in maximal contractions to NA were recorded in any of the 4 observations in the NCR. The degree of increase was inconsistent but seems greater in the SHR. Thus, in the presence of cocaine the pD_2 values of NA in the anococcygeus were 5.83 ± 0.06 and 5.61 ± 0.06 while those of PE were 6.68 ± 0.04 and 6.37 ± 0.07 in the NCR and SHR respectively (Figs. 1 & 2, Table 1). Between the NCR and the SHR the pD_2 values of each agonist were significantly different ($p < 0.05$) (Table 1). In the vas deferens the pD_2 values of NA in the presence of cocaine were 5.42 ± 0.03 and 5.86 ± 0.05 and PE, 5.72 ± 0.04 and 5.80 ± 0.05 in the NCR and SHR respectively (Figs. 3 & 4, Table 1). The NA values were

FIGURE 1

NA concentration-response curves of the anococcygeus muscle in the absence and presence of cocaine. Each point represents the mean ± S.E. of at least four observations.



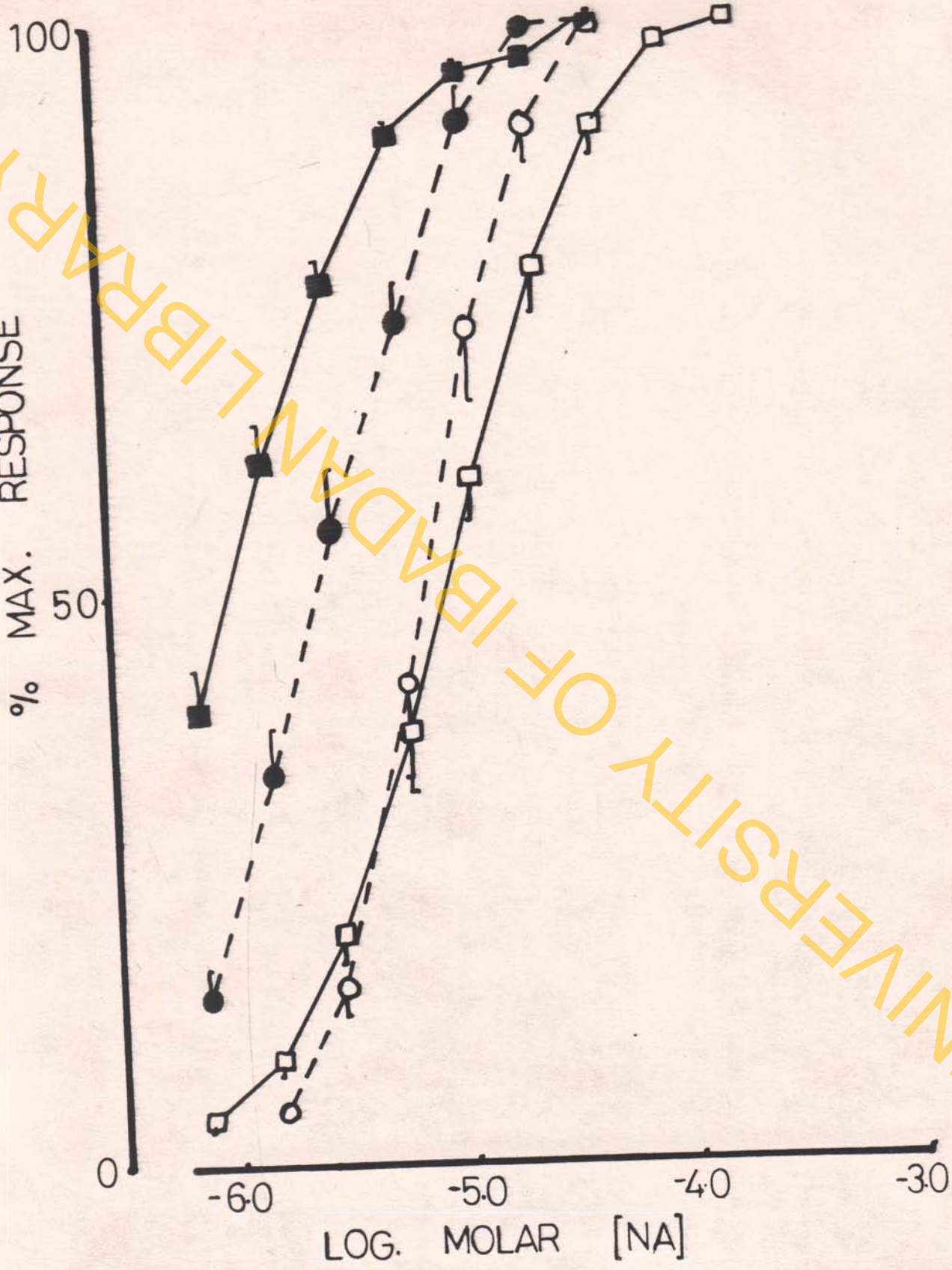
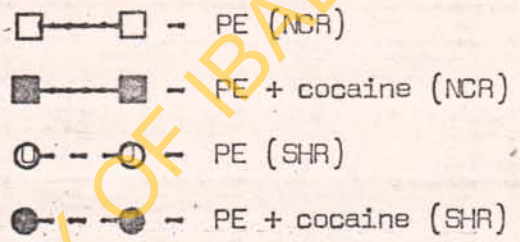


FIGURE 2

PE concentration-response curves of the anococcygeus muscle in the absence and presence of cocaine.



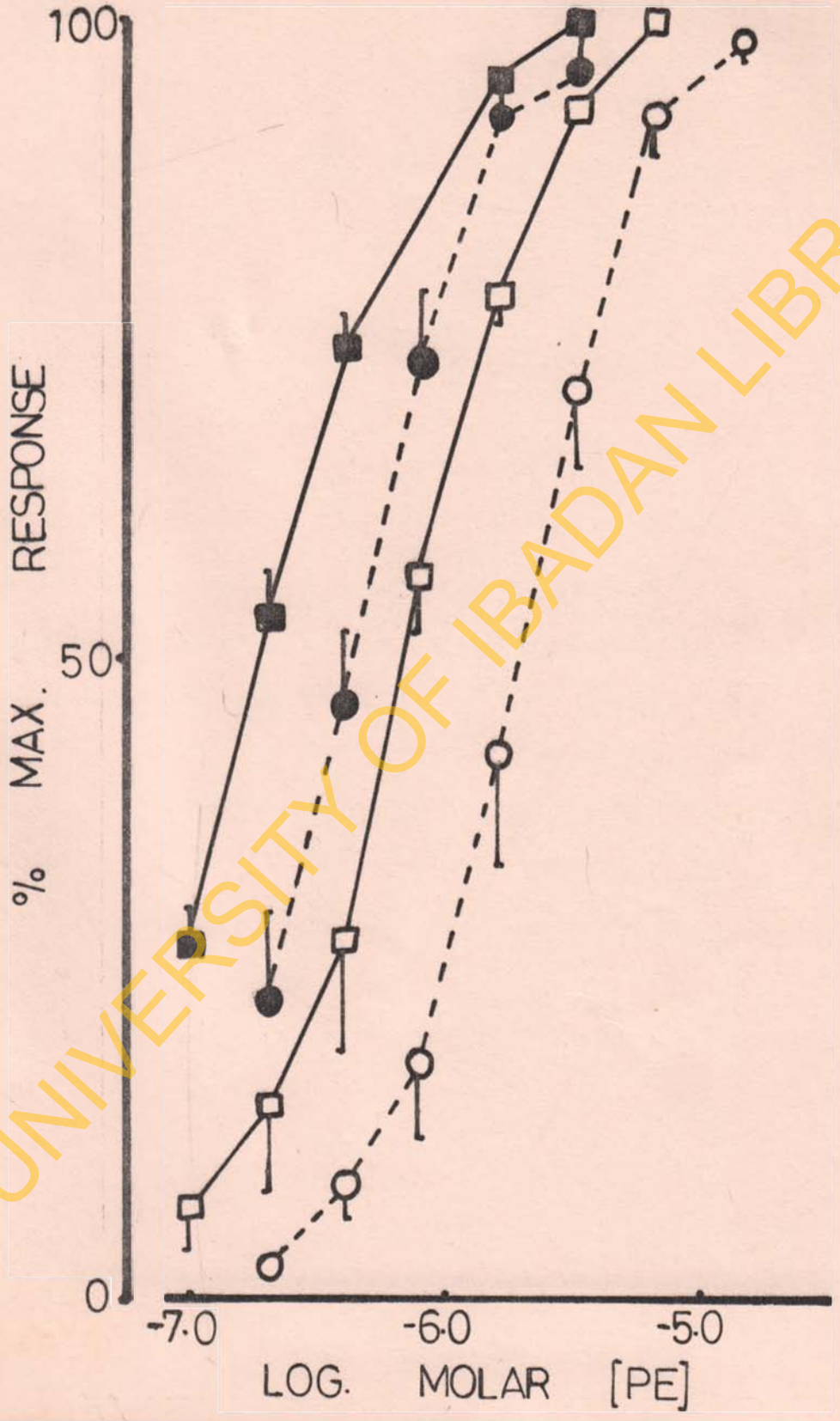
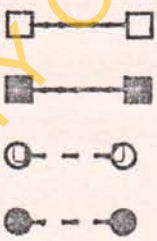


FIGURE 3

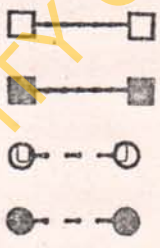
NA concentration response curves of the vas deferens in the absence and presence of cocaine. Each point represents the mean ± S.E of at least 4 observations.



- NA (NCR)
- NA + cocaine (NCR)
- NA (SHR)
- NA + cocaine (SHR)

FIGURE 4

PE concentration-response curves of the vas deferens in the absence and presence of cocaine. Each point represe the mean ± S.E of at least eight observations.



- PE (NCR)
- PE + cocaine (NCR)
- PE (SHR)
- PE + cocaine (SHR)

significantly different ($p < 0.05$) but the PE values were not ($p > 0.05$) (Table 1).

3.4. Antagonist Studies

The antagonists used were prazosin, phentolamine and yohimbine and all the experiments involving antagonists were done in the presence of 5.24×10^{-7}M cocaine. Phentolamine ($2.50 \times 10^{-8} - 5.0 \times 10^{-7}$M), prazosin ($1.1 \times 10^{-9} - 8.8 \times 10^{-7}$M or $1.77 \times 10^{-6} - 7.04 \times 10^{-6}$M) and yohimbine ($5.75 \times 10^{-8} - 4.50 \times 10^{-7}$M or $1.84 \times 10^{-6} - 1.1 \times 10^{-5}$M) produced appreciably parallel rightward shifts of the log-concentration - response curves of NA and PE in both NCR and SHR (Figs. 5 - 10). The antagonisms were analysed as indicated in Chapter 2, obtaining the parameters that were used to assess antagonism which were as follows:

3.4.1 PHENTOLAMINE

A. Anococcygeus (ANOC.) muscle: Phentolamine ($2.50 \times 10^{-8} - 5.0 \times 10^{-7}$M) produced competitive antagonism of both NA and PE in the NCR with pA_2 values of 8.11 ± 0.09 (slope = 0.82 ± 0.26) and 8.64 ± 0.18 (slope = 0.67 ± 0.28) respectively (Fig. 11 and Table 2a) and with corresponding Mackay plot regression co-efficient -0.18 ± 0.26 and -0.06 ± 0.19 which were not significantly different from zero ($p > 0.05$) (table 3). Against both agonists the phentolamine mean K_{diss} had no significant variation over the three antagonist concentrations (Table 4) and had grand mean values of $1.23 \pm 0.22 \times 10^{-8}$ and $1.00 \pm 0.16 \times 10^{-8}$ for NA and PE respectively (Table 4). In

FIGURE 5

A. Phentolamine shifts of the NA concentration-response curve in the NCR and SHR anococcygeus. Each point represents the mean ± S.E of four observations.

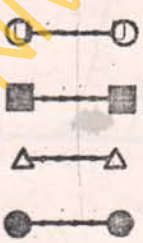
NA + cocaine

after 2.5×10^{-8} (NCR) or 6.25×10^{-8} M (SHR) phentolamine

after 6.25×10^{-8} (NCR) or 2.5×10^{-7} M (SHR) phentolamine

after 1.25×10^{-7} (NCR) or 5.0×10^{-7} M (SHR) phentolamine.

B. Phentolamine shifts of the PE concentration-response curve in the NCR and SHR anococcygeus. Each point represents the mean ± S.E of four observations.



PE + cocaine

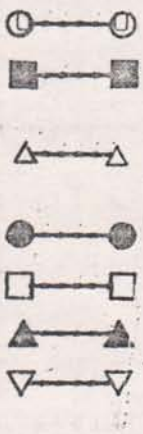
after 6.25×10^{-8} M phentolamine

after 2.5×10^{-7} M phentolamine

after 5.0×10^{-7} M phentolamine

FIGURE 6

A. Prazosin shifts of the NA concentration-response curve in the NCR and SHR anococcygeus. Each point represents the mean ± S.E of four observations.



NA + cocaine

after $1.1 \times 10^{-}$ (NCR) or 2.2×10^{-8} M (SHR) prazosin

after 5.5×10^{-8} (NCR) or 1.1×10^{-7} M (SHR) prazosin

after 2.2×10^{-7} M prazosin

after 1.8×10^{-6} M prazosin

after 3.5×10^{-6} M prazosin

after 5.3×10^{-6} (NCR) or 7.0×10^{-6} M (SHR) prazosin

B. Prazosin shifts of the PE concentration-response curve in the NCR and SHR anococcygeus. Each point represents the mean ± S.E of four observations.

PE + cocaine

after 5.5×10^{-9} M prazosin

after 2.2×10^{-8} M prazosin

after 1.1×10^{-7} M prazosin

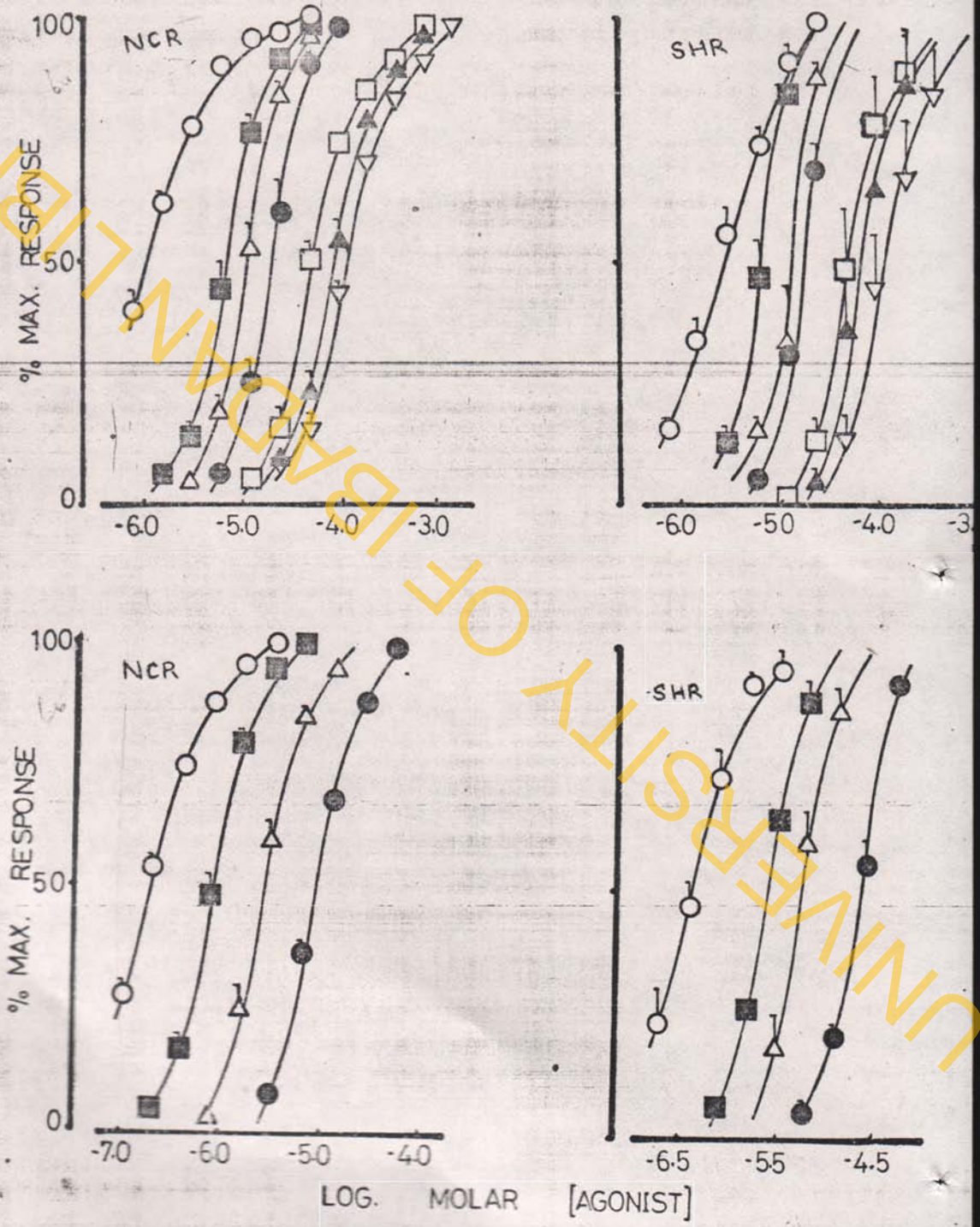
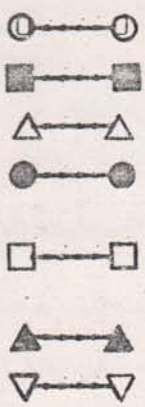


FIGURE 7

A. Yohimbine shifts of the NA concentration-response curve in the NCR and SHR anococcygeus. Each point represents the mean ± S.E of four observations.



NA + cocaine

after 5.75×10^{-8} M yohimbine

after 1.15×10^{-7} M yohimbine

after 2.30×10^{-7} (NCR) or $4.60 \times 4.60 \times 10^{-7}$ M (SHR) yohimbine

after 9.2×10^{-7} (NCR) or 1.84×10^{-6} M (SHR) yohimbine

after 3.68×10^{-6} M yohimbine

after 7.36×10^{-6} M yohimbine

B. Yohimbine shifts of the PE concentration-response curve in NCR and SHR anococcygeus. Each point represents the mean ± S.E of four observations

PE + cocaine

after 5.75×10^{-8} (NCR) or 1.15×10^{-7} M (SHR) yohimbine

after 1.15×10^{-7} (NCR) or 4.6×10^{-7} M (SHR) yohimbine

after 2.3×10^{-7} (NCR) or 9.2×10^{-7} M (SHR) yohimbine

after 2.76×10^{-6} M yohimbine

after 5.52×10^{-6} M yohimbine

after 1.10×10^{-5} M yohimbine

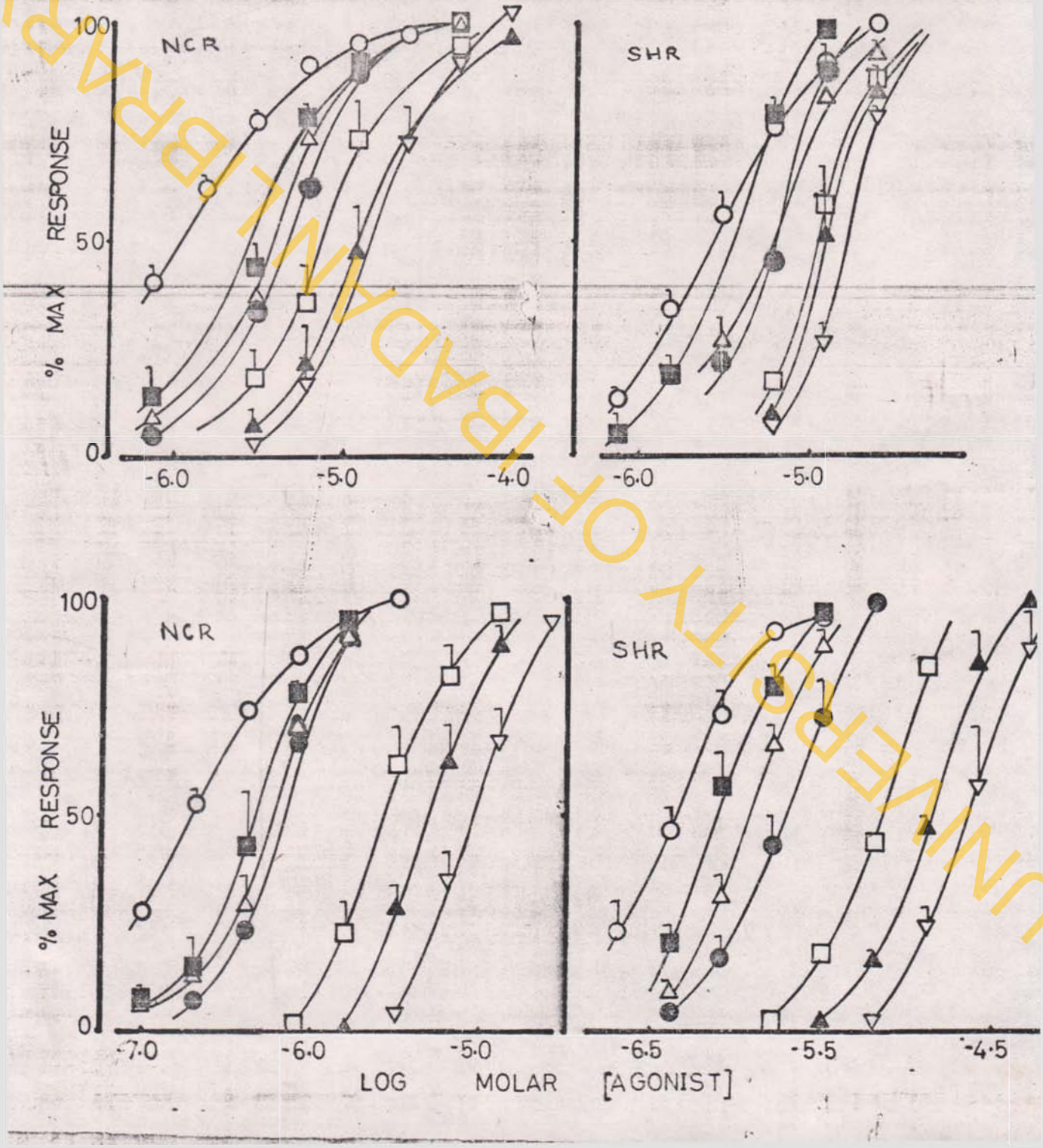
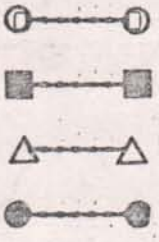


FIGURE 8

A. Phentolamine shifts of the NA concentration-response curve in NCR and SHR vas. Each point represents the mean ± S.E of four observations.



