A functional study of alpha-adrenoceptors in the rabbit ovarian vascular bed

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A Functional Study Of α-Adrenoceptors In The Rabbit Ovarian Vascular Bed

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Abstract

Blood flow to the ovary is an essential part of the ovulatory process. An increase in blood flow to the ovary precedes the process of ovulation. Therefore, any factor(s) that interfere with blood flow through the ovarian artery, would interfere with the ovulatory process and also development of the fertilized ovum. The objective of this study was to (1) identify α-adrenergic receptors in the rabbit ovarian vascular bed, (2) investigate if such adrenoceptor subtypes are linked to different signal transduction mechanisms and (3) study the influence of hormonal changes (induction of ovulation, oestrogen treatment and pregnancy) on α_1-adrenoceptor mediated responses.

The studies were conducted using an in vitro perfusion system, where the ovarian vascular bed (en bloc) was perfused with Krebs' solution delivered at a constant flow of 6 ml/min using a peristaltic pump. Changes in perfusion pressure which reflected peripheral resistance were measured.

The results showed that noradrenaline (NA) induced reproducible dose-dependent vasoconstrictor responses which were slightly but not significantly reduced by removal of the endothelium. The vasoconstrictor effects were however enhanced by L-NOARG but not aminoguanidine indicating modulation of NA responses by endothelial NO. Phentolamine (10^{-6} M) displaced NA dose-response curve to the right without suppressing the Emax. The pK_B value was calculated to be 7.7±0.08. The rank order of agonist potency was NA ≂ oxymetazoline > SDZ NVI 085 > phenylephrine > methoxamine. Prazosin (10^{-8}M-10^{-5}M) displaced agonist dose-response curves to the right. The pA_2/pK_B values ranged between 7.27-7.7 against NA, phenylephrine, methoxamine and SDZ NVI 085 and were not significantly different from each other. Prazosin was, however, significantly less potent against oxymetazoline (pA_2=6.38). Yohimbine was not effective against NA or phenylephrine. WB-4101 (10^{-8}M-10^{-5}M) displaced agonist dose-response curves to the right. The pA_2/pK_B values ranged between 7.1-7.9 against NA, phenylephrine, methoxamine and SDZ NVI 085. WB-4101 was significantly less potent against oxymetazoline (pK_B=6.9). SZL-49 ($5x10^{-6}$M) but not CEC ($3x10^{-5}$M-10^{-4}M) significantly reduced vasoconstrictor responses to all the agonists. Niguldipine

(10^{-9}M) significantly attenuated NA-induced vasoconstriction. These results would suggest that α_{1A}-subtype receptor mediated postjunctional effects of the adrenergic agonists.

Electrical stimulation of the perfused preparation produced frequency-dependent vasoconstrictor effects which were abolished by 6-OHDA indicating the involvement of adrenergic nerves. The responses were also concentration-dependently antagonized by prazosin and WB-4101, while yohimbine reduced the response to electrical stimulation by 20% at 10^{-5}M. The vasoconstrictor effect to electrical stimulation was also inhibited by SZL-49 but not CEC. These results would suggest that the vasoconstrictor responses of the ovarian vascular bed to electrical stimulation are mediated via the α_{1A}-adrenoceptor subtype.

The relative roles of extracellular calcium influx and intracellular calcium release in the coupling of α_1-adrenoceptor activation, were also investigated. Vasoconstrictor responses to NA were significnatly reduced in Ca^{2+}-free KH-solution (with or without EGTA) and after treatment with Ca^{2+} channel antagonists, verapamil and nifedipine. The difference in efficacy between NA and SDZ NVI 085 did not show that verapamil and nifedipine being more potent against SDZ NVI 085. The vasoconstrictor response to KCl was abolished by the Ca^{2+} channel inhibitors. Such results indicate that the major source of Ca^{2+} for mediating NA-induced vasoconstriction was from the extracellular calcium pool through the activation of ROCs with a probably minor role for the intracellular Ca^{2+} stores, which was found not to be the sarcoplasmic reticulum, since ryanodine did not produce any inhibitory effect on the vasoconstrictor responses to NA. In addition, TK but not PKC was found to be the intracellular second messenger which participates in the signal transduction mechanisms in this preparation.

The influence of the hormonal changes on the vascular reactivity to α_1-adrenergic agonists was also examined. The results showed an increased sensitivity of the ovarian vascular bed to NA and oxymetazoline but not to histamine in rabbits treated with 17ß-oestradiol propionate and also during ovulation and pregnancy. Except for oxymetazoline, there was no change in the maximum response. In pregnant rabbits, prazosin (10^{-7} M) but not yohimbine (10^{-5} M) displaced NA dose-response curve to the right. The pK_B value for prazosin against NA was 9.6+0.3. SZL-49 ($5x10^{-6}$ M)

but not CEC ($3x10^{-5}$ M) significantly reduced vasoconstrictor response to NA. These results are comparable with the corresponding results in control rabbits, indicating the predominance of α_{1A}-adrenoceptor subtype. In addition, the vasodilator responses to carbachol and SNP were maintained (though not enhanced), and L-NOARG was more effective in attenuating the vasodilator response to carbachol compared to contol situation, an indication of the enhanced role for EDRF (NO) in rabbits treated with 17ß-oestradiol propionate as well as during ovulation and pregnancy.

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Abbreviations

Abbreviations used in this thesis are those recomended by the "instructions to authors" for the Brithish Journal of Pharmacology 1995 (Br. J. Pharmac.114, 245-251), except for the following:

7-NI	7-nitro indazole
Ca^{2+}-CaM	calcium-calmodulin complex
CAM	calmodulin
cNOS	constitutive nitric oxide synthase
DAG	diacylglycerol
DHP	dihydropyridine
EDCF	endothelium-derived contracting factor
EDHF	endothelium-derived hyperpolarizing factor
EGF	epidermal growth factor
EGF-URO	epidermal growth factor-urogastrone
FAD	flavin-adenine dinucleotide
FMN	flavin mononucleotide
FSH	follicular stimulating hormone
iNOS	inducible nitric oxide synthase
$Ins(1,4,5)P_3$	inositol 1,4,5-triphosphate
$Ins1,4P_2$	inositol 1,4-biphosphate
L-NAME	N^G-nitro-L-arginine methyl ester
L-NIO	N-iminoethyl-L-ornithine
L-NMA	N^G-monomethyl-L-arginine
L-NMMA	N^G-monomethyl-L-arginine acetate
LH	luteinizing hormone
MAPK	mitogen activated protein kinase
MLC	myosin light chain
MLC_{20}	20 kDa myosin light chain
MLCK	myosin light chain kinase
NADPH	reduced nicotine amide adenosine dinucleotide phosphate

NO	nitric oxide
NOS	nitric oxide synthase
PA	phosphatidic acid
PDGF	platelet-derived growth factor
PK	protein kinase
PKC	protein kinase C
PLC	phospholipase C
PMA	phorbol 12-myristate 13-acetate
PtdIns	phosphatidylinositol
ROC	receptor-operated calcium channel
SOD	superoxide dismutase
TK	tyrosine kinase
TP	tyrphostins
URO	urogastrone
VOC	voltage-operated calcium channel

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CHAPTER ONE
INTRODUCTION

1.1 Ovarian vascular bed

The ovary is one of the most highly vascularized organs of the body. Both ovaries are supported by a mesentery called the mesovarium. An additional mesentery, the ovarian ligament attaches each ovary to the lateral edge of the uterus (Fig.1) (Wingerd, 1985). Each ovary is divisible into a central medulla which comes to the surface along the thin edge of the ovary; and a peripheral cortex, which invests all other portions of the medulla. In the ovary of the young adult rabbit there is very little if any interstitial tissue, and the mature follicles, with their bases bordering the medulla, occupy the entire thickness of the enveloping cortical tissue. The medulla is made up of loose connective (stromal) and interstitial tissue which support the major arteries, veins and lymphatics (Burr & Davies, 1951).