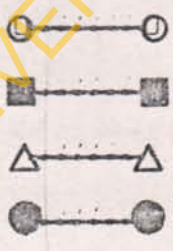
NA + cocaine

after 6.25×10^{-8}M phentolamine

after 2.5×10^{-7}M phentolamine

after 5.0×10^{-7}M phentolamine

B. Phentolamine shifts of the PE concentration-response curve in the NCR and SHR vas. Each point represents the mean ± S.E of four observations.



PE ± cocaine

after 6.25×10^{-8}M phentolamine

after 2.50×10^{-7} phentolamine

after 5.0×10^{-7} phentolamine

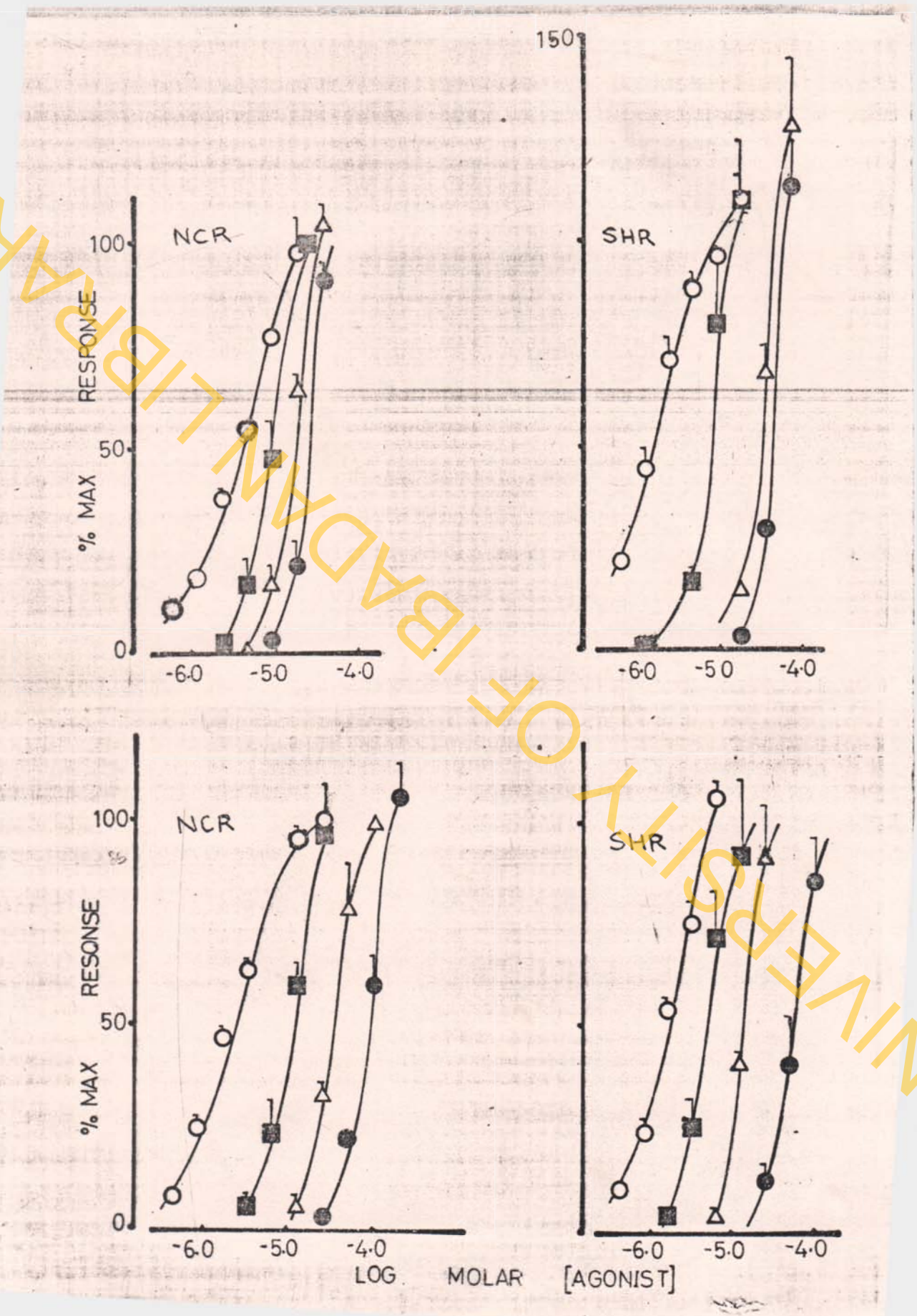
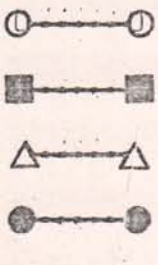


FIGURE 9

A. Prazosin shifts of the NA concentration-response curve in the NCR and SHR vas. Each point represents the mean ± S.E of four observations.



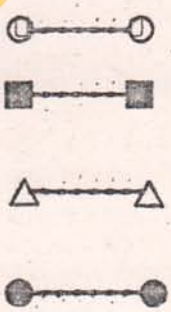
NA + cocaine

after 2.2×10^{-8}M prazosin

after 1.1×10^{-7}M prazosin

after 4.4×10^{-7} (NCR) or 2.2×10^{-7}M (SHR) prazosin

B. Prazosin shifts of the NA concentration-response curve in the NCR and SHR vas. Each point represents the mean ± S.E of four observations.



PE + cocaine

after 2.2×10^{-8} (NCR) or 5.5×10^{-9}M (SHR) prazosin.

after 1.1×10^{-7} (NCR) or 2.2×10^{-8}M (SHR) prazosin.

after 2.2×10^{-7} (NCR) or 1.1×10^{-7}M (SHR) prazosin

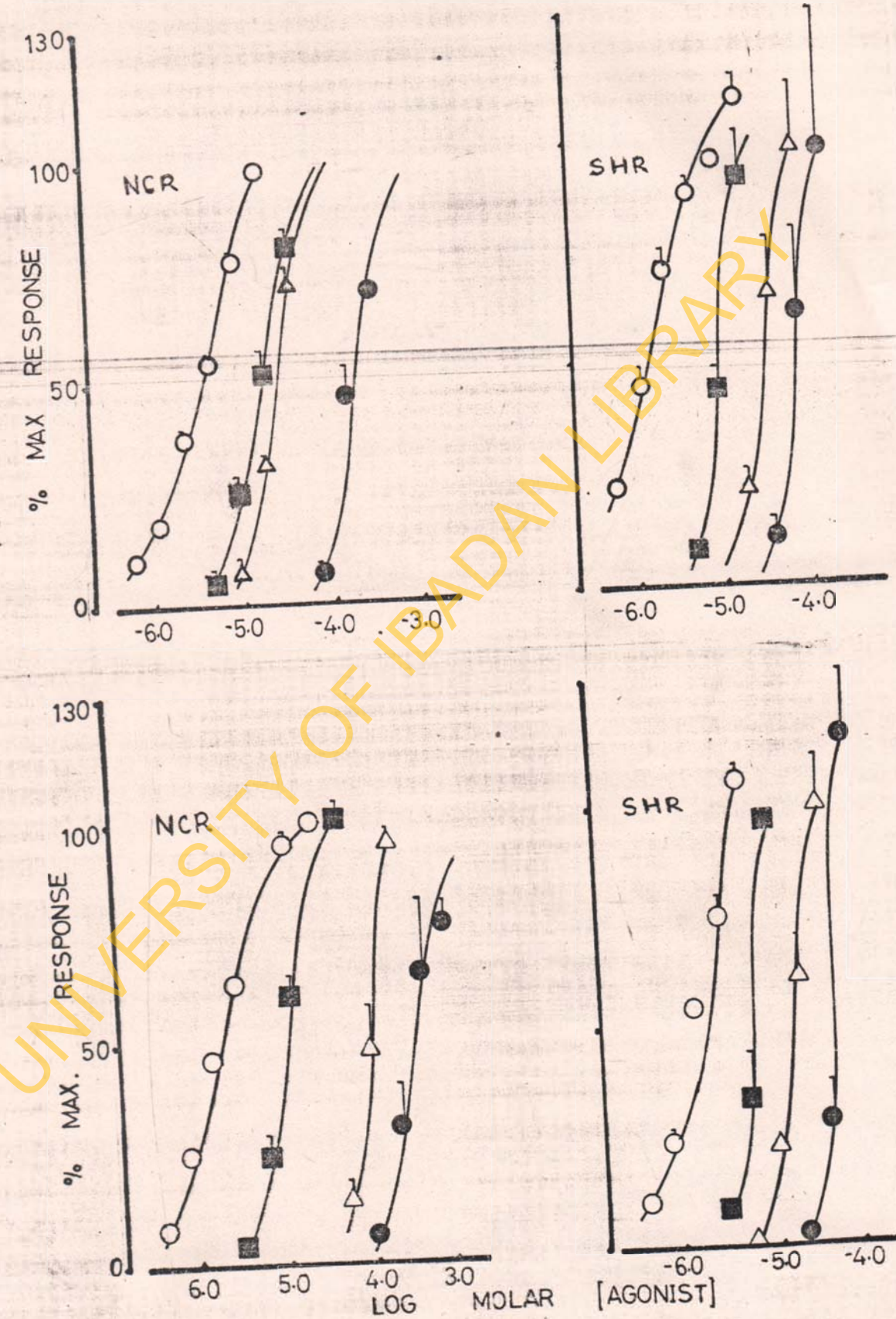
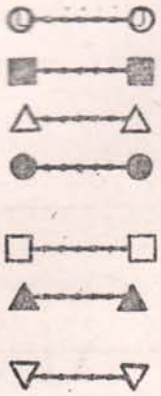


FIGURE 10

A. Yohimbine shifts of the NA concentration-response curve in the NCR and SHR vas. Each point represents the mean ± S.E of four observations



NA + cocaine

after 5.75×10^{-8}M yohimbine

after 2.3×10^{-7}M yohimbine

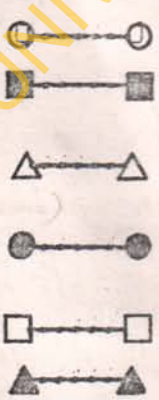
after 9.2×10^{-7}(NCR) or 4.6×10^{-7}M (SHR) yohimbine

after 1.84×10^{-6}M yohimbine

after 5.52×10^{-6} (NCR) or 3.68×10^{-6}M (SHR) yohimbine

after 1.10×10^{-5} (NCR) or 7.36×10^{-6}M (SHR) yohimbine

B. Yohimbine shifts of the PE concentration-response curve in the NCR and SHR vas. Each point represents the mean ± S.E of four observations.



PE + cocaine

after 5.75×10^{-8} (NCR) or 1.15×10^{-7}M (SHR) yohimbine

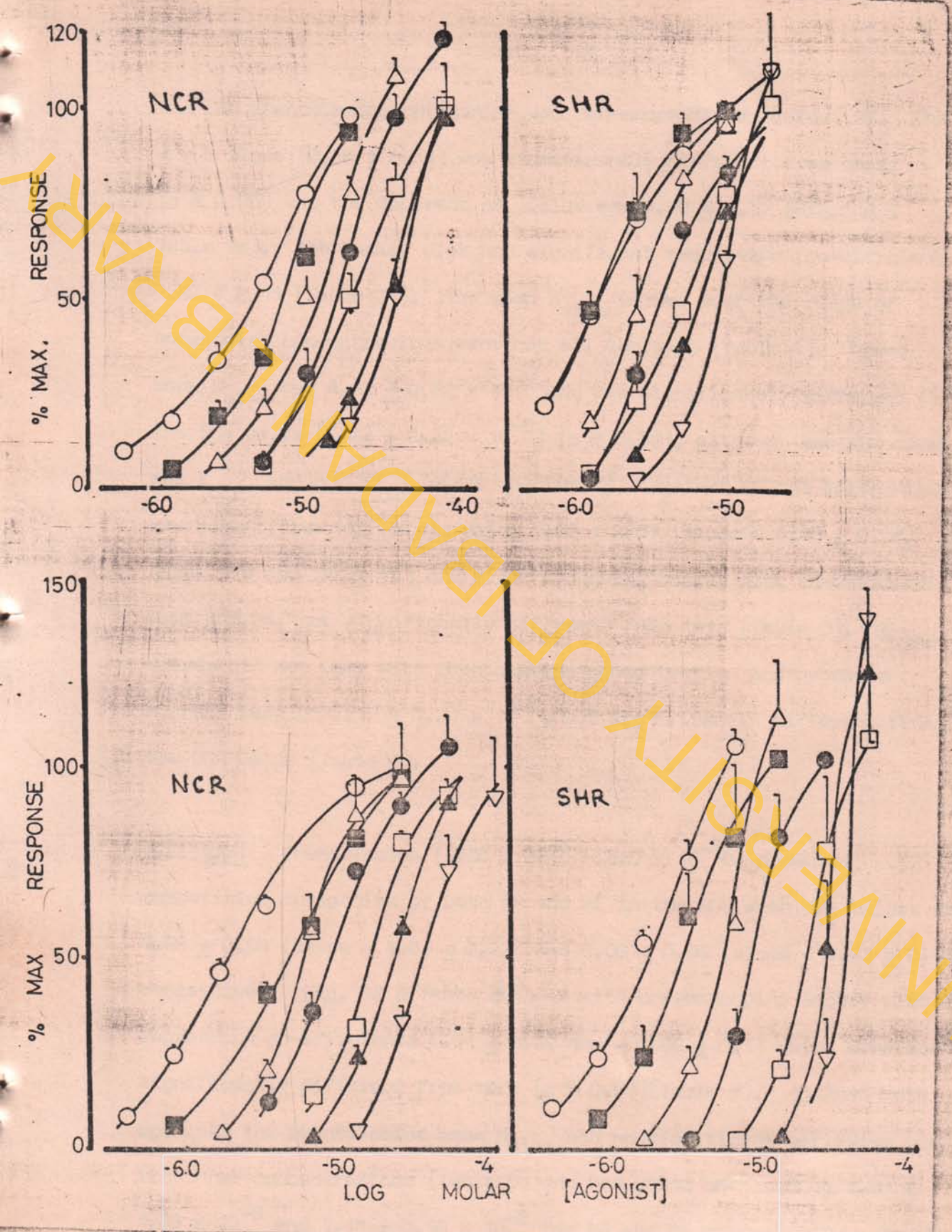
after 2.3×10^{-7} (NCR) or 4.6×10^{-7}M (SHR) yohimbine

after 4.6×10^{-7}M (NCR) or 9.2×10^{-7}M (SHR) yohimbine

after 2.76×10^{-6}M yohimbine

after 5.52×10^{-6}M yohimbine

after 1.1×10^{-5}M yohimbine



the SHR phentolamine antagonism was non-competitive against NA. The A - S slope (0.58 ± 0.10) was significantly different from unity ($p < 0.05$) and the apparent pA_2 value was 7.90 ± 0.07 (Fig. 11a & Table 2a). The Mackay plot had significant regression (co-efficient - -0.42 ± 0.11 (Table 3)). The mean K_{diss} values over the range of antagonist concentrations were not all the same (Table 4). Grand mean K_{diss} was $4.52 \pm 0.62 \times 10^{-8}$ and was significantly different from the NCR value ($1.23 \pm 0.22 \times 10^{-8}$) ($p < 0.05$), although the NCR case was K_{diss} and not K'_{diss} -(see chapter 2). Against PE the antagonism was competitive with a pA_2 value 7.66 ± 0.07 (slope = 1.38 ± 0.25) (Fig. 11b and Table 2a) and a corresponding Mackay plot co-efficient 0.38 ± 0.24, not significantly different from zero (Table 3). K_{diss} values did not vary with phentolamine concentration and produced a grand mean of $1.17 \pm 0.29 \times 10^{-8}$, not significantly different from the NCR value (Table 4).

B. VAS: Phentolamine (2.50×10^{-8} - 5.0×10^{-7}M) produced competitive antagonism of both NA and PE in the NCR with pA_2 values of 7.08 ± 0.08 (slope = 1.01 ± 0.22) and 8.06 ± 0.04 (slope = 0.93 ± 0.10) respectively (Fig. 12 & Table 2b) and with corresponding Mackay plot regression co-efficients 0.01 ± 0.22 and - 0.06 ± 0.11 which were not significantly different from zero ($p > 0.05$)(Table 5). Against both agonists the phentolamine mean K_{diss} had no significant variation over its three concentrations (Table 6) and had grand mean values 9.60 ± 1.39×10^{-8} and $1.11 \pm 0.10 \times 10^{-8}$ for NA and PE respectively. In the

FIGURE 11

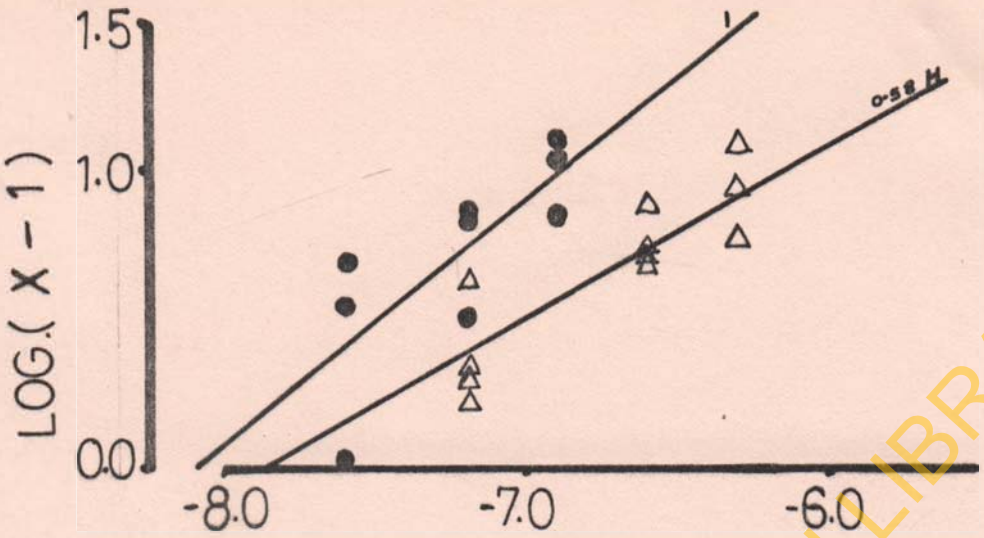
A. Arunlakshana and Schild (A – S) plot for the antagonism of NA induced responses of the anococcygeus by phentolamine.

B. A – S plot for the antagonism of PE induced responses of the anococcygeus by phentolamine.

"H" = the SHR line. Line marked 1 where the slope is not significantly different from 1 and the actual value where different from 1.

● – NCR

△ – SHR.