1.1.1 Blood supply to the ovary

Blood supply to the ovaries is through the ovarian arteries from the anterolateral aspects of the aorta. Each ovarian artery arises from the dorsal aorta about 3cm posterior the renal artery. About midway between the dorsal aorta and the ovary each ovarian artery gives rise to a branch which supplies the oviduct and the uterus (Burr & Davies, 1951). Before entering the ovary, the artery is often thrown into one or more loose coils. Intra-ovarian branches of the ovarian artery are spiralled. The spiral arteries control blood flow so that the cortex receives a uniform supply of blood under reduced pressure. It has also been hypothesized that this spiralling may facilitate extension of the artery to accommodate the growth of the ovary during the follicular phase of the cycle and during formation of the corpus luteum following ovulation (Reynolds, 1950).

1.1.2 Innervation of the ovary

Sympathetic nerves supplying the reproductive tract originate not only from pre- and paravertebral ganglia, but also from adrenergic ganglionic formations located in the immediate vicinity of the effector organs (short adrenergic nerves). The short adrenergic neurons have been found to differ functionally from ordinary long neurons (Euler & Lishajko, 1966), especially in relation to their sensitivity to reserpine and 6-OHDA. It is quite possible that this specific pattern of adrenergic innervation has a special physiologic significance in the female reproductive tract (Fig.2).