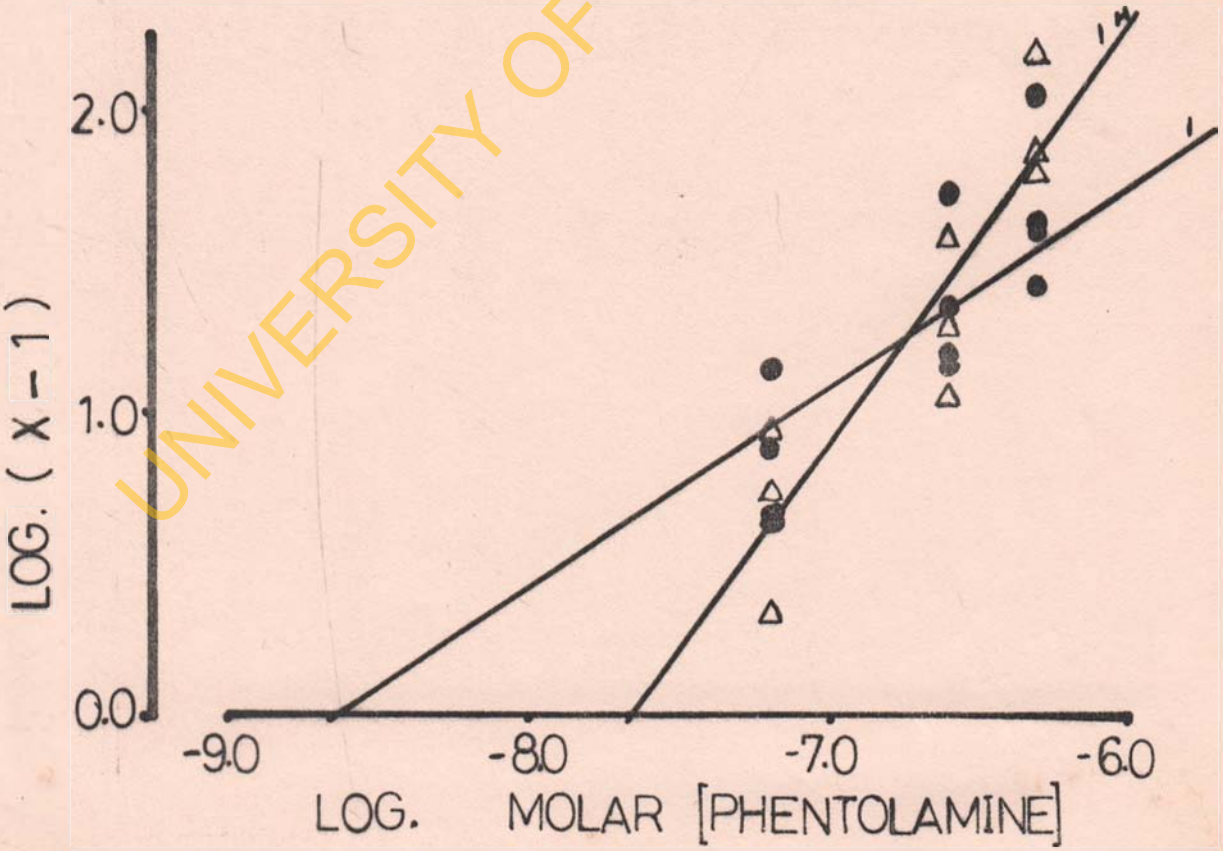


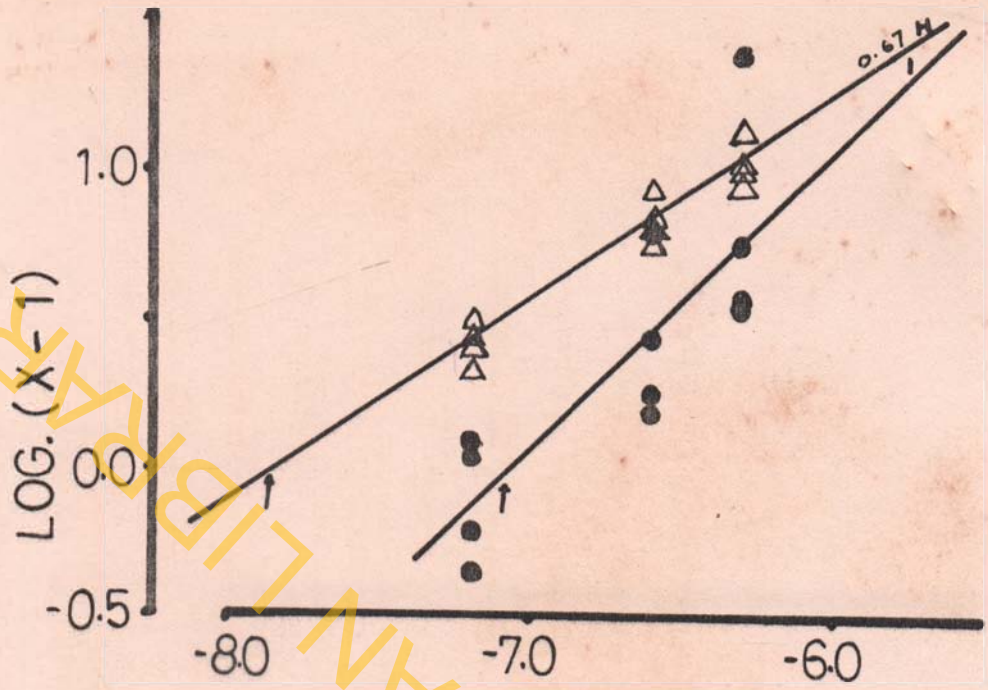
FIGURE 12

A. A – S plot for the antagonism of NA induced responses of the vas by phentolamine.

B A – S plot for the antagonism of PE induced responses of the vas by phentolamine.

"H" = the SHR line. Line marked 1 where the slope is not significantly different from 1 and the actual value where different from 1.

● – NCR. △ △ – SHR



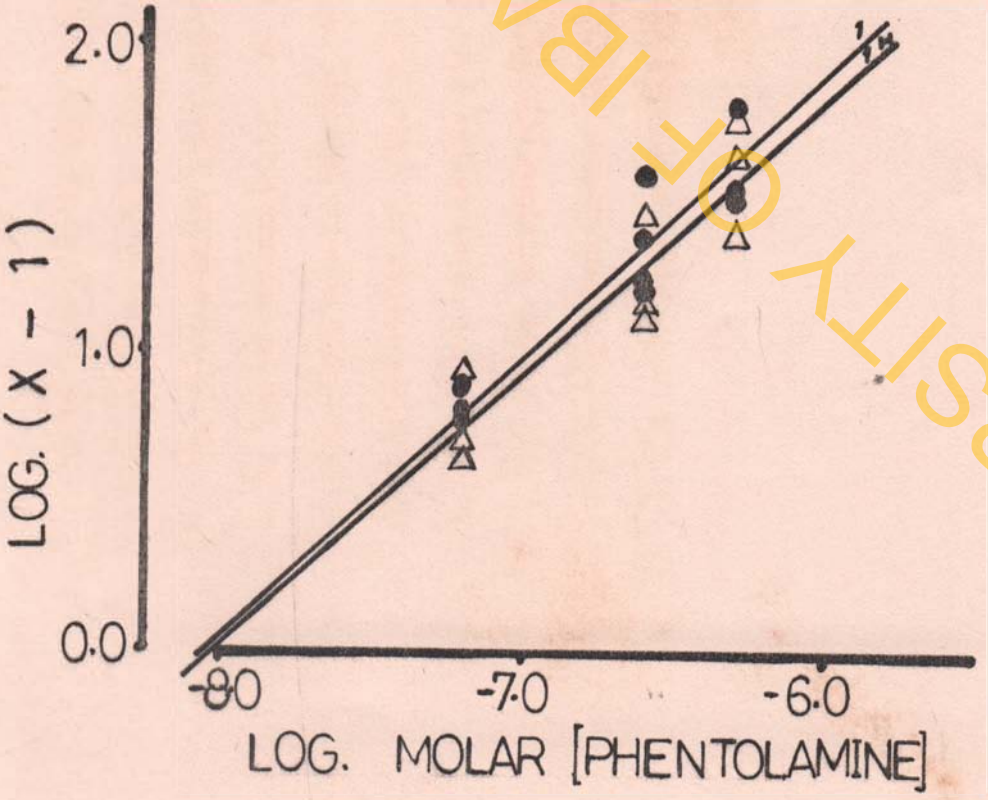


TABLE 2

A. Arunlakshana & Schild (A - S) plot parameters- slope and pA_2 values - for agonist: antagonist interactions in the anococcygeus muscle.

B. A - S plot parameters-slope and pA_2 values - for agonist: antagonist interactions in the vas deferens.

* = slope not significantly different from unity.

A – S PLOT PARAMETERS

ANOC.

		L – PRAZ.		PHENT.		H – PRAZ.		L – YOH.		H – YOH.	
		pA_2	slope	pA_2	slope	pA_2	slope	pA_2	slope	pA_2	slope
NCR	NA	8.45 ± 0.04	0.46 ± 0.03	8.11 ± 0.09	0.82 ± 0.26 *	7.58 ± 0.11	0.93 ± 0.09 *	7.08 ± 0.06	0.74 ± 0.19 *	6.27 ± 0.06	0.96 ± 0.14 *
	PE	8.93 ± 0.04	0.85 ± 0.07 *	8.64 ± 0.18	0.67 ± 0.28 *	–	–	7.62 ± 0.09	0.55 ± 0.18	6.64 ± 0.02	0.90 ± 0.06 *
SHR	NA	8.85 ± 0.06	0.52 ± 0.07	7.90 ± 0.07	0.58 ± 0.10	7.89 ± 0.14	0.65 ± 0.24 *	6.74 ± 0.06	0.65 ± 0.10	6.49 ± 0.03	0.61 ± 0.07
	PE	8.89 ± 0.06	0.80 ± 0.09 *	7.66 ± 0.07	1.38 ± 0.25 *	–	–	7.06 ± 0.35	0.65 ± 0.60 *	6.60 ± 0.06	0.96 ± 0.19 *

VAS

		L – PRAZ.		PHENT.		L – YOH.		H – YOH.	
		pA_2	slope	pA_2	slope	pA_2	slope	pA_2	slope
NCR	NA	8.00 ± 0.04	0.96 ± 0.08 *	7.08 ± 0.08	1.01 ± 0.22 *	6.80 ± 0.11	0.50 ± 0.12	6.36 ± 0.09	0.47 ± 0.11
	PE	8.35 ± 0.08	1.17 ± 0.23 *	8.06 ± 0.04	0.93 ± 0.10 *	7.44 ± 0.06	0.55 ± 0.09	6.79 ± 0.03	0.58 ± 0.06
SHR	NA	8.55 ± 0.04	0.80 ± 0.07	7.85 ± 0.04	0.67 ± 0.06	6.82 ± 0.04	1.10 ± 0.11 *	6.83 ± 0.08	0.53 ± 0.13
	PE	8.64 ± 0.02	0.88 ± 0.03	8.02 ± 0.07	0.91 ± 0.15 *	6.58 ± 0.08	1.04 ± 0.23 *	7.56 ± 0.15	0.39 ± 0.14

TABLE 3

Nature (competitive or non-competitive) of antagonism in the anococcygeus judging by the A - S or Mackay plot slopes and mean K_{diss} variance.

df1 = significantly different from one

1 = not significantly different from one

df0 = significantly different from zero

0 = not significantly different from zero

sd = mean K_{diss} not all the same over antagonist concentrations.

nd = mean K_{diss} steady

Comp = competitive

N-comp = non-competitive

TABLE 3

ANOC

Rat Strain	Agonist	A – S Slope	Remark	Mackay Slope	Remark	K_{diss} remark	Final Remark	Antag.
NCR	NA	0.46 ± 0.03	df1	−0.54 ± 0.02	df0	sd	N–Comp	L – PRAZ.
	PE	0.85 ± 0.07	1	−0.14 ± 0.07	0	nd	Comp	
SHR	NA	0.52 ± 0.07	df1	−0.47 ± 0.07	df0	sd	N–Comp	
	PE	0.80 ± 0.09	1	−0.2 ± 0.09	0	nd	Comp	
NCR	NA	0.82 ± 0.26	1	−0.18 ± 0.26	0	nd	Comp	PHENT.
	PE	0.67 ± 0.28	1	−0.06 ± 0.19	0	nd	Comp	
SHR	NA	0.58 ± 0.10	df1	−0.42 ± 0.11	df0	sd	N–Comp	
	PE	1.38 ± 0.25	1	0.38 ± 0.24	0	nd	Comp	
NCR	NA	0.98 ± 0.09	1	−0.01 ± 0.03	0	nd	Comp	H – PRAZ.
	PE	–	–	–	–	–	–	
SHR	NA	0.65 ± 0.24	1	−0.37 ± 0.24	0	nd	Comp	
	PE	–	–	–	–	–	–	
NCR	NA	0.74 ± 0.19	1	−0.26 ± 0.16	0	nd	Comp	L – YOH.
	PE	0.55 ± 0.18	df1	−0.45 ± 0.2	df0	nd	Comp	
SHR	NA	0.65 ± 0.10	df1	−0.35 ± 0.11	df0	nd	Comp	
	PE	0.65 ± 0.60	1	−0.06 ± 0.34	0	nd	Comp	
NCR	NA	0.96 ± 0.14	1	−0.03 ± 0.13	0	nd	Comp	H – YOH.
	PE	0.90 ± 0.06	1	−0.07 ± 0.07	0	nd	Comp	
SHR	NA	0.61 ± 0.07	df1	−0.35 ± 0.07	df0	sd	N–Comp	
	PE	0.96 ± 0.19	1	−0.02 ± 0.51	0	nd	Comp	

TABLE 4

Analysis of mean K_{diss} variance and grand mean K_{diss} values in the anococcygeus muscle.

nd = mean K_{diss} steady over antagonist concentrations

sd = mean K_{diss} not all the same over antagonist concentrations.

ND = grand mean K_{diss} not significantly different between NCR and SHR.

D = grand mean K_{diss} significantly different between NCR and SHR.

TABLE 4

Rat Strain	Agonist	Mean $K_d \pm$ S.E.(M) at three antagonist doses			Remark	Grand Mean $K_d \pm$ S.E. (M)	Antag.
		1st	2nd	3rd			
NCR	NA	$6.56 \pm 0.68 \times 10^{-9}$	$1.52 \pm 0.15 \times 10^{-8}$	$3.25 \pm 0.22 \times 10^{-8}$	sd	$1.81 \pm 0.35 \times 10^{-8}$	L - PRAZ.
	PE	$1.65 \pm 0.36 \times 10^{-9}$	$1.83 \pm 0.26 \times 10^{-9}$	$2.46 \pm 0.25 \times 10^{-9}$	nd	$1.98 \pm 0.18 \times 10^{-9}$	
SHR	NA	$5.45 \pm 0.44 \times 10^{-9}$	$1.21 \pm 0.15 \times 10^{-8}$	$1.65 \pm 0.25 \times 10^{-8}$	sd	$1.13 \pm 0.16 \times 10^{-8}$ ND	
	PE	$1.72 \pm 0.41 \times 10^{-9}$	$2.69 \pm 0.67 \times 10^{-9}$	$3.16 \pm 0.74 \times 10^{-9}$	nd	$2.52 \pm 0.35 \times 10^{-9}$ ND	
NCR	NA	$1.18 \pm 0.71 \times 10^{-8}$	$1.23 \pm 0.43 \times 10^{-8}$	$1.28 \pm 0.28 \times 10^{-8}$	nd	$1.23 \pm 0.22 \times 10^{-8}$	PHENT.
	PE	$1.10 \pm 0.27 \times 10^{-8}$	$8.44 \pm 3.86 \times 10^{-9}$	$1.16 \pm 0.32 \times 10^{-8}$	nd	$1.00 \pm 0.16 \times 10^{-8}$	
SHR	NA	$2.75 \pm 0.55 \times 10^{-8}$	$4.30 \pm 0.46 \times 10^{-8}$	$6.50 \pm 1.2 \times 10^{-8}$	sd	$4.52 \pm 0.62 \times 10^{-8}$ D	
	PE	$1.58 \pm 0.82 \times 10^{-8}$	$1.34 \pm 0.52 \times 10^{-8}$	$5.79 \pm 1.63 \times 10^{-9}$	nd	$1.17 \pm 0.29 \times 10^{-8}$ ND	
NCR	NA	$3.61 \pm 0.36 \times 10^{-8}$	$3.68 \pm 0.23 \times 10^{-8}$	$3.93 \pm 0.36 \times 10^{-8}$	nd	$3.77 \pm 0.15 \times 10^{-8}$	H - PRAZ.
	PE	-	-	-	-	-	
SHR	NA	$7.15 \pm 1.70 \times 10^{-8}$	$1.05 \pm 0.32 \times 10^{-7}$	$1.23 \pm 0.47 \times 10^{-7}$	nd	$9.99 \pm 1.68 \times 10^{-8}$ D	
	PE	-	-	-	-	-	
NCR	NA	$8.28 \pm 2.84 \times 10^{-8}$	$9.10 \pm 1.07 \times 10^{-8}$	$1.13 \pm 0.06 \times 10^{-7}$	nd	$9.52 \pm 0.85 \times 10^{-8}$	L - YOH.
	PE	$3.74 \pm 0.55 \times 10^{-8}$	$4.97 \pm 1.38 \times 10^{-8}$	$7.78 \pm 2.65 \times 10^{-8}$	nd	$5.36 \pm 0.92 \times 10^{-8}$	
SHR	NA	$1.27 \pm 0.18 \times 10^{-7}$	$2.17 \pm 0.54 \times 10^{-7}$	$2.60 \pm 0.35 \times 10^{-7}$	nd	$2.01 \pm 0.25 \times 10^{-7}$ D	
	PE	$3.54 \pm 3.70 \times 10^{-7}$	$3.17 \pm 3.18 \times 10^{-7}$	$2.61 \pm 2.29 \times 10^{-7}$	nd	$3.11 \pm 1.35 \times 10^{-7}$ D	
NCR	NA	$6.00 \pm 1.96 \times 10^{-7}$	$5.64 \pm 1.23 \times 10^{-7}$	$6.23 \pm 0.88 \times 10^{-7}$	nd	$5.92 \pm 0.68 \times 10^{-7}$	H - YOH.
	PE	$2.94 \pm 0.15 \times 10^{-7}$	$3.05 \pm 0.28 \times 10^{-7}$	$3.36 \pm 0.22 \times 10^{-7}$	nd	$3.12 \pm 0.12 \times 10^{-7}$	
SHR	NA	$6.12 \pm 0.47 \times 10^{-7}$	$8.87 \pm 0.41 \times 10^{-7}$	$1.01 \pm 0.08 \times 10^{-6}$	sd	$8.37 \pm 0.99 \times 10^{-7}$ D	
	PE	$2.89 \pm 0.33 \times 10^{-7}$	$2.64 \pm 0.46 \times 10^{-7}$	$3.22 \pm 0.96 \times 10^{-7}$	nd	$2.92 \pm 0.29 \times 10^{-7}$ ND	

SHR the antagonism was non-competitive against NA since the slope of the Schild regression line (0.67 ± 0.06) was significantly different from unity ($p < 0.05$)(Fig. 12a) and the Mackay plot regression co-efficient - 0.33 ± 0.05 was significantly different from zero (Table 5). The apparent pA_2 was 7.85 ± 0.04 (Table 2b) and the grand mean K'_{diss}, 3.57 ± 0.34 x 10^{-8} was significantly different from the NCR value. The mean K_{diss} values were not all the same over the three antagonist concentrations (Table 6). Against PE the antagonism was competitive producing a pA_2 of 8.02 ± 0.07 (slope = 0.91 ± 0.15) (Fig. 12b & Table 2b). The Mackay plot regression co-efficient of -0.09 ± 0.15 was not significantly different from zero (Table 5) and likewise the mean K_{diss} did not vary with phentolamine concentration (Table 6). Grand mean K_{diss} was 1.33 ± 0.17 x 10^{-8} but was not significantly different from the NCR value (Table 6).

3.4.2. PRAZOSIN

Two ranges of prazosin concentrations (Low (L) and High (H)) were used against NA in the anococcygeus muscle. This was not possible with PE because of the very high concentrations of PE required to surmount the antagonism of the higher concentrations of prazosin (H-Praz). On the vas deferens the H-praz produced an unsurmountable antagonism. Thus, in experiments involving PE or the vas deferens, only the lower prazosin concentrations (L-Praz) was used.

TABLE 5

Nature (competitive or non-competitive) of antagonist in the vas judging by the A - S or Mackay plot slopes and mean K_{diss} variance.

df1	=	significantly different from one
1	=	not significantly different from one
df0	=	significantly different from zero
0	=	not significantly different from zero
sd	=	mean K_{diss} not all the same over antagonist concentrations
nd	=	mean K_{diss} steady
Comp	=	competitive
N-comp	=	non-competitive

TABLE 5

VAS

Rat Strain	Agonist	A – S Slope	Remark	Mackay Slope	Remark	K_{diss} remark	Final Remark	Antag.
NCR	NA	0.96 ± 0.08	1	−0.1 ± 0.08	0	nd	Comp	L – PRAZ.
	PE	1.17 ± 0.23	1	0.17 ± 0.23	0	nd	Comp	
SHR	NA	0.80 ± 0.07	df1	−0.19 ± 0.06	df0	sd	N–Comp	
	PE	0.88 ± 0.01	df1	−0.12 ± 0.04	df0	sd	N–Comp	
NCR	NA	1.01 ± 0.22	1	0.01 ± 0.22	0	nd	Comp	PHENT.
	PE	0.93 ± 0.10	1	−0.06 ± 0.11	0	nd	Comp	
SHR	NA	0.67 ± 0.06	df1	−0.33 ± 0.05	df0	sd	N–Comp	
	PE	0.91 ± 0.15	1	−0.09 ± 0.15	0	nd	Comp	
NCR	NA	0.50 ± 0.12	df1	−0.97 ± 0.10	df0	sd	N–Comp	L – YOH
	PE	0.55 ± 0.09	df1	−0.47 ± 0.11	df0	sd	N–Comp	
SHR	NA	1.10 ± 0.11	1	0.10 ± 0.11	0	nd	Comp	
	PE	1.04 ± 0.23	1	0.01 ± 0.26	0	nd	Comp	
NCR	NA	0.47 ± 0.11	df1	−0.44 ± 0.11	df0	sd	N–Comp	H – YOH.
	PE	0.58 ± 0.06	df1	−0.40 ± 0.05	df0	sd	N–Comp	
SHR	NA	0.53 ± 0.13	df1	−0.46 ± 0.12	df0	sd	N–Comp	
	PE	0.39 ± 0.14	df1	−0.67 ± 0.19	df0	sd	N–Comp	

TABLE 6

Analysis of mean K_{diss} variance and grand mean K_{diss} values in the vas deferens.

nd = mean K_{diss} steady over antagonist concentrations.

sd = mean K_{diss} not all the same over antagonist concentrations.

ND = grand mean K_{diss} not significantly different between NCR and SHR.

D = grand mean K_{diss} significantly different between NCR and SHR.

TABLE 6

VAS

Rat Strain	Agonist	Mean $K_d \pm$ S.E.(M) at three antagonist doses			Remark	Grand Mean $K_d \pm$ S.E.(M)	Antag.
		1st	2nd	3rd			
NCR	NA	$1.11 \pm 0.29 \times 10^{-8}$	$1.23 \pm 0.25 \times 10^{-8}$	$1.21 \pm 0.22 \times 10^{-8}$	nd	$1.17 \pm 0.13 \times 10^{-8}$	L - PRAZ.
	PE	$3.84 \pm 1.36 \times 10^{-9}$	$3.72 \pm 1.44 \times 10^{-9}$	$2.69 \pm 1.16 \times 10^{-9}$	nd	$3.42 \pm 0.65 \times 10^{-9}$	
SHR	NA	$4.22 \pm 0.47 \times 10^{-9}$	$6.48 \pm 0.97 \times 10^{-9}$	$6.31 \pm 0.47 \times 10^{-9}$	sd	$5.67 \pm 0.46 \times 10^{-9}$ (D)	
	PE	$2.73 \pm 0.13 \times 10^{-9}$	$2.69 \pm 0.20 \times 10^{-9}$	$3.92 \pm 0.35 \times 10^{-9}$	sd	$3.11 \pm 0.21 \times 10^{-9}$ (ND)	
NCR	NA	$8.68 \pm 2.39 \times 10^{-8}$	$9.43 \pm 2.37 \times 10^{-8}$	$9.59 \pm 3.21 \times 10^{-8}$	nd	$9.60 \pm 1.39 \times 10^{-8}$	PHENT
	PE	$9.80 \pm 0.59 \times 10^{-9}$	$1.17 \pm 0.23 \times 10^{-8}$	$1.17 \pm 0.24 \times 10^{-8}$	nd	$1.11 \pm 0.10 \times 10^{-8}$	
SHR	NA	$2.37 \pm 0.24 \times 10^{-8}$	$3.62 \pm 0.36 \times 10^{-8}$	$4.71 \pm 0.44 \times 10^{-8}$	sd	$3.57 \pm 0.34 \times 10^{-8}$ (D)	
	PE	$1.15 \pm 0.27 \times 10^{-8}$	$1.52 \pm 0.40 \times 10^{-8}$	$1.33 \pm 0.43 \times 10^{-8}$	nd	$1.33 \pm 0.17 \times 10^{-8}$ (ND)	
NCR	NA	$1.10 \pm 0.34 \times 10^{-7}$	$1.82 \pm 0.35 \times 10^{-7}$	$4.02 \pm 0.90 \times 10^{-7}$	sd	$2.31 \pm 0.53 \times 10^{-7}$	L - YOH
	PE	$4.89 \pm 1.33 \times 10^{-8}$	$8.63 \pm 1.91 \times 10^{-8}$	$1.19 \pm 0.15 \times 10^{-7}$	sd	$8.45 \pm 1.17 \times 10^{-8}$	
SHR	NA	$1.83 \pm 0.46 \times 10^{-7}$	$1.42 \pm 0.29 \times 10^{-7}$	$1.49 \pm 0.25 \times 10^{-7}$	nd	$1.58 \pm 0.17 \times 10^{-7}$ (ND)	
	PE	$3.60 \pm 1.72 \times 10^{-7}$	$4.42 \pm 1.65 \times 10^{-7}$	$3.06 \pm 1.02 \times 10^{-7}$	nd	$3.69 \pm 0.73 \times 10^{-7}$ (D)	
NCR	NA	$1.13 \pm 0.25 \times 10^{-6}$	$1.59 \pm 0.23 \times 10^{-6}$	$2.36 \pm 0.26 \times 10^{-6}$	sd	$1.69 \pm 0.20 \times 10^{-6}$	H - YOH
	PE	$5.20 \pm 0.17 \times 10^{-7}$	$7.34 \pm 0.47 \times 10^{-7}$	$9.22 \pm 0.71 \times 10^{-7}$	sd	$7.25 \pm 0.57 \times 10^{-7}$	
SHR	NA	$5.83 \pm 1.39 \times 10^{-7}$	$6.92 \pm 0.97 \times 10^{-7}$	$9.49 \pm 0.68 \times 10^{-7}$	sd	$7.10 \pm 0.74 \times 10^{-7}$ (D)	
	PE	$4.77 \pm 0.97 \times 10^{-7}$	$6.92 \pm 1.45 \times 10^{-7}$	$1.15 \pm 0.21 \times 10^{-6}$	sd	$7.72 \pm 1.25 \times 10^{-7}$ (ND)	

A. ANOC. Muscle: The Schild plot for L-Praz and H-Praz against NA produced two distinct (statistically attested by the method of Armitage, 1974) regression lines.

L-Praz (1.1×10^{-8} - 8.8×10^{-7}M) was non-competitive against NA in both NCR and SHR. The slopes of the Schild lines were 0.46 ± 0.03 and 0.52 ± 0.07 respectively with corresponding apparent pA_2 values of 8.45 ± 0.04 and 8.85 ± 0.06. Both slope values were significantly different from one ($p < 0.05$) Fig. 13a and Table 2a). In addition, Mackay plot regression co-efficients - 0.54 ± 0.02 and - 0.47 ± 0.07 (for NCR and SHR respectively) were significantly different from zero ($p < 0.05$)(Table 3). Also, in both NCR and SHR, the mean K_{diss} values over the range of prazosin concentrations were not all the same. The grand mean K^{1}_{diss} $1.13 \pm 0.16 \times 10^{-8}$ obtained in the SHR was not significantly different from the value $1.81 \pm 0.35 \times 10^{-8}$ in the NCR (Table 4). L-Praz produced competitive antagonism of PE - induced contractions in both NCR and SHR with pA_2 values of 8.93 ± 0.04 (slope = 0.85 ± 0.07) in the NCR and 8.89 ± 0.06 (slope = 0.80 ± 0.09) in the SHR (Fig. 13b, Table 2a). The corresponding grand mean K_{diss} values of $1.98 \pm 0.18 \times 10^{-9}$ and $2.52 \pm 0.35 \times 10^{-9}$ were not significantly different from each other (Table 4). The mean K_{diss} values did not vary with antagonist concentration in both rat strains. Mackay plot regression co-efficient was - 0.14 ± 0.07 in the NCR and - 0.22 ± 0.09 in SHR and in both cases were not significantly different from zero (Table 3).

H-Praz (1.77×10^{-6} - 7.04×10^{-6}M) produced competitive antagonism

FIGURE 13

A. A – S plot for the antagonism of NA induced contractions of the anococcygeus by prazosin.

B. A – S plot for the antagonism of PE induced contractions of the anococcygeus by prazosin.

"H" = the SHR line. Line marked 1 where the slope is not significantly different from 1 and the actual value where different from 1.

● ● NCR, △ △ SHR.

TABLE 3

ANOC

Rat Strain	Agonist	A – S Slope	Remark	Mackay Slope	Remark	K_{diss} remark	Final Remark	Antag.
NCR	NA	0.46 ± 0.03	df1	−0.54 ± 0.02	df0	sd	N-Comp	L – PRAZ.
	PE	0.85 ± 0.07	1	−0.14 ± 0.07	0	nd	Comp	
SHR	NA	0.52 ± 0.07	df1	−0.47 ± 0.07	df0	sd	N-Comp	
	PE	0.80 ± 0.09	1	−0.2 ± 0.09	0	nd	Comp	
NCR	NA	0.82 ± 0.26	1	−0.18 ± 0.26	0	nd	Comp	PHENT.
	PE	0.67 ± 0.28	1	−0.06 ± 0.19	0	nd	Comp	
SHR	NA	0.58 ± 0.10	df1	−0.42 ± 0.11	df0	sd	N-Comp	
	PE	1.38 ± 0.25	1	0.38 ± 0.24	0	nd	Comp	
NCR	NA	0.98 ± 0.09	1	−0.01 ± 0.03	0	nd	Comp	H – PRAZ.
	PE	–	–	–	–	–	–	
SHR	NA	0.65 ± 0.24	1	−0.37 ± 0.24	0	nd	Comp	
	PE	–	–	–	–	–	–	
NCR	NA	0.74 ± 0.19	1	−0.26 ± 0.16	0	nd	Comp	L – YOH.
	PE	0.55 ± 0.18	df1	−0.45 ± 0.2	df0	nd	Comp	
SHR	NA	0.65 ± 0.10	df1	−0.35 ± 0.11	df0	nd	Comp	
	PE	0.65 ± 0.60	1	−0.06 ± 0.34	0	nd	Comp	
NCR	NA	0.96 ± 0.14	1	−0.03 ± 0.13	0	nd	Comp	H – YOH.
	PE	0.90 ± 0.06	1	−0.07 ± 0.07	0	nd	Comp	
SHR	NA	0.61 ± 0.07	df1	−0.35 ± 0.07	df0	sd	N-Comp	
	PE	0.96 ± 0.19	1	−0.02 ± 0.51	0	nd	Comp	

TABLE 4

Analysis of mean K_{diss} variance and grand mean K_{diss} values in the anococcygeus muscle.

nd = mean K_{diss} steady over antagonist concentrations

sd = mean K_{diss} not all the same over antagonist concentrations.

ND = grand mean K_{diss} not significantly different between NCR and SHR.

D = grand mean K_{diss} significantly different between NCR and SHR.

TABLE 4

Rat Strain	Agonist	Mean $K_d \pm$ S.E.(M) at three antagonist doses			Remark	Grand Mean $K_d \pm$ S.E.(M)	Antag.
		1st	2nd	3rd			
NCR	NA	$6.56 \pm 0.68 \times 10^{-9}$	$1.52 \pm 0.15 \times 10^{-8}$	$3.25 \pm 0.22 \times 10^{-8}$	sd	$1.81 \pm 0.35 \times 10^{-8}$	L - PRAZ.
	PE	$1.65 \pm 0.36 \times 10^{-9}$	$1.83 \pm 0.26 \times 10^{-9}$	$2.46 \pm 0.25 \times 10^{-9}$	nd	$1.98 \pm 0.18 \times 10^{-9}$	
SHR	NA	$5.45 \pm 0.44 \times 10^{-9}$	$1.21 \pm 0.15 \times 10^{-8}$	$1.65 \pm 0.25 \times 10^{-8}$	sd	$1.13 \pm 0.16 \times 10^{-8}$ ND	
	PE	$1.72 \pm 0.41 \times 10^{-9}$	$2.69 \pm 0.67 \times 10^{-9}$	$3.16 \pm 0.74 \times 10^{-9}$	nd	$2.52 \pm 0.35 \times 10^{-9}$ ND	
NCR	NA	$1.18 \pm 0.71 \times 10^{-8}$	$1.23 \pm 0.43 \times 10^{-8}$	$1.28 \pm 0.28 \times 10^{-8}$	nd	$1.23 \pm 0.22 \times 10^{-8}$	PHENT.
	PE	$1.10 \pm 0.27 \times 10^{-8}$	$8.44 \pm 3.86 \times 10^{-9}$	$1.16 \pm 0.32 \times 10^{-8}$	nd	$1.00 \pm 0.16 \times 10^{-8}$	
SHR	NA	$2.75 \pm 0.55 \times 10^{-8}$	$4.30 \pm 0.46 \times 10^{-8}$	$6.50 \pm 1.2 \times 10^{-8}$	sd	$4.52 \pm 0.62 \times 10^{-8}$ D	
	PE	$1.58 \pm 0.82 \times 10^{-8}$	$1.34 \pm 0.52 \times 10^{-8}$	$5.79 \pm 1.63 \times 10^{-9}$	nd	$1.17 \pm 0.29 \times 10^{-8}$ ND	
NCR	NA	$3.61 \pm 0.36 \times 10^{-8}$	$3.68 \pm 0.23 \times 10^{-8}$	$3.93 \pm 0.36 \times 10^{-8}$	nd	$3.77 \pm 0.15 \times 10^{-8}$	H - PRAZ.
	PE	-	-	-	-	-	
SHR	NA	$7.15 \pm 1.70 \times 10^{-8}$	$1.05 \pm 0.32 \times 10^{-7}$	$1.23 \pm 0.47 \times 10^{-7}$	nd	$9.99 \pm 1.68 \times 10^{-8}$ D	
	PE	-	-	-	-	-	
NCR	NA	$8.28 \pm 2.84 \times 10^{-8}$	$9.10 \pm 1.07 \times 10^{-8}$	$1.13 \pm 0.06 \times 10^{-7}$	nd	$9.52 \pm 0.85 \times 10^{-8}$	L - YOH.
	PE	$3.74 \pm 0.55 \times 10^{-8}$	$4.97 \pm 1.38 \times 10^{-8}$	$7.78 \pm 2.65 \times 10^{-8}$	nd	$5.36 \pm 0.92 \times 10^{-8}$	
SHR	NA	$1.27 \pm 0.18 \times 10^{-7}$	$2.17 \pm 0.54 \times 10^{-7}$	$2.60 \pm 0.35 \times 10^{-7}$	nd	$2.01 \pm 0.25 \times 10^{-7}$ D	
	PE	$3.54 \pm 3.70 \times 10^{-7}$	$3.17 \pm 3.18 \times 10^{-7}$	$2.61 \pm 2.29 \times 10^{-7}$	nd	$3.11 \pm 1.35 \times 10^{-7}$ D	
NCR	NA	$6.00 \pm 1.96 \times 10^{-7}$	$5.64 \pm 1.23 \times 10^{-7}$	$6.23 \pm 0.88 \times 10^{-7}$	nd	$5.92 \pm 0.68 \times 10^{-7}$	H - YOH.
	PE	$2.94 \pm 0.15 \times 10^{-7}$	$3.05 \pm 0.28 \times 10^{-7}$	$3.36 \pm 0.22 \times 10^{-7}$	nd	$3.12 \pm 0.12 \times 10^{-7}$	
SHR	NA	$6.12 \pm 0.47 \times 10^{-7}$	$8.87 \pm 0.41 \times 10^{-7}$	$1.01 \pm 0.08 \times 10^{-6}$	sd	$8.37 \pm 0.99 \times 10^{-7}$ D	
	PE	$2.89 \pm 0.33 \times 10^{-7}$	$2.64 \pm 0.46 \times 10^{-7}$	$3.22 \pm 0.96 \times 10^{-7}$	nd	$2.92 \pm 0.29 \times 10^{-7}$ ND	

SHR the antagonism was non-competitive against NA since the slope of the Schild regression line (0.67 ± 0.06) was significantly different from unity ($p < 0.05$)(Fig. 12a) and the Mackay plot regression co-efficient - 0.33 ± 0.05 was significantly different from zero (Table 5). The apparent pA_2 was 7.85 ± 0.04 (Table 2b) and the grand mean K'_{diss}, $3.57 \pm 0.34 \times 10^{-8}$ was significantly different from the NCR value. The mean K_{diss} values were not all the same over the three antagonist concentrations (Table 6). Against PE the antagonism was competitive producing a pA_2 of 8.02 ± 0.07 (slope = 0.91 ± 0.15) (Fig. 12b & Table 2b). The Mackay plot regression co-efficient of -0.09 ± 0.15 was not significantly different from zero (Table 5) and likewise the mean K_{diss} did not vary with phentolamine concentration (Table 6). Grand mean K_{diss} was $1.33 \pm 0.17 \times 10^{-8}$ but was not significantly different from the NCR value (Table 6).

3.4.2. PRAZOSIN

Two ranges of prazosin concentrations (Low (L) and High (H)) were used against NA in the anococcygeus muscle. This was not possible with PE because of the very high concentrations of PE required to surmount the antagonism of the higher concentrations of prazosin (H-Praz). On the vas deferens the H-praz produced an unsurmountable antagonism. Thus, in experiments involving PE or the vas deferens, only the lower prazosin concentrations (L-Praz) was used.

TABLE 5

Nature (competitive or non-competitive) of antagonist in the vas judging by the A - S or Mackay plot slopes and mean K_{diss} variance.

df1	=	significantly different from one
1	=	not significantly different from one
df0	=	significantly different from zero
0	=	not significantly different from zero
sd	=	mean K_{diss} not all the same over antagonist concentrations
nd	=	mean K_{diss} steady
Comp	=	competitive
N-comp	=	non-competitive

TABLE 6

Analysis of mean K_{diss} variance and grand mean K_{diss} values in the vas deferens.

nd = mean K_{diss} steady over antagonist concentrations.

sd = mean K_{diss} not all the same over antagonist concentrations.

ND = grand mean K_{diss} not significantly different between NCR and SHR.

D = grand mean K_{diss} significantly different between NCR and SHR.

TABLE 6

VAS

Rat Strain	Agonist	Mean $K_d \pm$ S.E.(M) at three antagonist doses			Remark	Grand Mean $K_d \pm$ S.E.(M)	Antag.
		1st	2nd	3rd			
NCR	NA	$1.11 \pm 0.29 \times 10^{-8}$	$1.23 \pm 0.25 \times 10^{-8}$	$1.21 \pm 0.22 \times 10^{-8}$	nd	$1.17 \pm 0.13 \times 10^{-8}$	L - PRAZ.
	PE	$3.84 \pm 1.36 \times 10^{-9}$	$3.72 \pm 1.44 \times 10^{-9}$	$2.69 \pm 1.16 \times 10^{-9}$	nd	$3.42 \pm 0.65 \times 10^{-9}$	
SHR	NA	$4.22 \pm 0.47 \times 10^{-9}$	$6.48 \pm 0.97 \times 10^{-9}$	$6.31 \pm 0.47 \times 10^{-9}$	sd	$5.67 \pm 0.46 \times 10^{-9}$ (D)	
	PE	$2.73 \pm 0.13 \times 10^{-9}$	$2.69 \pm 0.20 \times 10^{-9}$	$3.92 \pm 0.35 \times 10^{-9}$	sd	$3.11 \pm 0.21 \times 10^{-9}$ (ND)	
NCR	NA	$8.68 \pm 2.39 \times 10^{-8}$	$9.43 \pm 2.37 \times 10^{-8}$	$9.59 \pm 3.21 \times 10^{-8}$	nd	$9.60 \pm 1.39 \times 10^{-8}$	PHENT
	PE	$9.80 \pm 0.59 \times 10^{-9}$	$1.17 \pm 0.23 \times 10^{-8}$	$1.17 \pm 0.24 \times 10^{-8}$	nd	$1.11 \pm 0.10 \times 10^{-8}$	
SHR	NA	$2.37 \pm 0.24 \times 10^{-8}$	$3.62 \pm 0.36 \times 10^{-8}$	$4.71 \pm 0.44 \times 10^{-8}$	sd	$3.57 \pm 0.34 \times 10^{-8}$ (D)	
	PE	$1.15 \pm 0.27 \times 10^{-8}$	$1.52 \pm 0.40 \times 10^{-8}$	$1.33 \pm 0.43 \times 10^{-8}$	nd	$1.33 \pm 0.17 \times 10^{-8}$ (ND)	
NCR	NA	$1.10 \pm 0.34 \times 10^{-7}$	$1.82 \pm 0.35 \times 10^{-7}$	$4.02 \pm 0.90 \times 10^{-7}$	sd	$2.31 \pm 0.53 \times 10^{-7}$	L - YOH
	PE	$4.89 \pm 1.33 \times 10^{-8}$	$8.63 \pm 1.91 \times 10^{-8}$	$1.19 \pm 0.15 \times 10^{-7}$	sd	$8.45 \pm 1.17 \times 10^{-8}$	
SHR	NA	$1.83 \pm 0.46 \times 10^{-7}$	$1.42 \pm 0.29 \times 10^{-7}$	$1.49 \pm 0.25 \times 10^{-7}$	nd	$1.58 \pm 0.17 \times 10^{-7}$ (ND)	
	PE	$3.60 \pm 1.72 \times 10^{-7}$	$4.42 \pm 1.65 \times 10^{-7}$	$3.06 \pm 1.02 \times 10^{-7}$	nd	$3.69 \pm 0.73 \times 10^{-7}$ (D)	
NCR	NA	$1.13 \pm 0.25 \times 10^{-6}$	$1.59 \pm 0.23 \times 10^{-6}$	$2.36 \pm 0.26 \times 10^{-6}$	sd	$1.69 \pm 0.20 \times 10^{-6}$	H - YOH
	PE	$5.20 \pm 0.17 \times 10^{-7}$	$7.34 \pm 0.47 \times 10^{-7}$	$9.22 \pm 0.71 \times 10^{-7}$	sd	$7.25 \pm 0.57 \times 10^{-7}$	
SHR	NA	$5.83 \pm 1.39 \times 10^{-7}$	$6.92 \pm 0.97 \times 10^{-7}$	$9.49 \pm 0.68 \times 10^{-7}$	sd	$7.10 \pm 0.74 \times 10^{-7}$ (D)	
	PE	$4.77 \pm 0.97 \times 10^{-7}$	$6.92 \pm 1.45 \times 10^{-7}$	$1.15 \pm 0.21 \times 10^{-6}$	sd	$7.72 \pm 1.25 \times 10^{-7}$ (ND)	

A. ANOC. Muscle: The Schild plot for L-Praz and H-Praz against NA produced two distinct (statistically attested by the method of Armitage, 1974) regression lines.