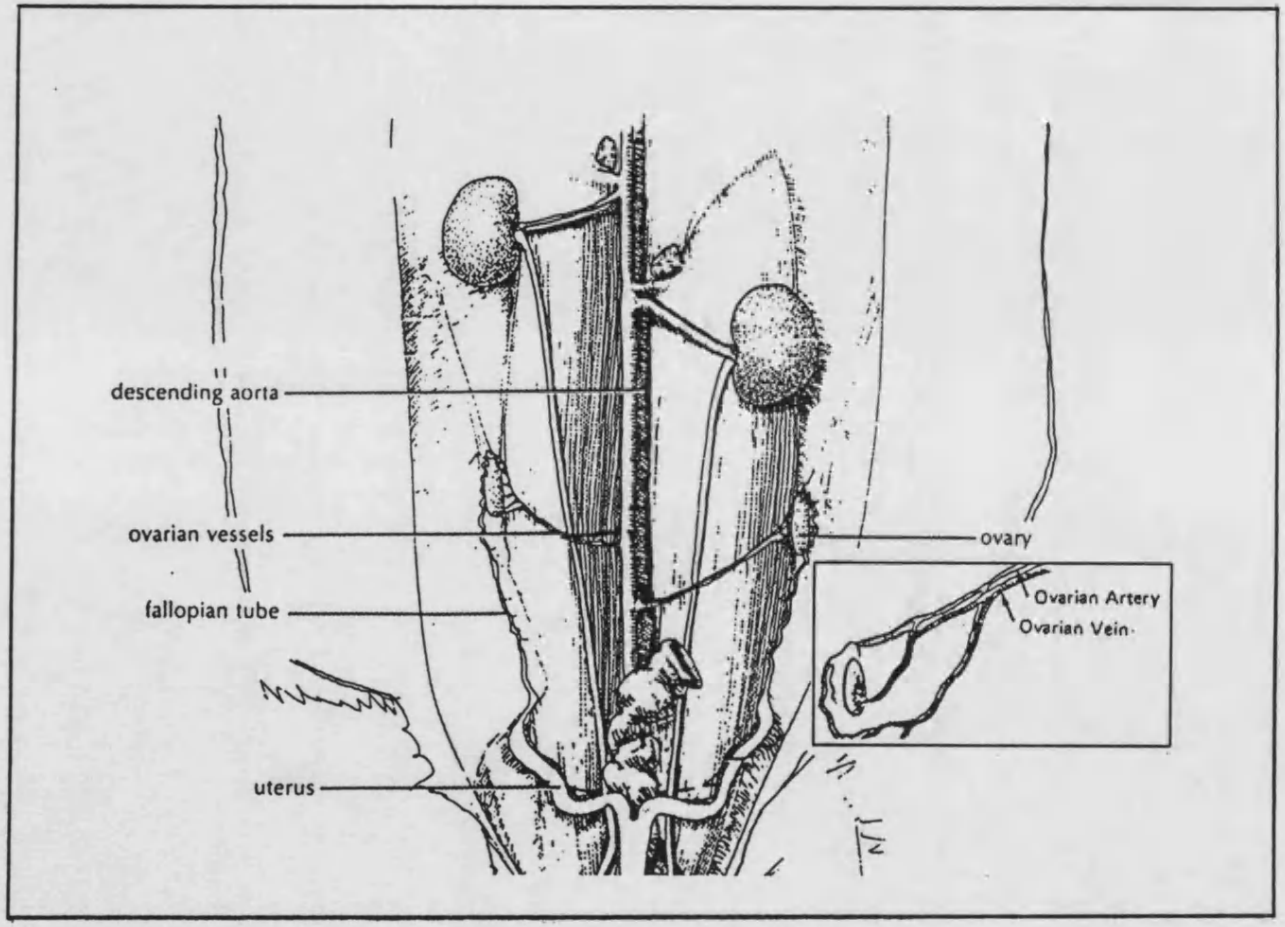


Fig.1 The reproductive system of the female rabbit. (modified from Wingerd, 1985)