L-Praz (1.1×10^{-8} - 8.8×10^{-7}M) was non-competitive against NA in both NCR and SHR. The slopes of the Schild lines were 0.46 ± 0.03 and 0.52 ± 0.07 respectively with corresponding apparent pA_2 values of 8.45 ± 0.04 and 8.85 ± 0.06. Both slope values were significantly different from one ($p < 0.05$) Fig. 13a and Table 2a). In addition, Mackay plot regression co-efficients -0.54 ± 0.02 and -0.47 ± 0.07 (for NCR and SHR respectively) were significantly different from zero ($p < 0.05$)(Table 3). Also, in both NCR and SHR, the mean K_{diss} values over the range of prazosin concentrations were not all the same. The grand mean K_{diss} $1.13 \pm 0.16 \times 10^{-8}$ obtained in the SHR was not significantly different from the value $1.81 \pm 0.35 \times 10^{-8}$ in the NCR (Table 4). L-Praz produced competitive antagonism of PE - induced contractions in both NCR and SHR with pA_2 values of 8.93 ± 0.04 (slope = 0.85 ± 0.07) in the NCR and 8.89 ± 0.06 (slope = 0.80 ± 0.09) in the SHR (Fig. 13b, Table 2a). The corresponding grand mean K_{diss} values of $1.98 \pm 0.18 \times 10^{-9}$ and $2.52 \pm 0.35 \times 10^{-9}$ were not significantly different from each other (Table 4). The mean K_{diss} values did not vary with antagonist concentration in both rat strains. Mackay plot regression co-efficient was -0.14 ± 0.07 in the NCR and -0.22 ± 0.09 in SHR and in both cases were not significantly different from zero (Table 3).

H-Praz (1.77×10^{-6} - 7.04×10^{-6}M) produced competitive antagonism

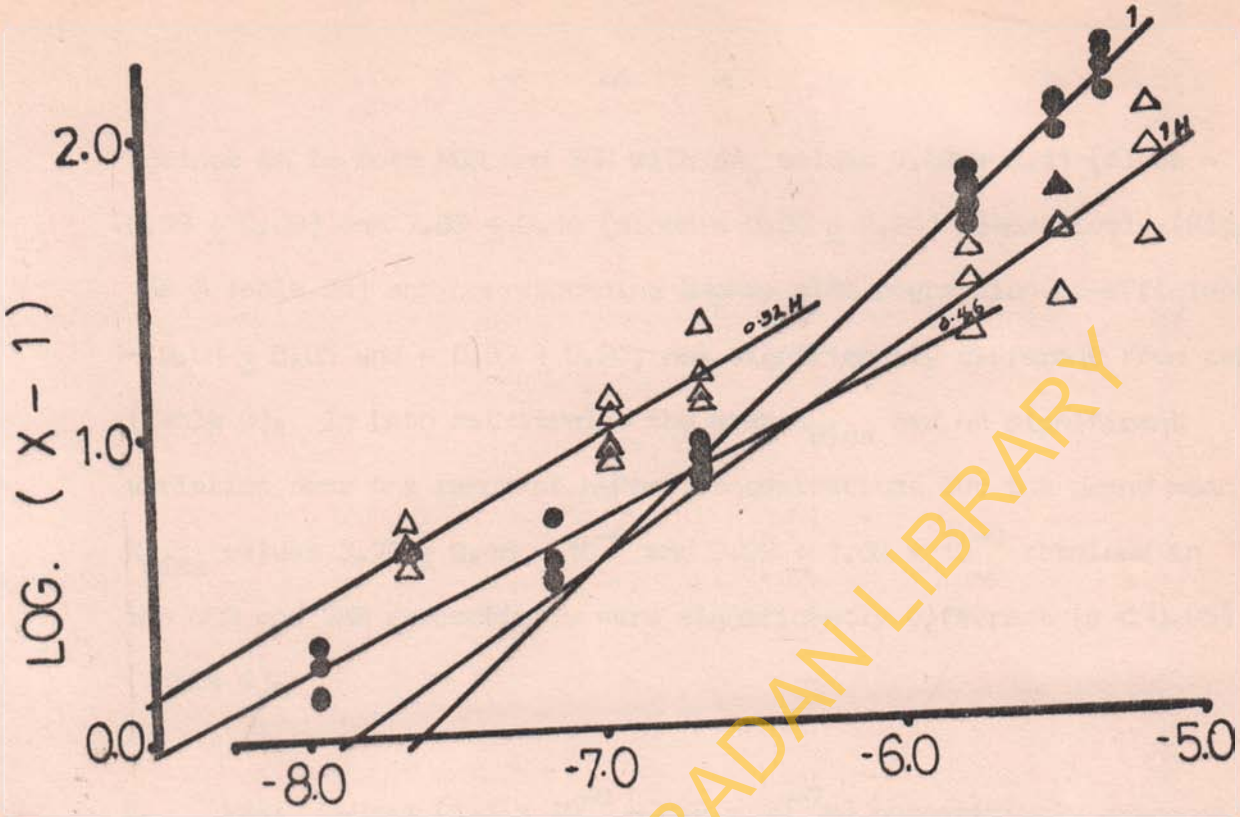
FIGURE 13

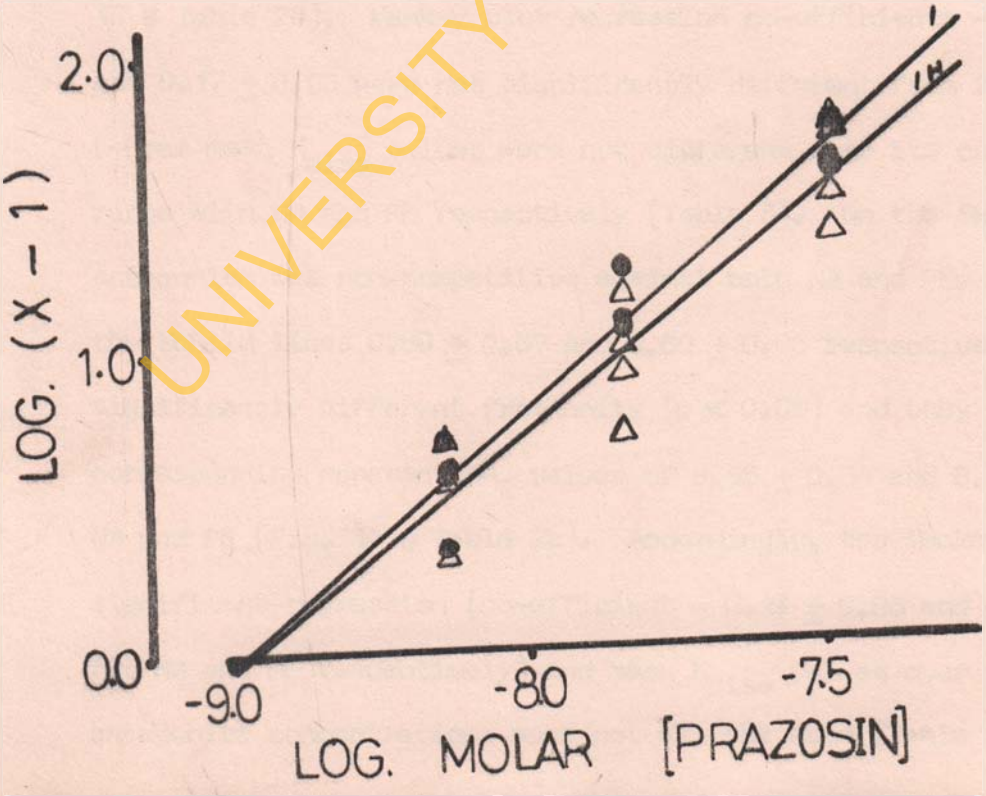
A. A – S plot for the antagonism of NA induced contractions of the anococcygeus by prazosin.

B. A – S plot for the antagonism of PE induced contractions of the anococcygeus by prazosin.

"H" = the SHR line. Line marked 1 where the slope is not significantly different from 1 and the actual value where different from 1.

● ● NCR, △ △ SHR.





against NA in both NCR and SHR with pA_2 values 7.58 ± 0.11 (slope = 0.93 ± 0.09) and 7.89 ± 0.14 (slope = 0.65 ± 0.24) respectively (Fig. 13a & Table 2a) and corresponding Mackay plot regression co-efficient - 0.01 ± 0.03 and - 0.37 ± 0.24, not significantly different from zero (Table 3). In both rat strains the mean K_{diss} had no significant variation over the range of H-Praz concentrations but the grand mean K_{diss} values $3.77 \pm 0.15 \times 10^{-8}$ and $9.99 \pm 1.68 \times 10^{-8}$ obtained in the NCR and SHR respectively were significantly different ($p < 0.05$) (Table 4).

B. VAS: L-Praz (1.1×10^{-8} - 8.8×10^{-7}M) competitively antagonised both NA and PE in the NCR with pA_2 values of 8.00 ± 0.04 (slope = 0.96 ± 0.08) and 8.35 ± 0.08 (slope = 1.17 ± 0.23) respectively (Fig. 14 & Table 2b). Mackay plot regression co-efficients - 0.10 ± 0.08 and 0.17 ± 0.23 were not significantly different from zero and the L-Praz mean K_{diss} values were not different over its concentration range with NA and PE respectively (Table 5). On the SHR vas, L-Praz antagonism was non-competitive against both NA and PE. The slopes of the Schild lines 0.80 ± 0.07 and 0.88 ± 0.03 respectively were significantly different from unity ($p < 0.05$) and they yielded corresponding apparent pA_2 values of 8.55 ± 0.04 and 8.64 ± 0.02 for NA and PE (Fig. 14 & Table 2b). Accordingly, the Mackay plots had significant regression (co-efficient - 0.19 ± 0.06 and - 0.12 ± 0.04 for NA and PE respectively) and mean K_{diss} values over the range of antagonist concentrations were not all the same (Table 5). The grand

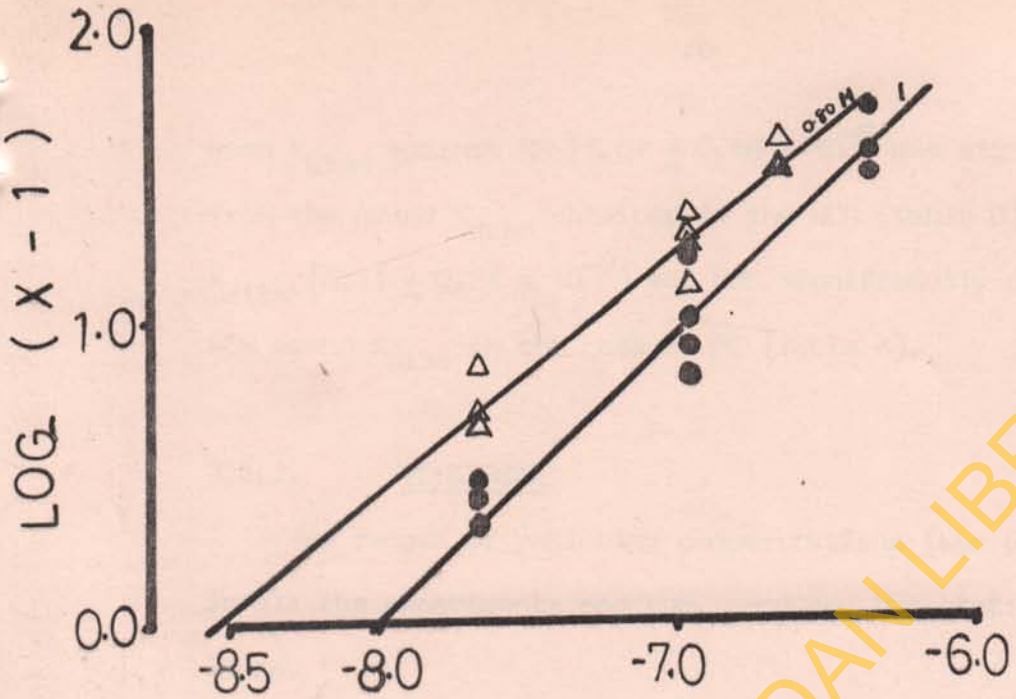
FIGURE 14

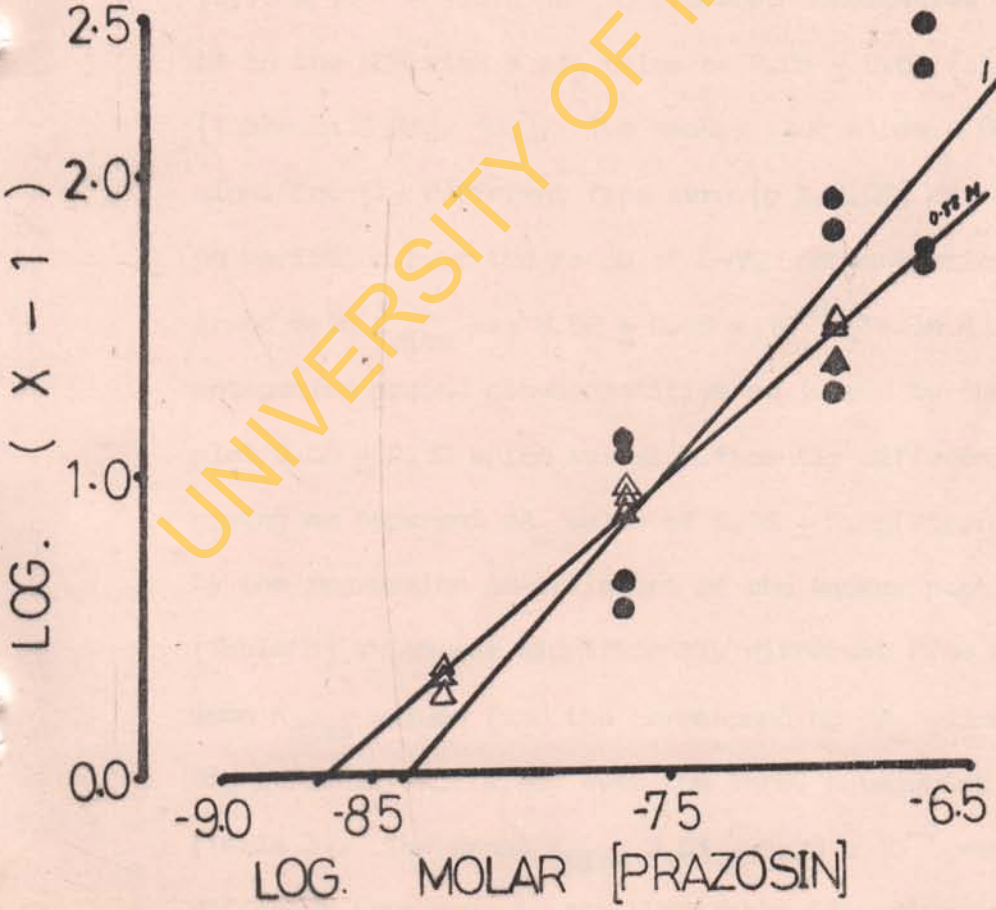
A. A – S plot for the antagonism of NA induced contractions of the vas by prazosin.

B. A – S plot for the antagonism of PE induced contractions of the vas by prazosin.

"H" = the SHR line. Line marked 1 where the slope is not significantly different from 1 and the actual value where different from 1.

● NCR, △ SHR.





mean K_{diss} against NA ($5.67 \pm 0.46 \times 10^{-9}$ was significantly different from the grand K_{diss} obtained in the NCR (Table 6) whereas SHR grand K_{diss} ($3.11 \pm 0.21 \times 10^{-9}$) was not significantly different from the NCR grand K_{diss} in the case of PE (Table 6).

3.4.3. YOHIMBINE.

Two ranges of yohimbine concentrations (Low & High) were used in all the experiments and they produced two statistically distinct lines.

A. ANOC Muscle: The lower concentrations of yohimbine (L-YOH) ($5.75 \times 10^{-8} - 4.5 \times 10^{-7}$M) produced competitive antagonism against NA in the NCR with a pA_2 value of 7.08 ± 0.06 (slope, 0.74 ± 0.19) (Table 2a & Fig. 15a). The Mackay plot slope -0.26 ± 0.16 was not significantly different from zero ($p > 0.05$) and the mean K_{diss} showed no variation over the range of L-YOH concentrations (Table 3). The grand mean K_{diss} was $9.52 \pm 0.85 \times 10^{-8}$ (Table 4). In the SHR, L-YOH antagonism proved non-competitive as judged by the slope of the A - S plot 0.65 ± 0.10 which was significantly different from one ($p < 0.05$), giving an apparent pA_2 value of 6.74 ± 0.06(Fig. 15a & Table 2b) and also by the regression co-efficient of the Mackay plot -0.35 ± 0.11 (Table 3) which was significantly different from zero. However, the mean K_{diss} values (and the corresponding pA_2 values) were not significantly different over the three antagonist concentrations (Table 3). The grant K_{diss} $2.01 \pm 0.25 \times 10^{-7}$ was significantly different from that in the NCR (Table 4). Also, in the NCR, L-YOH

FIGURE 15

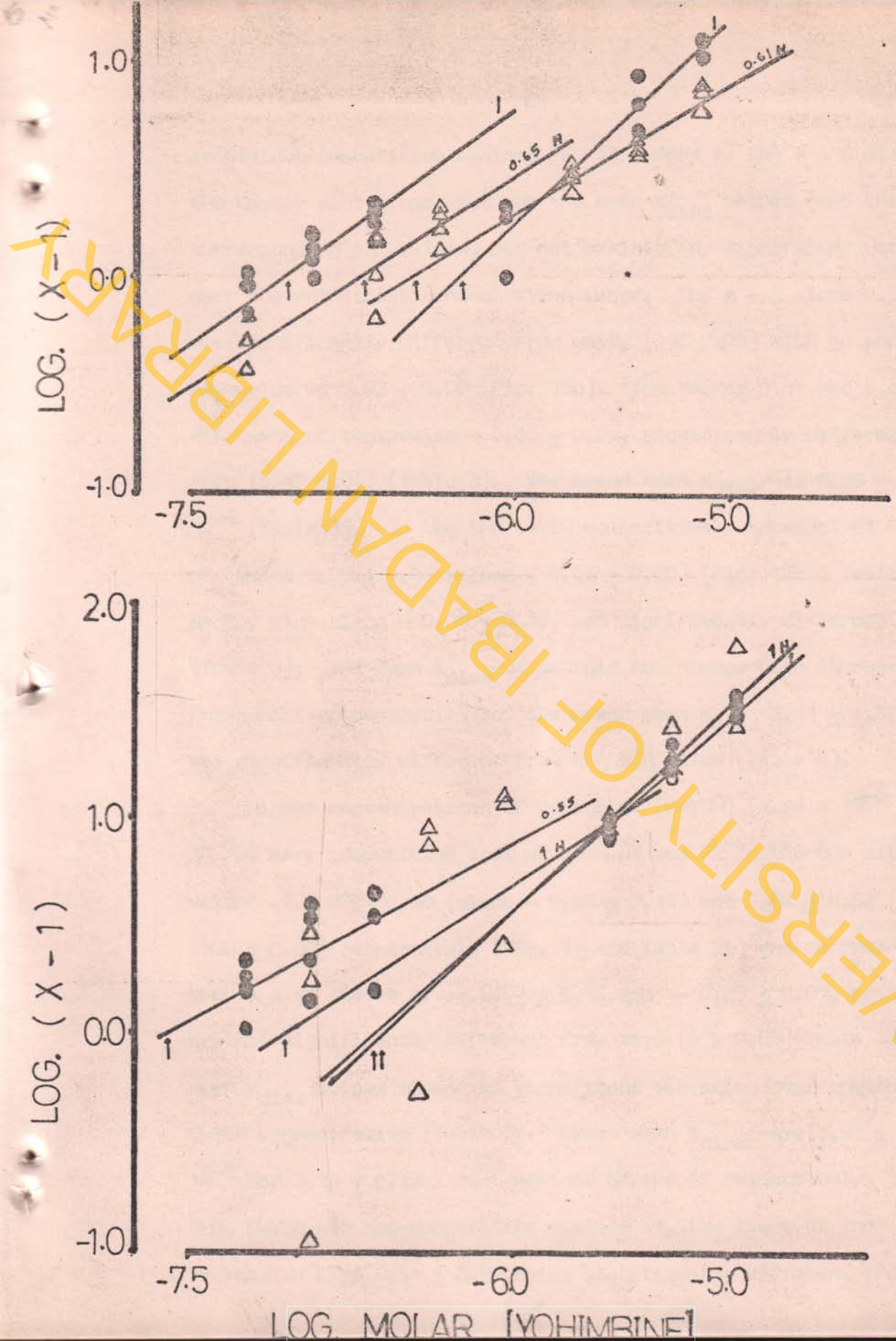
A. A – S plot for the antagonism of NA induced contractions of the anococcygeus by yohimbine.

B. A – S plot for the antagonism of PE induced contractions of the anococcygeus by yohimbine.

"H" = the SHR line. Line marked 1 where the slope is not significantly different from 1 and the actual value where different from 1.

● NCR

△ SHR



proved non-competitive against PE, if judged by the A - S plot and the Mackay plot slopes but yet the mean K_{diss} values (and the corresponding pA_2 values) did not exhibit any significant variation over the antagonist concentration range. The A - S slope 0.55 ± 0.18 was significantly different from unity ($p < 0.05$) with an apparent pA_2 value of 7.62 ± 0.09 (Fig. 15b). The Mackay plot had a coefficient of regression - 0.45 ± 0.20, significantly different from zero ($p < 0.05$) (Table 3). The grand mean K_{diss} was 5.36 ± 0.92 x 10^{-8} (Table 4). In the SHR L-YOH competitively antagonised PE with a pA_2 value 7.06 ± 0.35 (slope = 0.65 ± 0.60) (Fig. 15b & Table 2a) and Mackay plot slope - 0.06 ± 0.34, not significantly different from zero (Table 3). The mean K_{diss} values did not change with increasing antagonist concentration and the grand mean K_{diss} 3.11 ± 1.35 x 10^{-7} was significantly different from the NCR value (Table 4).

Higher concentrations of yohimbine (H-YOH) (1.84 z 10^{-6} - 1.1 x 10^{-5}M) were competitive against both NA and PE in the NCR with pA_2 values of 6.27 ± 0.06 (slope = 0.96 ± 0.14) and 6.64 ± 0.02 (slope = 0.90 ± 0.06) respectively (Fig. 15 and Table 2a) and corresponding Mackay plot slopes of - 0.03 ± 0.13 and - 0.07 ± 0.07, both of which are not significantly different from zero ($p > 0.05$)(Table 3). The mean K_{diss} values showed no significant variation over the range of H-YOH concentration (Table 3). Grand mean K_{diss} were 5.92 ± 0.68 x 10^{-7} and 3.12 ± 0.12 x 10^{-7} against NA and PE respectively. In the SHR, H-YOH was non-competitive against NA, the slope of the regression line 0.61 ± 0.07 being significantly different from unity

FIGURE 16

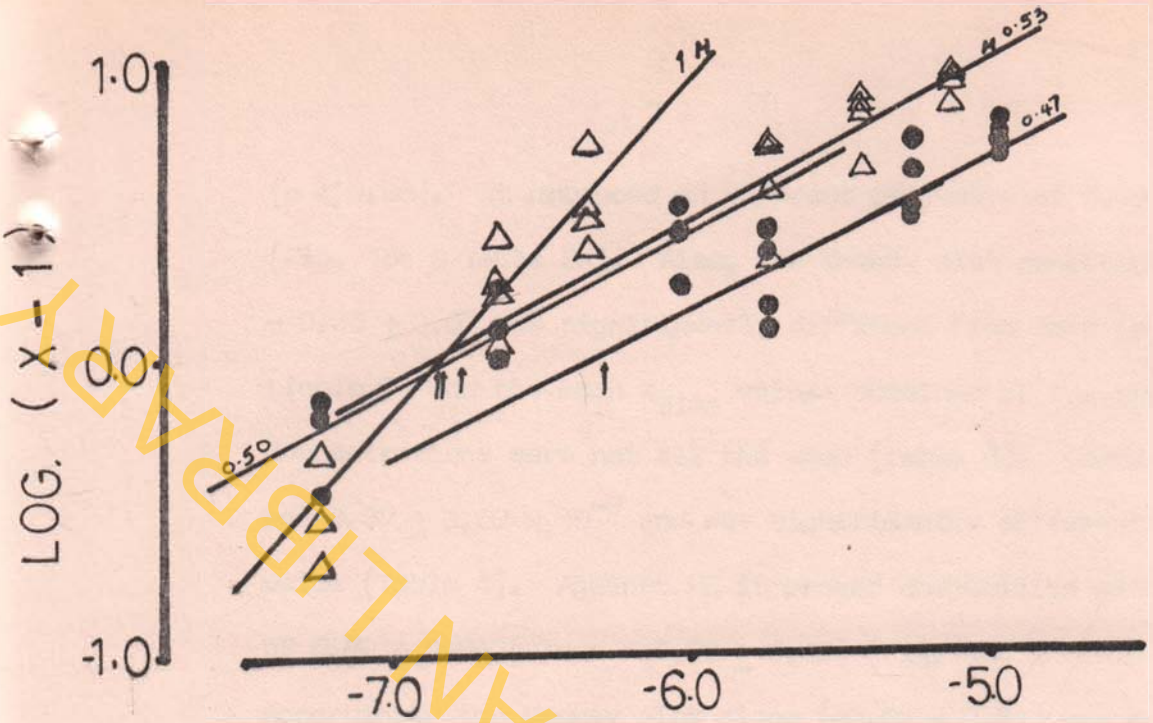
A. A – S plot for the antagonism of NA induced contractions of the vas by yohimbine.

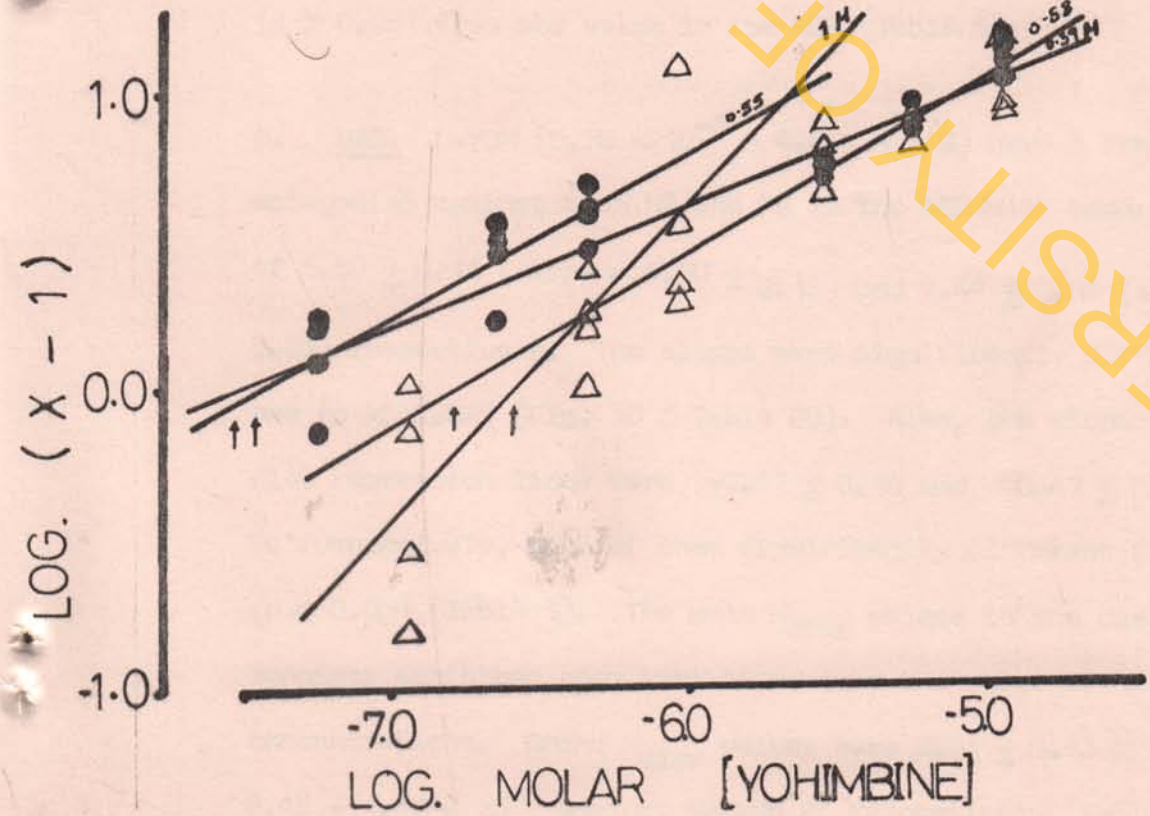
B. A – S plot for the antagonism of PE induced contractions of the vas by yohimbine.

"H" = the SHR line. Line marked 1 where the slope is not significantly different from 1 and the actual value where different from 1.

● NCR

△ SHR





($p < 0.05$). It produced an apparent pA_2 value of 6.49 ± 0.03 (Fig. 15a & Table 2a). Also, the Mackay plot co-efficient - 0.35 ± 0.07 was significantly different from zero ($p < 0.05$) (Table 3) and the mean K_{diss} values obtained at the three antagonist concentrations were not all the same (Table 4). Grand mean K_{diss} was 8.37 ± 0.59 x 10^{-9} and was significantly different from the NCR value (Table 4). Against PE it proved competitive with a pA_2 value of 6.60 ± 0.06 (slope = 0.96 ± 0.19) (Fig. 15b & Table 2a). Accordingly the Mackay plot slope (-0.02 ± 0.51) was not significantly different from zero ($p > 0.05$)(Table 3) and the mean K_{diss} values did not vary over the three concentrations of H-YOH (Table 4). The grand mean K_{diss} 2.92 ± 0.29 x 10^{-7} was not significantly different ($p > 0.05$) from the value in the NCR (Table 4).

B. VAS: L-YOH (5.75 x 10^{-8} - 4.5 x 10^{-7}M) gave a non-competitive antagonism against both NA and PE in the NCR with apparent pA_2 values of 6.80 ± 0.11 (slope = 0.50 ± 0.12) and 7.44 ± 0.06 (slope = 0.55 ± 0.09) respectively. The slopes were significantly different from one ($p < 0.05$) (Fig. 16 & Table 2b). Also, the slopes of the Mackay plot regression lines were -0.47 ± 0.10 and -0.47 ± 0.11 for NA and PE respectively, both of them significantly different from zero ($p < 0.05$) (Table 5). The mean K_{diss} values in the cases of both agonists exhibited some variations over the range of antagonist concentrations. Grand K_{diss} values were 2.31 ± 0.53 x 10^{-7} and 8.45 ± 1.17 x 10^{-8} against NA and PE respectively (Table 6). In the

SHR the antagonism was competitive against both NA and PE with pA_2 values of 6.82 ± 0.04 (slope = 1.10 ± 0.11) and 6.58 ± 0.08 (slope = 1.04 ± 0.23) (Fig. 16 & Table 2b). Mackay plot slopes were 0.10 ± 0.11 and 0.01 ± 0.26 (for NA and PE respectively) (Table 5). These values were not significantly different from zero ($p > 0.05$) and accordingly the mean K_{diss} values did not vary with increasing L-YOH concentration. The grand K_{diss} $1.58 \pm 0.17 \times 10^{-7}$ obtained against NA was not significantly different from that obtained in the NCR whereas the value $3.69 \pm 0.73 \times 10^{-7}$ obtained with PE was significantly different from that in the NCR ($p < 0.05$)(Table 6).

H-YOH ($1.84 \times 10^{-6} - 1.1 \times 10^{-5}$) produced non-competitive antagonism against both NA and PE in both NCR and SHR. The slopes of the Schild regression lines were significantly different from unity ($p < 0.05$). Slopes of the Mackay plot lines were significantly different from zero ($p < 0.05$) and the mean K_{diss} values were not all the same over the antagonist concentration range in the cases of both agonists and in both NCR and SHR. In the NCR H-YOH produced apparent pA_2 values of 6.36 ± 0.09 (A - S slope = 0.47 ± 0.11 and Mackay plot slope, - 0.44 ± 0.11) and 6.79 ± 0.03 (A - S slope = 0.58 ± 0.06 and Mackay plot slope = -0.40 ± 0.11) against NA and PE respectively (Fig. 16, Table 5 & Table 2b). It gave apparent pA_2 values of 6.83 ± 0.08 (A - S slope = 0.53 ± 0.13 and Mackay plot slope - 0.46 ± 0.12) against NA and 7.56 ± 0.15 (A - S slope = 0.39 ± 0.14 and plot slope - 0.67 ± 0.14) against PE in the SHR (Fig. 16, Table 2b

and Table 5).

The grand K_{diss} values of H-YOH against NA were $1.69 \pm 0.02 \times 10^{-6}$ and $7.10 \pm 0.74 \times 10^{-7}$ in the NCR and SHR respectively (Table 6). The values were significantly different ($p < 0.05$). Corresponding values $7.25 \pm 0.57 \times 10^{-7}$ and $7.72 \pm 1.25 \times 10^{-7}$ obtained against PE in the two rat strains were not significantly different (Table 6).

CHAPTER FOUR

DISCUSSION

5 DISCUSSION

NOR ANOC.

Noradrenaline (NA) and phenylephrine (PE) produced concentration-related contractions of the rat anococcygeus muscle with pD_2 values of 5.03 ± 0.05 and 6.14 ± 0.08 respectively in the absence of cocaine. In the presence of cocaine respective pD_2 values of 5.83 ± 0.06 and 6.68 ± 0.04 were obtained. These values are similar to the values (5.83 and 6.54 respectively) computed from the EC_{50} values reported by Brown et al. (1980) for these agonists on the anococcygeus muscle. The difference in the pD_2 values obtained in the absence and presence of cocaine reveal the influence of neuronal uptake on the potency of these agonists. The values gave dose ratios of 6.31 for NA and 3.48 for PE showing that the influence of neuronal uptake is more pronounced on NA than on PE. This is in accordance with the observation that NA has a greater affinity than PE for the neuronal uptake mechanism (Iversen, 1967). The higher pD_2 value of PE cannot, therefore, be due to the influence of neuronal uptake but that PE is actually more potent than NA in this preparation. This suggests that the post-junctional α_1-adrenoceptors in the anococcygeus muscle might be more sensitive than in some other preparations. For instance NA was more potent than PE in the rabbit pulmonary artery (Starke et al., 1975a) and aorta (Wikberg, 1978) and guinea pig ileum (Wikberg, 1978) even though PE was more selective for α_1-adrenoceptors.

Low concentrations of prazosin (L-Praz.) produced reversible antagonism of NA. This antagonism was however non-competitive as

indicated by the slopes of the Schild and Mackay plot regression lines. The A – S slope was different from one and the Mackay slope different from zero. It was observed that, as concerns competitive nature or otherwise the A – S and Mackay analyses will usually give the same indication. Furthermore, it was observed that, empirically, the regression co-efficient of the A – S plot will always add up to the negative value of the co-efficient of the Mackay plot to give approximately one and that the standard errors of the two co-efficients are very similar (see Table 7). For this reason the actual value of the dissociation constant (K_{diss}) was calculated directly from the dose ratios at each antagonist concentration – as another test for competitive antagonism. (Hereafter, reference will be made to only either of the A – S or Mackay analyses apart from the K_{diss} analysis). The L – Praz. K_{diss} values varied with the antagonist concentrations indicating non-competitive antagonism. Higher concentrations of the antagonist (H – Praz.) still produced reversible antagonism to NA but the antagonism was competitive, with a pA_2 value of 7.58 ± 0.11. This difference in the nature of antagonism shown by L – Praz. and H – Praz. may be what is reflected on the prazosin family of curves where the H – Praz. curves exhibited better parallelism than the L – Praz. curves (Fig. 6a). Both low and high concentrations of yohimbine (L – YOH and H – YOH respectively) competitively antagonised the NA evoked contractions with two significantly different ($p < 0.05$) pA_2 values of 7.08 ± 0.06 and 6.27 ± 0.06 respectively. These results showed that the NA evoked contractions were due to α-adrenoceptor

TABLE 2

Addition of the A – S slope and the negative value of the Mackay plot slope ($\simeq$ 1).

TABLE 7

ANOC.

	A - S slope	- {Mackay slope}	Addition
NA	0.46 ± 0.03	0.54 ± 0.02	1
PE	0.85 ± 0.07	0.14 ± 0.07	0.99
NA	0.52 ± 0.07	0.47 ± 0.07	0.99
PE	0.80 ± 0.09	0.20 ± 0.09	1
NA	0.82 ± 0.26	0.18 ± 0.26	1
PE	1.38 ± 0.25	-0.38 ± 0.24	1
NA	0.74 ± 0.19	0.26 ± 0.16	1
PE	0.55 ± 0.18	0.45 ± 0.20	1
NA	0.65 ± 0.10	0.35 ± 0.11	1
PE	0.90 ± 0.06	0.07 ± 0.07	0.97
NA	0.61 ± 0.07	0.35 ± 0.07	0.96
PE	0.96 ± 0.19	0.02 ± 0.51	0.98

stimulation in agreement with previously reported observations by many workers. The results also suggest that there are more than one α-adrenoceptor subtype located post-junctionally in the rat anococcygeus muscle. One receptor subtype might be the conventional post-junctional α_1-adrenoceptor and the other a sub-population with similar characteristics to the α_2-adrenoceptor as also suggested by Docherty & McGrath (1980) from an in-vivo study on the rat anococcygeus muscle. Based on this suggestion, the above presented results can be explained as follows:

NA produces its contractions by an equipotent stimulation of the two receptor subtypes. L - Praz (a selective α_1-adrenoceptor antagonist, Cavevo et al., 1977; Timmermans & van Zwieten, 1980) blocked only the α_1-mediated component of the NA evoked contractions with little or no effect on the α_2- like population. In such a case - where an antagonist selectively inhibits the involvement of one of two receptor subtypes stimulated by a non-selective agonist - the agonist: antagonist interaction will not fit in with the bi-molecular model assumed by Arunlakshana & Schild (1959). This suggestion was made by Furchgott (1976) and has been supported by experimental results. Thus, there have been a number of reports in which the slopes of the Schild plots were significantly less than unity for presumably competitive antagonists in preparations with both β_1 and β_2 adrenoceptors present post-junctionally (O'Donell & Wanstall, 1980; Taylor, 1982). Accordingly, L - Praz produced a Schild regression co-efficient of 0.46 ± 0.03 against NA in the anococcygeus muscle. H - Praz, which

produced a competitive antagonism against NA might be acting on both α_1- and α_2- adrenoceptors - because its "selective antagonism" is a relative term which may not be true at high doses. In support of this is the observation by Timmermans, Van Meel & van Zwieten(1980) that relatively higher concentrations of prazosin produced a blockade of post-junctional α_2 adrenoceptors with a pA_2 value of 5.91 in normotensive pithed rats.

The two pA_2 values obtained from the yohimbine experiment seem to be further evidence in support of a dual occurrence of post-junctional α-adrenoceptor subtypes. Yohimbine is an α-adrenoceptor antagonist with preference for the α_2- subtype (Starke et al., 1975b; Doxey et al., 1977, Brown et al., 1980). Thus, L - YOH preferentially inhibited the α_2- mediated component of the NA induced contraction producing the pA_2 of 7.08. At higher concentrations, yohimbine might not be able to discriminate any more and therefore antagonised both the α_1 and α_2- sub-populations with the lower pA_2 of 6.27. However L - YOH did not behave like L-Praz (presented above) in that it produced competitive antagonism, its Schild plot slope being not significantly different from unity. This can be explained as due to the fact that yohimbine is less selective for the α_2- subtype than prazosin is for the α_1- subtype (Brown et al., 1980). Thus, the L - YOH action might involve a small proportion of α_1-adrenoceptor subtype, enough to render the antagonism competitive (i.e. make the A - S plot slope $\simeq$ 1). With this explanation it will be expected that the antagonism by L-Praz of a selective α_1-adrenoceptor agonist

and that by L - YOH of a selective α_2- agonist should yield A - S slope values that are not significantly different from unity. Accordingly, L-Praz. competitively antagonised (A - S slope not different from 1) contractions evoked by PE, a selective α_1-adrenoceptor agonist with a pA_2 value of 8.93. The effect of L - YOH on a selective α_2- agonist could not be studied because the α_2- agonists available were either partial agonists (UK, 14, 304-18, guanfacine) or they produced erratic response (guanabenz) or could not evoke any contrations (B-HT 920). The non-availability of an "ideal" α_2-adrenoceptor agonist has been the main difficulty in in-vitro studies of post-junctional α_2-adrenoceptors using end-organ responses (see Drew, 1982 and McGrath, 1982 for commentaries).