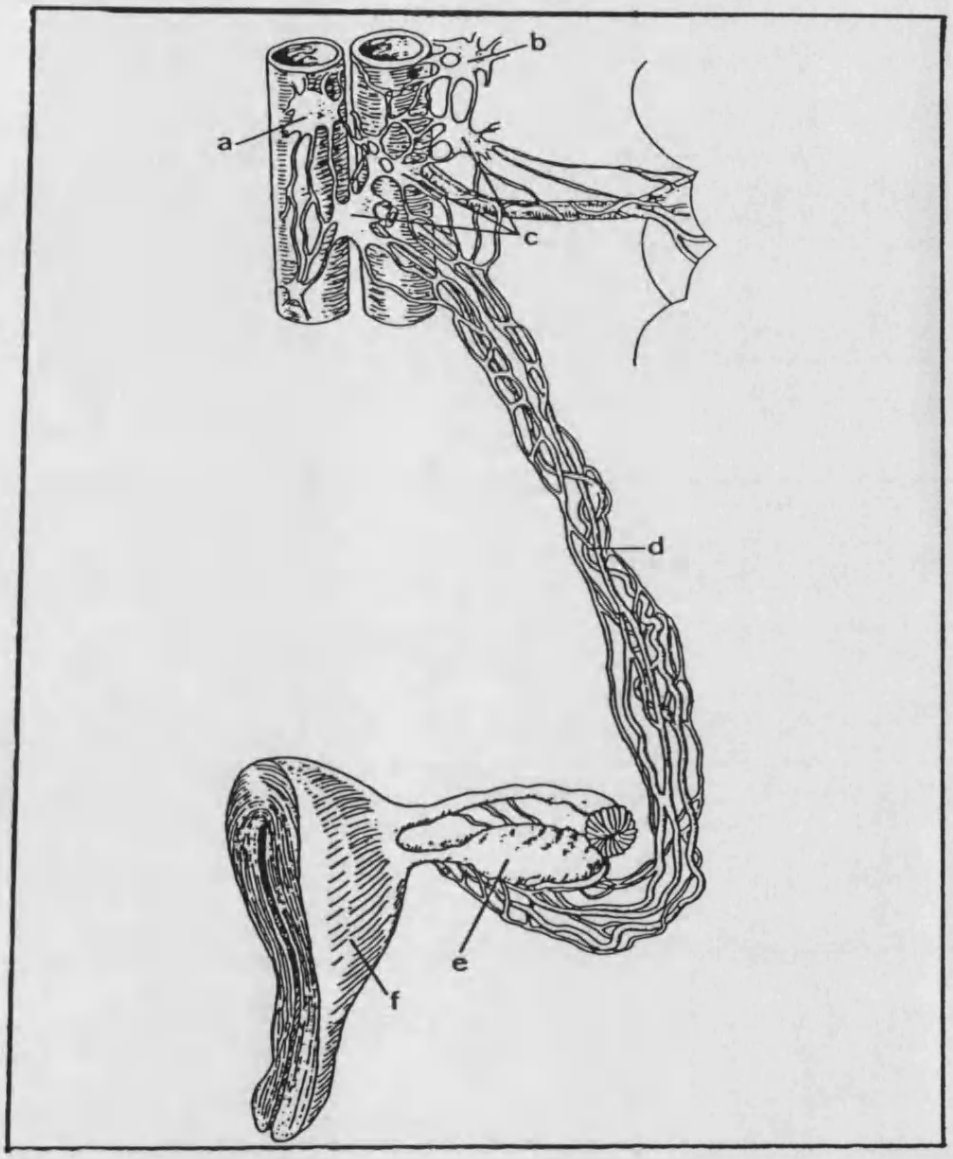


Fig. 2 Anatomy and distribution of the major sympathetic nerve supply to the human ovary. The superior mesenteric (a) and celiac (b) ganglia connect with the aortico-renal plexus (c), from which the ovarian plexus (d) arises to run along the ovarian vessels to the ovary (e). The corresponding half of the uterus is indicated (f). (cited from Owman et al., 1991)

The density of adrenergic innervation agrees well with the amount of NA contained in the ovaries (Rosengen & Sjoberg, 1967). Noradrenaline is present in the ovaries of many mammalian species. There is considerable species variation in ovarian noradrenaline concentration, ranging from 0.2 μg/g in the rat to 4.2 μg/g in the cat (Mohsin & Pennefather, 1979). Ovarian noradrenaline is not significantly altered during pregnancy, after treatment with 17-ß-oestradiol and or progesterone, or with HCG, in contrast to the marked alterations that can be induced in short adrenergic neurons supplying the uterus, vagina and oviduct under these conditions (Owman et al., 1979).

1.1.3 Adrenergic regulation of ovarian function

One of the main functions of the ovary is the release of matured ova which then pass along the fallopian tube, where they are fertilized, to the uterus where implantation takes place. Anatomical and physiological studies have suggested that ovarian smooth muscle nerves may function to facilitate the process of ovulation (Owman et al., 1979). Pharmacological experiments have indicated the presence of α- and ß- adrenergic receptors in the follicular wall which mediate contractile and dilator responses (Walles et al., 1975), suggesting that the neuromuscular complex in the theca externa may be involved in the ovulatory process.

A role of ovarian innervation to modulate steroidogenesis was suggested on basis of electron microscopic observations and fluorescent histochemical studies (Burden, 1972). In rat and guinea pig ovaries the interstitial cells receive a dense adrenergic innervation. These cells are believed to secrete androgen and oestrogens. Their particular anatomical location suggests that a process of neuroendocrine transduction (Wurtman, 1973) may be possible within the ovary. Lawrence and Burden (1976) observed that the density of adrenergic innervation of interstitial cells of rat ovary increases during pregnancy, supporting the possibility of an autonomic influence on steroidogenesis. Since adrenergic fibres do not penetrate into the granulosa layer of the follicles, or into corpora lutea, it seems unlikely that they directly influence steroid production by the latter structures (Mohsin and Pennefather, 1979).

Adrenergic antagonists, such as phenoxybenzamine, and denervation of the ovary have been shown to modify ovarian function (Bahr et al., 1974).

1.1.4 Ovulation

Immediately after the onset of menstruation, and by a mechanism still unknown, one follicle is selected for further development. This follicle, under the influence of FSH begins to differentiate rapidly becoming the dominant follicle. At the same time, the other follicles undergo atresia. There is increased vascularization of the dominant follicle which allows for a more efficient delivery of cholesterol and circulating gonadotrophins. The dominant follicle is the main source of circulating oestradiol. At about mid-cycle, the elevated levels of oestradiol exert a positive feed back effect on the secretion of LH and FSH. The LH surge leads to a series of coordinated biochemical and morphological events including activation of proteolytic enzymes, granulosa cell differentiation, release of histamine, prostaglandins and other hormones (Rhoades & Tanner, 1995). The ultimate result is follicular rupture and ovulation. The physiological role of the midcycle FSH surge is not clear, it is however not essential for ovulation as ovulation can be induced, before the midcycle surge of endogenous gonadotrophins, by injecting LH or HCG (Rhoades & Tanner, 1995).