The significant regression (- 0.45, Table 3) obtained in the Mackay plot analysis of the L - YOH: PE interaction indicated that the antagonism was non-competitive whereas the steady mean K_{diss} of the same interaction indicated competitive antagonism (Table 4). This paradoxy cannot be easily explained. Nevertheless, a possible explanation may be as follows: The mean K_{diss} values are compared by analysis of variance - a statistical method by which it is tested whether the means of several (at least three) sets of observations are the same. In this particular case it tested whether the mean K_{diss} at different antagonist concentrations are not different. In other words, if the mean K_{diss} is denoted by "y" analysis of variance (ANOVA) tests whether dy (change in y) is significantly different from zero or not. Both the A - S and Mackay analyses are log - log plots (i.e. both the abscissa and ordinate values are in

logarithm) and the slope of the line is a measure of the ratio of the relative change in ordinates (ys) to the change in abscissae (xs). In other words the slope equals $\frac{dy}{dx}$. Thus, even if dy is very small and judged not to be different from zero by analysis of variance a much smaller dx, for instance, will render the slope dy/dx a big value which may become significantly different from zero in the Mackay plot. To buttress this explanation the mean pA_2 value obtained at each antagonist concentration were compared by ANOVA and the values proved not to be different even though the pA_2 regressed significantly on the log-antagonist concentration in the Mackay plot. This may suggest that a comparison of the mean K_{diss} may be a more precise way of testing for competition in drug antagonism. However, the information obtained from the A - S plot is still useful especially as the NA: L - YOH pair yielded a slope that is not different from one. It shows some differences between NA and PE in their interaction with L-YOH. PE, unlike NA produces its contractions chiefly via the α_1-adrenoceptor subtype while L-YOH acts mainly at the α_2-subpopulation. Thus, the two drugs act at different loci in the preparation. The competition between such a pair might not be "perfect". This may be what was reflected as the low A - S slope value 0.55 $\pm$ 0.18 (Fig. 15b & Table 2a) (which was significantly different from unity) and the slope - 0.45 $\pm$ 0.2 (Table 3) of the Mackay plot. According to the above propounded hypothesis this means a change dx in the abscissae (log B) is much bigger than the corresponding change dy in the ordinates (log x - 1) - on the A - S

plot - which is not the case in the ideal competitive antagonism. In a log - log plot like the A - S plot,

Small change $dy = \log y_2 - \log y_1 = y_2/y_1$ and likewise $dx = \log x_1 - \log x_2 = x_1/x_2$. Thus, a change dy or dx on such a logarithmic plot represents a ratio of the corresponding numbers (from which the logarithm was obtained) and is an estimate of the multiplication factor between two dose-ratios or antagonist doses, whichever the case is. In the ideal competitive antagonism the multiplication of a certain antagonist concentration by a certain factor results in a corresponding multiplication of the dose ratio by the same factor, making the slope of the A - S plot one. But in a case where the Schild slope is less than one, it implies that a certain multiplication of the antagonist concentration does not result in a similar multiplication in the dose ratio but by some lower factor. Practically, this implies that L - YOH is so much "more than a match" for PE, in their competition for α_2-adrenoceptors, that after its first dose there remained little or no more α_2-adrenoceptors to compete for ; as it were, the reaction has reached saturation. At such a point, large increases in any of the reactants do not result in any appreciable change in the products. The Mackay plot also portrays this observation. The ordinates on the Mackay plot are pA_2 values. pA_2 itself is a logarithm value. Log $(x - 1)$ - log B yields a log value pA_2, the antilog of which equals $\frac{x - 1}{B}$. That is, pA_2 is the logarithm of a ratio. Thus dy in the Mackay plot =

$$\log y_2 - \log y_1 = \log \frac{a_2}{b_2} - \log \frac{a_1}{b_1}$$

(where a and b represent the numerator and denominator respectively, of the ratio that yielded y) or

$$\frac{\frac{a_2^2}{b_2^2}}{\frac{a_1}{b_1}} = \frac{a_2}{b_2} \times \frac{b_1}{a_1} = dy$$

On the abscissa $dx = \log x_1 - \log x_2$ or $\frac{x_1}{x_2}$. The abscissa values (xs) are the same as the denominator b(antagonist molar concentration) in the above equations. Thus,

$\frac{x_1}{x_2}$ can also be written as $\frac{b_1}{b_2}$.

The slope of the Mackay plot regression line

$$\frac{dy}{dx} = \frac{a_2}{b_2} \times \frac{b_1}{a_1} \times \frac{b_2}{b_1} = \frac{a_2}{a_1} \quad \text{or } \log a_2 - \log a_1$$

which is the ratio of the dose ratios. The dose ratio was said (earlier on) not to vary much (compared with the antagonist concentrations). Thus, a_2 and a_1 will not be very different and the ratio $\frac{a_2}{a_1}$, the slope, will not be too far from one even though dy (dpA_2) is not significantly different from zero.

VAS: NA and PE produced concentration related contractions of the rat vas deferens (RVD) with pD_2 values of 5.16 ± 0.03 and 5.47 ± 0.05 respectively in the absence of cocaine. In the presence of cocaine respective pD_2 values of 5.42 ± 0.03 and 5.72 ± 0.04 were obtained

(Table 1). The pD_2 5.42 obtained for NA was similar to the value 5.4 obtained by Ariens (1963) and Pratesi & Grana (1965) but they did not compare with PE. Patil, LePidus, Campbell & Tye (1967) obtained a pD_2 value of 5.2 for PE on the RVD which, though low compared with the value obtained in the present study, produced a PE: NA potency ratio 1.08 similar to 1.06, obtained in this study. These values gave dose ratios of 1.82 ± 0.13 and 1.78 ± 0.06 for NA land PE respectively, as a result of cocaine potentiation. These cocaine potentiation ratios are much lower than those obtained in the anococcygeus muscle despite the fact that the vas is also densely innervated. This might be due to the very close juxtaposition in the vas of nerve terminal and smooth muscle membranes. The width of the junctional gaps in the rat vas deferens ranged from 180 ^{o}A – 250 ^{o}A (Richardson, 1962) whilst in the anococcygeus muscle they were never less than 550 ^{o}A with a mean value of 2600 ^{o}A (Gillespie & Lullman-Rauch (1974), that is, about a ten-fold difference. The tightness of the junction in the vas might make the $uptake_1$ mechanism in the vas deferens an especially efficient one which exhibits only little sensitivity to cocaine. Furthermore, unlike in the anococcygeus there is little or no difference between the NA and PE dose ratios and this is inconsistent with the observation in the anococcygeus. It may also be due to the high $uptake_1$ efficiency in the preparation.

Low concentrations of prazosin (L-Praz) produced competitive antagonism to both NA and PE with pA_2 values of 8.00 ± 0.04 and 8.35 ± 0.08 respectively (Table 2b). These same concentrations of prazosin

gave a non-competitive antagonism of NA in the anococcygeus muscle. At higher concentrations prazosin produced an irreversible blockade of NA, which same concentrations gave a competitive antagonism in the anococcygeus. This suggests that NA does not evoke its anococcygeus and RVD contractions by identical receptor mechanisms. It seems that NA, like PE evokes its contractions of the RVD via a predominance of α_1-adrenoceptors. Yohimbine produced two Schild lines corresponding to its low and high concentration ranges (Fig. 16) against both NA and PE as it did in the anococcygeus muscle. However, unlike in the anococcygeus muscle the antagonism was not competitive at either YOH concentration range as judged by the slopes of the Schild lines which were significantly different from one and the mean K_{diss} values which varied with YOH doses (Table 5) in the cases of both agonists. It seems that YOH at both low and high concentrations could not produce a perfect competition against the agonists at the receptor sites. This may be so if the receptor site is α_1- for which yohimbine exhibits less preference. This supports the above suggestion that the contractions of the RVD to exogenously applied NA and PE are predominantly α_1-adrenoceptor mediated. This has also been suggested by other workers (Docherty et al., 1977; Doggrell & Waldron, 1982). Nevertheless, the presence of a small proportion of the α_2-subtype cannot be ruled out since the double A - S line phenomenon was also exhibited in the vas deferens. One would expect that high YOH should produce a competitive antagonism even if only the α_1-adrenoceptors are present, since high YOH does not discriminate. This seems rather difficult to explain at present. It may be that the α_1-adrenoceptor

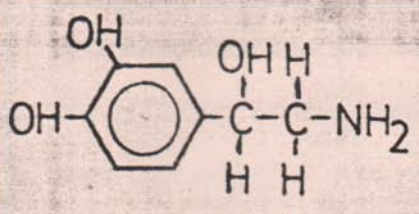
population of the vas deferens is not exactly the same as in the anococcygeus muscle. A similar suggestion to this was made by MacDonald & McGrath (1980) that the post-junctional α_1-adrenoceptor population in the isolated RVD may not be the same as in the anococcygeus muscle and yet does not possess α_2-adrenoceptor characteristics.

It should be noted that in many experiments remarkable enhancement in maximal responses (to both NA and PE) of both NCR and SHR vas was observed especially in H-YOH experiments (Fig. 10). This kind of observation has been reported by Jurkiewicz & Jurkiewicz (1976) but it seems some more detailed experiments need be performed before this could be satisfactorily explained.

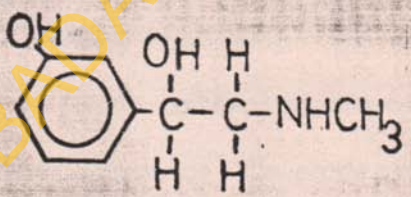
SHR

ANOC. Muscle: In the SHR NA and PE produced concentration related contractions with pD_2 values of 5.17 ± 0.07 and 5.80 ± 0.01 respectively in the absence of cocaine. The NA value was not significantly different from that obtained in the NCR. This is consistent with the result obtained from some similar comparative studies on isolated blood vessel preparations (Webb & Vanhontte, 1979; Mulvany, Aalkjaer & Christensen, 1979; Harris, Swamy, Triggle & Waters, 1980) in which NA sensitivity showed no significant difference between S.H.R. and normotensive rats. However, the PE pD_2 value was different in the SHR. In the presence of cocaine the responses were enhanced and NA and PE had pD_2 values of 5.61 and 6.37 respectively, both values were significantly different

from the NCR values. These values gave dose ratios of 2.75 ± 0.09 for NA, significantly different from the 6.31 ± 0.08 in the NCR and 3.72 ± 0.12 for PE, not significantly different from 3.47 ± 0.09 obtained in the NCR. This seems to indicate some differences in the uptake mechanism between the SHR and NCR, to which NA is sensitive and PE is not. It may not be a general defect in the uptake mechanism since the two agonists are not equally affected but rather some change in uptake specificity which became "unfavourable" for NA. NA and PE structures are given below:



NA



PE

The lower affinity of PE than NA for uptake$_1$ in the NCR was adduced to the presence on the nitrogen atom of the methyl ($-CH_3$) group (Iversen, 1967). A change in the stereo-specificity of the uptake process that makes it more crucial that the OH load on the benzene ring be minimal will affect NA more than PE. It may well be that it is this difference in uptake mechanism that rendered the NA pD_2 in the SHR similar to that in the NCR. It might have been a lower value (than in the NCR) as with PE but the lesser effect of uptake$_1$ on the exogenously applied NA makes its concentration higher in the biophase, rendering the observed pD_2 higher than it should have been. Thus, the values obtained after cocaine has eliminated the influence of the

uptake mechanism may be more representative of what obtains.

The lower pD_2 of both agonists in the SHR suggests that they are less potent in the SHR than in the NCR in the anococcygeus muscle. This, in turn, might imply that the α-adrenoceptors in the SHR are less sensitive. However, it should be mentioned that in some of the above cited studies on isolated blood vessel preparations (Mulvany et al., 1979; Webb & Vanhontte, 1979) it was observed that NA was more potent in the SHR after the elimination of the uptake$_1$ mechanism (either by cocaine treatment or acute chemical denervation). They concluded that the uptake$_1$ mechanism in SHR is more efficient than in the normotensive and that it is this effect that masks the greater sensitivity of the SHR preparations to NA in untreated preparations. This observation (and consequently the inference) is contrary to that presented in this report. This may not be easy to explain. Nevertheless, it is not unlikely that there exist differences between vascular and non-vascular tissues in the pathophysiologic alterations in SHR tissues. For instance neuronal uptake of NA is said to be reduced in the heart of hypertensive rats (Salt & Iversen, 1973; Howe, Provis & Chalmers, 1979). Furthermore, the agonist potency ratios are not the same. PE is 2.04 times and NA 1.66 times less potent in the SHR than in the NCR. It may be that there is an increase in the proportion of post-junctional α_2-adrenoceptors in the muscle, lowering the chances of PE in evoking a response, since PE was said to produce its contractions mainly by α_1-adrenoceptor stimulation (Starke et al., 1975a, Brown et al, 1980).

Prazosin antagonism had similar characteristics in SHR and NCR.

L-Praz. was non-competitive against NA but competitive against PE. H-Praz. was competitive against NA in both SHR and NCR. The NA versus H-Praz. curves were also more parallel to the control curve than the L-Praz curves. The grand mean K_{diss} values $1.98 \pm 0.18 \times 10^{-9}$ and $2.52 \pm 0.35 \times 10^{-9}$ for the PE: L-Praz pair in the NCR and SHR respectively were not significantly different (Table 4). Grand mean K_{diss} value $3.77 \pm 0.15 \times 10^{-8}$ for the NA: H-Praz pair in the NCR was significantly different from $9.99 \pm 1.68 \times 10^{-8}$ obtained in the SHR (Table 4). Comparison between SHR and NCR of the L-Praz : NA pair interaction may not be valid because the antagonisms were non-competitive (mean K_{diss} varied with L-Praz concentrations). The grand mean K_{diss} obtained therefore represents, as it were, a grand mean of three means obtained from samples drawn from dissimilar "populations". All such cases where any one of the two rat strains shows a non-competitive antagonism with any one agonist: antagonist pair had their comparison disregarded. Phentolamine versus PE had K_{diss} values $1.00 \pm 0.16 \times 10^{-8}$ in the NCR and $1.17 \pm 0.29 \times 10^{-8}$ in the SHR which were not significantly different. Comparison between the SHR and NCR of the phentolamine: NA pair was disregarded (See Table 4). L-Praz. and PE are relatively selective for the α_1-adrenoceptor while H-Praz, phentolamine and NA are not. Thus, it seems that differences only occur in interactions involving α_2-adrenoceptors. This, again, suggests that there are differences between NCR and SHR in their α_2-adrenoceptor populations. Thus, L-YOH, which is relatively selective for the α_2-adrenoceptor gave grand K_{diss} values $9.52 \pm$

0.85×10^{-6} (NCR) and $2.01 \pm 0.25 \times 10^{-7}$ (SHR) against NA, which were significantly different. It should be noted however, that in the SHR, NA vs. L-YOH behaved like PE did in the NCR in that the Schild regression co-efficient was significantly different from one despite the fact that the mean K_{diss} did not vary with antagonist concentration. This may not be explained as done for PE above because NA, unlike PE, does not discriminate between α_1- and α_2- receptors. L-YOH was competitive (A - S slope was not different from 1 and the mean K_{diss} constant) against PE in the SHR unlike in the NCR where the A - S slope was different from unity. This inconsistency cannot be easily explained. Nevertheless, it may be that the α_2-adrenoceptors in the SHR are also somewhat qualitatively different. Alternatively it may be explained as due to the increased α_2-adrenoceptor proportion which increases the involvement of α_2-adrenoceptors in the PE contractions. The proportion is now high enough to render the PE vs. L-YOH interaction competitive (as in the case of NA vs. L-YOH in the NCR). Against PE the L-YOH K_{diss} were significantly different between the NCR and the SHR but the H-YOH K_{diss} were not (Table 4). H-YOH was suggested to act on both α_1- and α_2-adrenoceptors without preference for any, so it behaved in a similar way to H-Praz and phentolamine. The different K_{diss} values obtained (between NCR and SHR) with L-YOH might be disregarded because its action is more on the α_2-adrenoceptor subtype while the agonist PE produces its effect via α_1-receptors, that is (as mentioned above) the antagonism was not perfect. Comparison between NCR and SHR of the NA: H-YOH pair was disregarded.

Phentolamine, L-YOH and H-YOH behaved differently (from the NCR) against NA in the SHR in terms of the A - S slopes (Table 4). This is not expected even if the proportion of any or both receptor subtypes is altered. Thus, it may be that there is a qualitative change in the receptor population apart from the change in proportions. In a general sense, this discrepancy seems to occur less in interactions involving PE than in the case of NA. This may suggest that the alteration affects some part of the receptor molecule that is not so crucial to PE in evoking a stimulus, for example α_2-adrenoceptor-like component.

VAS: In the SHR both agonists produced concentration-related contractions of the RVD with pD_2 value of 5.46 ± 0.04 for NA and 5.32 ± 0.06 for PE in the absence of cocaine. In the presence of cocaine NA and PE produced respective pD_2 values of 5.86 ± 0.05 and 5.80 ± 0.05. Both NA values were significantly different from those in the NCR whereas the PE values were not. This suggests that there might be an increase in α-adrenoceptor population or sensitivity which influences NA but not PE. It was suggested earlier that the RVD post-junctional adrenoceptor population is predominantly of the α_1-subtype with the possibility of a very small α_2- proportion. It should therefore be possible, if in spite of the increase the α_2- is still relatively of considerably low proportion, that PE evokes its contraction largely by α_1-receptor stimulation. The α_2-subtype might form a part of the "spare" receptor population especially if PE evokes its maximum contraction utilising a very small α_1-receptor proportion. NA may be

more sensitive to an increase in α_2-adrenoceptor population since it is non-selective for either subtype. However, if it acts in this preparation as full an agonist as PE it would be expected to produce its own action also treating the "additional" α_2-adrenoceptors as spare. This does not seem to be the case. NA reflected the probable increase in α_2-subpopulation in the SHR in its higher pD_2. Would this suggest that NA produces its effects in the RVD by utilising almost all the receptors such that the increased α_2-subpopulation in the SHR was useful and provided for greater responses? The maximum responses were not compared between SHR and NCR. Responses were recorded as percentage of maximum. These results are not consistent with that of Corbett et al. (1980) who observed no significant difference between SHR and normotensive rat vas deferens in NA EC_{50} values. However, they observed a greater maximum tension in the SHR.

The cocaine potentiation factors 2.51 $\pm$ 0.06 and 3.02 $\pm$ 0.08 for NA and PE respectively were significantly different from those obtained in the NCR. This may indicate that there is a reduction in the efficiency of the uptake process in the SHR. However, the higher potentiation factor (than with NA) obtained with PE cannot be easily explained especially considering the fact that the PE potentiation factor was not different between NCR and SHR in the anococcygeus muscle.

In the antagonist studies, only in the case of phentolamine vs. PE was the comparison (between SHR and NCR) of the grand mean K_{diss} values regarded. In all other cases either one or both of the two rat strains had the agonist; antagonist interaction being non-competitive

and the comparison of all such cases were disregarded for the reasons given earlier on. The phentolamine: PE pair produced competitive antagonism in both NCR and SHR with respective grant mean K_{diss} values of $1.11 \pm 0.10 \times 10^{-8}$ and $1.33 \pm 0.17 \times 10^{-8}$, the difference between these was not statistically significant. This single information may not be strong enough a premise upon which to make an adduction (as was done in the case of the anococcygeus muscle). However, if a similar alteration in the receptor population is present in the two preparations (as the agonist studies seem to show), it can be said that the similar characteristics (between the NCR and SHR) of the phentolamine: PE pair show that there is no change in the α_1-mediated component of the contractions.

L-Praz. was not competitive against NA in the SHR whereas L-YOH was competitive as opposed to the characteristics of the antagonists in the NCR (Table 5). This may be as a result of a probable increase in α_2-adrenoceptor population. L-Praz. now behaved as it did against NA in the anococcygeus muscle (explained above) and the increased α_2-population brought more α_2-receptors into play in the NA contractions, rendering L-YOH antagonism now competitive. But also, L-Praz was non-competitive against PE even though they both act preferentially on the α_1-adrenoceptor subtype. Similarly phentolamine was non-competitive against NA despite both of them being non-selective and yet it was competitive against PE (Table 5). L-YOH also proved competitive against the predominantly α_1-mediated PE responses despite L-YOH being α_2-selective. These may suggest that there is some kind of qualitative alteration of the post-junctional

α-adrenoceptors in SHR population apart from the probable increase in α_2-proportion. In the case of the RVD it is difficult to say which of the agonists is more sensitive to the alteration. This is unlike the case in the anococcygeus. However, it should be noted that these observations are due to the interactions of the drugs with the "resultant" receptor (after the alteration). Thus, the slight differences (between ANOC and VAS) might be due to different "resultants". This may not be unexpected, especially if the alteration is qualitatively the same, since the α_1-adrenoceptor in the vas is not exactly the same as in the anococcygeus muscle (suggested earlier on in this report and also by MacDonald and McGrath, 1980). The action of H-YOH in the vas deferens might involve some other mechanisms that are yet to be elucidated. For instance yohimbine has been shown to block dopamine (Tayo, 1979b) and 5-HT_2 (Kauman, 1983) receptors.

The present results seem to suggest that physio-pathological alterations in the SHR may not be identical in various tissues or organs. Thus, it may not be possible to extrapolate the information obtained from any one preparation to others. However, if - as suggested for the preparations in this study - an apparent increase in the post-junctional α_2-adrenoceptors occurs in SHR blood vessels, it may possibly be responsible for the conflicting reports obtained in <u>in-vitro</u> studies of isolated vascular preparations. <u>In vitro</u> studies of the post-junctional α_2-adrenoceptors have not yielded very consistent result although <u>in vivo</u> studies seem more consistent. Thus, McGrath (1982) opined that the precise conditions are not yet known under which post-

junctional α_2-adrenoceptors can be well demonstrated *in vitro*. He presented evidence showing that changes in the blood acid/base balance (due to changes in gas tensions) resulted in changes in vascular post-junctional α_2-adrenoceptor characteristics. Also, Drew (1982), citing the studies of other workers (Starke et al, 1974, 1975; Ruffolo et al, 1979) showed that some agonists like tramazoline and clonidine which are selective α_2-agonists act as full agonists *in vivo* but as partial agonists *in vitro*. This shows how crucial the composition of the physiological environment is to α_2-adrenoceptor characteristics. It may account for the difference between the observations of different groups of workers who are using similar preparations but with slight variations in experimental conditions.

APPENDIX

APPENDIX I

The theoretical background of pA_2 and pD_2

A. The interaction between a drug, D, and its receptor, R, can be expressed as

Drug + Receptor $\rightleftharpoons$ Drug-Receptor Complex.

D + R $\rightleftharpoons$ D - R ..... (eq. 1.1)

It is believed that the observed response occurs as a result of the formation of the D-R complex. The magnitude of the response depends upon the degree of interaction between the drug molecules and the receptors.

D + R $\rightleftharpoons$ D - R $\Longrightarrow$ response.

By the Law of Mass Action, this (D - R complex) itself is a function of concentration of the drug and the receptors available for interaction. Thus, if a concentration A of the drug combines and forms the complex with a proportion, r, of the total number of receptors present (which cannot be easily determined), the proportion of receptors remaining unoccupied during the action of the drug will be 1 - r ("total proportion" being unity).

These concentrations can be written out in the form of an equation

A + 1 - r $\rightleftharpoons$ r ..... (eq. 1.2)

The concentration A of the drug is assumed to be in excess so that the difference in concentration after forming the complex is negligible. K_1 and K_2 are the rate constants for the forward and backward reactions respectively, that is, the formation and dissociation of the complex.

rate of association = $K_1 A (1 - r)$.

rate of dissociation = $K_2 r$.

At equilibrium, the two rates are equal.

Thus, $\frac{K_1}{K_2} = K_a = \frac{r}{A(1-r)}$ ..... (eq. 1.3)

where K_a is the Affinity Constant. The affinity constant is a measure of the readiness with which the drug forms complexes with the receptors. Thus, differences in affinity constant with the same agonist (under the same experimental conditions) on different preparations will indicate the presence of a different type of receptor or the same receptor with different binding characteristics. K_a will therefore be useful in comparative studies on receptors.

A. J. Clark (1937) suggested that the magnitude of the response is proportional to the fraction of receptors occupied and that the maximum response is obtained when there is a hundred per cent occupation of receptors. A 50% occupation will produce half the maximum response. From eq. 1.3

r will be $\frac{1}{2}$ at 50% receptor occupation. If the molar concentration of the agonist producing half of the maximum response (i.e. 50% receptor occupation) is A_{50}

$$K_a \; A_{50} \; (1 - r) = r$$

A_{50} is commonly referred to as the EC_{50}

$$K_a \; (EC_{50}) \; (\tfrac{1}{2}) = \tfrac{1}{2}$$

$$K_a \; (EC_{50}) = 1$$

$$\frac{1}{EC_{50}} = K_a$$

$$-\log EC_{50} = \log K_a$$

$-\log EC_{50}$ is what was described in Chapter 2 as the pD_2. It is thus an estimate of K_a which can be used in receptor differentiation.

The original assumption by Clarke has since been modified (Ariens, 1954; Stephenson, 1956; Nickerson, 1956; Furchgott, 1966) and it is now known that the maximum response may be obtained without a necessary 100% receptor occupation. Nevertheless, a requisite fraction of the total receptor population needs be occupied to obtain a maximum response. The "excess" fraction of receptors forms a "receptor reserve" or "spare receptors" (Nickerson, 1956). Thus, in some tissues, a significant proportion of the receptor pool could be irreversibly inactivated, and yet a maximum response to a strong agonist could still be obtained if higher concentrations of the agonist were administered. The maximum would be depressed only when the total number of receptors was reduced (by inactivation) below the minimum proportion required (i.e. when the receptor number becomes the limiting factor in maximum attainable response).

Thus, the EC_{50} (molar concentration of drug required for half maximal response) of an agonist may not necessarily be the amount of drug required to occupy 50% of receptor population (which, according to the Law of Mass Action is the dissociation constant). Therefore EC_{50} (and pD_2, its negative logarithm) may not be accurate estimates of the dissociation constant. Furchgott (1966) developed a more accurate method of estimating the dissociation constant of an agonist. This disharmony between EC_{50} and the dissociation constants of full agonists

was discussed in detail by Ruffolo (1982) in a recent review.

Nevertheless, the EC_{50} (or pD_2) is still useful especially in comparative studies like the present one. The EC_{50} of an agonist on a particular preparation is a constant characteristic of the agonist: tissue interaction, likewise the proportion of receptors occupied (at EC_{50}) - never mind the actual value. Thus, if from two different strains of animal the same preparation yielded different EC_{50} values for a certain agonist, it suggests the existence of differences between the two strains in the reactivity of the preparation to the agonist. A change in EC_{50} indicates a corresponding change in the proportion of receptors occupied, which in turn indicates a change in the total receptor number. This is evidenced by the fact that EC_{50} changed as the total number of "active" receptors was reduced by an irreversible antagonist even though there were still more than enough receptors to reproduce maximal response (Ariens, Van Rossum & Koopman, 1960; Furchgott, 1966). It is however admitted that it is largely assumed that the processes between receptor activation and the observed response are the same in the two subjects being compared and that any difference in reactivity reflects differences at the receptor level. An alternative theory of drug action was proposed by Paton (1961). The Rate Theory, as it is known, believes that the response is a function of the rate of association of drug molecules and receptors i.e. Stimulation is seen as a succession of quantal events each corresponding to the formation of a single drug-receptor complex. In other words the excitation is not due to occupation but due to the "process of occupation".

The rate of interaction of drug and receptors however still depends on their individual concentrations and the Law of Mass Action is therefore still applicable. At equilibrium conditions there is little or no difference between the two theories. The rates of association and dissociation become equal, the number or proportion of receptors taking part in the interaction at any time during the equilibrium being constant. One may then talk of the proportion of receptors being "utilized" instead of receptors occupied. The response is therefore still dependent on K_a as is shown mathematically below.

$$A + 1 - r \underset{k_2}{\overset{k_1}{\rightleftharpoons}} r$$

rate of combination of drug with receptor i.e. rate of forward reaction

$$= Z = K_1 (1 - r) A \quad \ldots\ldots\ldots\ldots (1.4).$$

By the rate theory the response depends on Z.

at equilibrium $K_2 r = Z$ ..... 1.5.

From eq. 1.3

$$K_1 A (1 - r) = K_2 r \quad \ldots\ldots\ldots\ldots 1.6$$

After mathematical re-arrangement

$$r = \frac{A}{K_2 / K_1 + A} \quad \ldots\ldots\ldots\ldots 1.7$$

From eq. 1.5

$$r = \frac{Z}{K_2}$$

$$\frac{Z}{K_2} = \frac{A}{K_2 / K_1 + A}$$

$$Z = \frac{AK_2}{K_2 / K_1 + A} \text{ or } \frac{AK_2}{1/K_a + A} \quad \ldots\ldots\ldots 1.8$$

Thus Z, which determines the magnitude of the response is directly proportional to K_a. Accordingly, all responses in these studies were equilibrium values.

The equation $K_a = \frac{r}{A(1-r)}$ ..... (eq. 1.3)

is general and it assumes that one molecule (or a mole) of the agonist combines with a molecule (or a mole) of the receptor. When 2 molecules of the drug combines with one of the receptor the rate of association becomes $K_1A^2(1-r)$.

At equilibrium $K_a = \frac{r}{A^2(1-r)}$

When 'n' number of drug molecules combine with a receptor molecule

$$K_a = \frac{r}{A^n(1-r)} \quad \text{..... (eq. 1.3a)}$$

B. The affinity of a competitive antagonist for a receptor can also be used in classifying receptors or for assessing differences in characteristics of the same receptor when present in different effectors or in the same effector in different physiological conditions. These uses are based on two principles:

(i) that a competitive antagonist should produce a constant value of affinity constant when tested against various agonists if all the agonists and the antagonist are acting on the same receptor.

(ii) that a competitive antagonist should produce a constant value of affinity constant when used against a single agonist on different effectors, if the receptors on the different effectors are the same.

The interaction between an agonist D and a competitive antagonist G, each interacting with receptor R, follow the model

$$D + R \rightleftharpoons D.R \Rightarrow \text{response}$$

$$G + R \rightleftharpoons G.R \Rightarrow \text{no response.}$$

The affinity constant of the agonist reaction is K_a and that of the antagonist K_b. K_b cannot be determined as done above for an agonist because it does not produce a response of its own. Its effect is shown only in its ability to reduce the effect of an agonist by competing with the agonist for the receptors. Thus, if curve (a) of Fig. 1 is the log. concentration-response curve of an agonist, curve $(a + b_1)$ typically represents

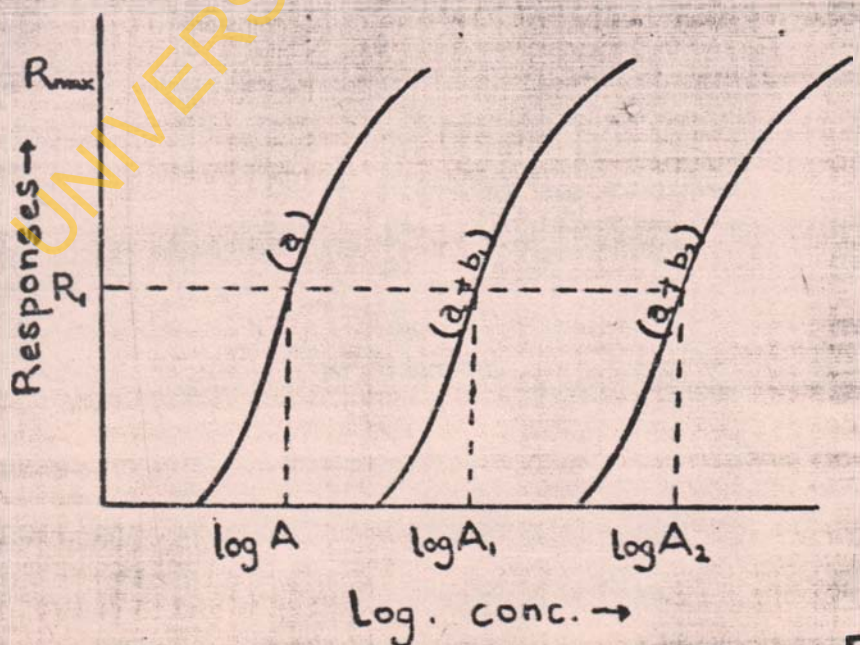
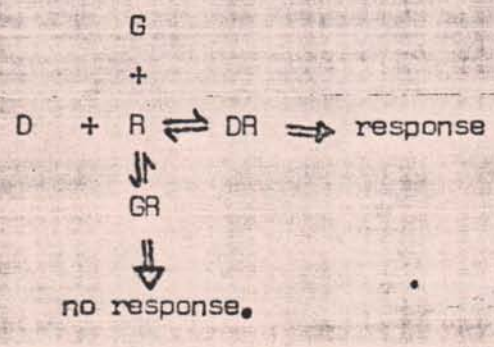


Fig. 1

the agonist dose-response curve in the presence of some constant concentration of the antagonist. Curve $(a + b_2)$ represents the curve in the presence of a higher concentration of the antagonist. The antagonist shifts the curve further to the right, the higher its concentration. The degree of the shift (an indication of the concentration and therefore the effect of the antagonist) is shown by the "dose ratio" A_1/A or A_2/A. The dose ratio is the ratio of the new concentration (A_1 or A_2) of the agonist required to achieve a certain level of response R_1 to the initial dose of the agonist (A) that achieved R_1 in the absence of the antagonist. The dose ratio is determined in the mid-range (linear portion of the curve) of the responses thereby avoiding errors near the physiologic extremes of response. The affinity constant of an antagonist can therefore be determined from a quantity like the dose ratio which indicates the effect (a type of "response") of each concentration of an antagonist.

The interaction of an agonist and its antagonist with a receptor when they are concurrently present in the system can be shown thus



In concentrations

$$\begin{array}{c} B \\ + \\ A + (1 - r^1 - q) \rightleftharpoons r^1 \\ \Downarrow \\ q \end{array}$$

where A is the agonist concentration, B is the antagonist concentration, r^1 is the proportion of the receptors bound to the agonist, q, the proportion bound to the antagonist and $(1 - r^1 - q)$ is the remaining (unbound) receptor proportion at equilibrium. All concentrations are molar values. If the affinity constant of the agonist is K_a and the antagonist K_b

$$K_a = \frac{r^1}{A(1 - r^1 - q)} \qquad \text{.....} \quad 1.9$$

$$K_b = \frac{q}{B(1 - r^1 - q)} \qquad \text{.....} \quad 1.10$$

eliminating $1 - r^1 - q$

$$\frac{r^1}{K_a A} = \frac{q}{K_b B} \qquad \text{.....} \quad 1.11$$

From eq. 1.10

$$K_b B(1 - r^1) = q(1 + K_b B)$$

$$q = \frac{K_b B(1 - r^1)}{1 + K_b B} \qquad \text{.....} \quad 1.12$$

Substituting into eq. 1.11

$$\frac{r^1}{K_a A} = \frac{BK_b\ (1 - r)}{1 + BK_b} \times \frac{1}{BK_b} \qquad \text{.....} \quad 1.13$$

After re-arrangement

$$\frac{1 + K_b B}{K_a A} + 1 = \frac{1}{r^1} \qquad \text{.....} \quad 1.14$$

which is the reciprocal of the new proportion of receptors occupied by the agonist in the presence of the antagonist. If the agonist concentration is increased to a certain value A^1 so as to obtain the same response as concentration A in the absence of the antagonist, a new value of r^1 (r^{11}) achieved which is equal to r.

Thus,

$$\frac{1 + K_b B}{K_a A^1} + 1 = \frac{1}{r^{11}} = \frac{1}{r}$$

From eq. 1.7

$$\frac{1/K_a + A}{A} = 1/r$$

Therefore

$$\frac{1 + K_b B}{K_a A^1} + 1 = \frac{1/K_a + A}{A}$$

re-arranging

$$\frac{A^1}{A} - 1 = K_b B \qquad \text{.....} \quad 1.15$$

The ratio A^1/A was described as the dose ratio. Schild (1947) defined and denoted the ratio by "X" and defined a term pA_x as ;the negative logrithm of a value of B (molar concentration of antagonist) which produces a dose ratio of X.

$$pA_x = -\log Bx$$

Taking common logarithms of eq. 1.15

$$\log (x - 1) = \log K_b + \log B \quad \text{.....} \quad 1.16$$

$$= \log K_b - pA_x \quad \text{.....} \quad 1.17$$

when $X = 2$, $\log (x - 1)$ becomes zero

$$pA_x = pA_2 = \log K_b \quad \text{.....} \quad 1.18$$

Thus, the pA_2 is related to K_b, the affinity constant of the antagonist. The pA_2 value can therefore be used to assess the potency of different antagonists against the same agonist on the same receptor, or to differentiate receptors when a single antagonist is used against a single agonist on different receptors. An antagonist producing different pA_2 values against a single agonist on different effectors will suggest the presence of two different receptors or differences in the sensitivities of similar receptors.

pA_2 Determination

The equation

$$\log (x - 1) = \log K_b + \log B \quad \text{.....} \quad 1.16$$

is similar to the general equation of a straight line

$$y = C + mx$$

where y represents the ordinate values, x, the abscissa values, m, the slope and C the intercept on the ordinate. A plot of log $(x - 1)$ against log B will be a straight line with an intercept log K_b on the ordinate and a slope of unity. When X is 2, log $(x - 1)$ (the y co-ordinate) is zero and the corresponding value of log B is the intercept on the abscissa. From eq. 1.17, the negative value of the intercept (log B) on the abscissa gives the pA_2. Also from eq. 1.18 $pA_2 = \log K_b$. This means the intercept on the ordinate and the negative value of the intercept on the abscissa should be the same if the antagonism is competitive. Practically, this is found to be true only when the slope is exactly one. Thus, the graphical estimation of $K_{diss} (= 1/K_b)$ is accurate only when the A - S slope = 1.

From eq. 1.16

$$\log (x - 1) = \log K_b + \log B$$

when $\log (x - 1) =$ zero

$$-\log K_b = \log B \qquad \text{.....} \quad 1.19$$

From eq. 1.18 we know that $pA_2 = \log K_b$. Thus, a plot of pA_2 against log B will be a straight line with a zero slope if (as with the ideal) the pA_2 (and therefore the K_{diss}) does not vary with antagonist concentration B. This is referred to in this report as the Mackay plot.

APPENDIX II

STATISTICAL ANALYSIS

1. Measure of location

The measure of location used in this study is the mean.

$$\text{Mean} = \frac{\text{Sum of observations}}{\text{Number of observations}} \quad \text{or}$$

$$\bar{X} = \frac{\Sigma X}{n}$$

where n denotes the number of observations, $\bar{X}$, the mean, X, each observation and ΣX, the sum of n observations.

2. Measure of variation

The standard error (S.E) was employed as the measure of variation and it is given by

$$S.E = \frac{S.D}{\sqrt{n}}$$

where S.D is the square root of the mean squared deviation from the mean.

i.e.

$$S.D = \sqrt{\frac{(x - \bar{x})^2}{n - 1}}$$

3. Regression Analysis

The regression line was fitted by calculation. The equation of

this line is defined by

$$y = b_o x + (\bar{y} - b_o \bar{x})$$

where $\bar{x}$ is the mean $\frac{\Sigma x}{n}$ of the x - (abscissa) coordinates and $\bar{y}$ is the mean $\frac{\Sigma y}{n}$ of the y -(ordinate) co-ordinates.

b is the slope of the line (otherwise known as the regression co-efficient) and is given by

$$b = \frac{\Sigma (x - \bar{x})y}{\Sigma (x - \bar{x})^2} = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

$(\bar{y} - b_o \bar{x})$ is the intercept on the ordinate (d). Standard error of estimate = S

$$S = \sqrt{\frac{(y - \bar{y})^2}{n - 2}}$$

where n - 2 = degrees of freedom.

$$\Sigma (y - y)^2 = \left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right] - b_o \left(\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}\right)$$

$$\therefore S = \sqrt{\frac{\left[\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right] - b_o \left(\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}\right)}{n - 2}}.$$

Standard error of regression co-efficient = S_b

$$S_b = \sqrt{\frac{S^2}{\sum x^2 - \frac{(\sum x)^2}{n}}}$$

Standard error of x - intercept (for the determination of standard error of pA_2) =

$$S_x = \left|\frac{S}{b}\right| \sqrt{\frac{1}{n} + \frac{\bar{y}/d}{\sum x^2 - \frac{(\sum x)^2}{n}}}$$

4. Test for significance in differences between two(pD_2, K_{diss}, slope or mean)

The t test was used.

$$t = \frac{\left|\bar{M}_A - \bar{M}_B\right|}{S_{A-B}}$$

$\left|\bar{M}_A - \bar{M}_B\right|$ is the absolute value of the difference between the two values and S_{A-B} the standard error of the difference between the two values.

$$S_{A-B} = \sqrt{(\text{S.E. of } M_A)^2 + (\text{S.E. of } M_B)^2}$$

$$= \sqrt{(S_A)^2 + (S_B)^2}$$

$$t = \frac{|\bar{M}_A - \bar{M}_B|}{\sqrt{S_A^2 + S_B^2}}$$

The degree of freedom = $(n_A + n_B) - 2$ where n_A and n_B denote the number of observations in samples A and B respectively. When one M value (say M_B) is assumed (i.e. having neither n nor S.E), S_{A-B} will be S_A and the degree of freedom $n_A - 2$.

5. Analysis of Variance (ANOVA)

This was used to test for significance in the differences between mean K_{diss} values in this study. The statistical formulae used in this analysis are presented below, arranged in the usual tabular form in which it is done.

For equal sample sizes

Source	d.f	Sum of Squares	Mean Square	F
Among columns	$k-1$	$SSC = \sum \frac{Ti.^2}{r} - \frac{T..^2}{rk}$	$MSC = \frac{SSC}{k-1}$	$\frac{MSC}{MSE}$
Error (within columns)	$k(r-1)$	$SSE = SST - SSC$	$MSE = \frac{SSE}{k(r-1)}$	
Total	$rk-1$	$SST = \sum\sum x_{ij}^2 = \frac{T..^2}{rk}$		

For unequal sample sizes

Source	d.f.	Sum of Squares	Mean Square	F
Among columns	$k-1$	$SSC = \sum \frac{Ti.^2}{n_i} - \frac{T..^2}{n}$	$MSC = \frac{SSC}{k-1}$	$\frac{MSC}{MSE}$
Error (Within)	$n-k$	$SSE = SST - SSC$	$MSE = \frac{SSE}{n-k}$	
Total	$n-1$	$SST = \sum\sum x_{ij}^2 - \frac{T..^2}{n}$		

Both among and within the columns, this statistical test measures the errors. Thus SSC means sum of the squares of the errors among the columns and SSE means the sum of the squares of the errors within each column. But in practice the word "error" is dropped off. Thus,

SSC = Column sum of squares

SSE = Error (within each column) sum of squares

SST = Total sum of squares

MSC = Column Mean square

MSE = Error mean square

k = number of columns

r = number of observations in each column

n_i = number of observation in the i^{th} column

n = total number of observations

$T_{i.}$ = Sum of the values in the i^{th} column

$T_{..}$ = Sum of the $T_{i.}$'s (sum of all the values)

x_{ij} = the j^{th} value in the i^{th} column.

d.f = degrees of freedom.

Typical example

K_{diss} values against PE on ANOC, at three L - YOH concentrations.

1st	2nd	3rd
3.16×10^{-8}	3.41×10^{-8}	6.44×10^{-8}
2.66	2.95	4.98
5.53	8.21	5.11
3.53	5.32	14.60
3.81		

H_o = mean K_{diss} not different, H_1 = mean K_{diss} different.

Let α-level be 0.05.

$k = 3$, $n = 13$.

$F\ (k-1, n-k) = F(0.05)(2, 10) = 4.10$ (from F-tables)

$T_{1\bullet} = 18.89$

$T_{2\bullet} = 19.89$

$T_{3\bullet} = 31.13$

$T_{\bullet\bullet} = 69.71$

$\frac{T_{\bullet\bullet}^2}{n} = 373.81$

$\sum\sum x^2_{ij} = 496.20$

$\sum \frac{Ti^2}{n} = 411.04$

SST = 496.20 - 373.81 = 122.39

SSC = 411.04 - 373.81 = 37.23

SSE = 122.39 - 37.23 = 85.16

Table

Source	d.f	Sum of Sq.	Mean Sq.	F
Among	2	37.23	$\frac{37.23}{2}$ →	18.62
Within	10	85.16	$\frac{85.16}{10}$ →	8.52 ⇓ 2.19
Total	12	122.39		

$2.19 < 4.10$, reject H_1

Mean K_{diss} values, not different.

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