Most female mammals exhibit a definite period of sexual receptivity near the time of ovulation, referred to as oestrus (heat). This is the only period when copulation can take place, ensuring that the female is mated at the time of ovulation (Rhoades & Tanner, 1995). On the other hand, female rabbits and cats are in a state of persistant oestrus and are sexually receptive to males. These are the reflex ovulators. In these animals, ovulation takes place within a definite period after mating (Hadley, 1984). In female rabbits, ovulation usually takes place 10-12hr after mating or HCG injection (Virutamasen et al., 1971; Lambertsen et al., 1976).

1.1.4.1 Vascular dynamics during ovulation

At ovulation, surge of LH causes a conspicuous change in the ovarian vasculature. Within a few minutes after gonadotropin stimulation there is a significant increase in ovarian blood flow (Wurtman, 1964; Lee & Novy, 1978) resulting from an LH-mediated release of vasodilator substances such as histamine, bradykinin and prostaglandins. The elevation in ovarian blood flow lasts for at least 9 hours, with a peak at about 4 hours after gonadotropin stimulation (Blasco et al., 1975). This increase in circulation is associated with the hyperaemic condition that develops in the dominant follicles to LH (Burr & Davis, 1951; Espey, 1974). Along with

vasodilatation, there is an increase in vascular permeability in the follicles (Zacchariae, 1958; Espey 1978). These vascular changes cause the follicle to become oedematous, a condition which persists until the time of follicular rupture (Bjersing & Cajander, 1974; Cherney et al., 1975). Thus LH induces ovarian hyperemia, vasodilatation, oedema and extravasation of blood in ovulatory follicles (Espey, 1980).

The two phenomenon most often observed in connection with ovulation are the engorgement of the ovarian blood vessels and the distension of the follicles due to the accumulation of fluid in them (Burr & Davies, 1951).

1.2 Classification of alpha-adrenoceptors

1.2.1 Concept of α_1- and α_2-adrenoceptors

Brown and Gillespie (1957) observed that α-adrenoceptor antagonists increased the overflow of noradrenaline from the perfused cat nictating membrane evoked by sympathetic nerve stimulation. This was thought to be due to the presence of α-adrenoceptors on the nerve terminals, blockade of which was responsible for the increased noradrenaline overflow (Langer et al., 1971; Starke et al., 1971b). It was proposed that these receptors exist predominantly pre-synaptically, constituting a negative feed back mechanism to regulate neurotransmitter release. This is in contrast to post-junctionally located α-adrenoceptors which mediate end-organ responses to noradrenaline. Because the receptors differ in their sensitivities to α-adrenergic agonists and antagonists, it was suggested that these adrenoceptors should not be characterized based on their anatomical distribution but rather on their functional activities (Berthelsen & Pettinger, 1977). On this basis, it was concluded that α_1-adrenoceptors were excitatory while α_2-adrenoceptors were inhibitory. These receptors were designated α_1-(post-junctional) and α_2-(pre-junctional) adrenoceptors. Later studies have shown that α_2-adrenoceptors located post-junctionally, could also mediate excitatory responses. Drew and Whiting (1979) observed that NA-induced vasoconstrictor responses in rats and cats, were inhibited by both selective α_1- and α_2-adrenoceptor antagonists. Therefore, the universally accepted method of classification, is that based solely on the relative affinities of highly selective antagonists. α_1-Adrenoceptors were found to have high affinity for agonists such as phenylephrine, methoxamine and 6-fluoronorepinephrine, while α_2-adrenoceptors have higher

selectivity for drugs such as clonidine and UK 14,304. The subdivision of α-adrenoceptors into α_1-and α_2-adrenoceptor subtypes was further substantiated by the development of selective antagonists for each subtype. α_1-Adrenoceptors were found to be antagonized selectively by prazosin, WB-4101, corynathine, phenoxybenzamine, benoxathian and benextramine; and α_2-adrenoceptors being selectively inhibited by drugs like rauwolscine, yohimbine and idazoxan (Table 1) (Ruffolo, 1990).

1.2.2 Subclassification of α_1-adrenoceptors

α_1-Adrenoceptors are by definition potently antagonized by prazosin and show low affinity for selective α_2-adrenoceptor antagonists such as yohimbine or rauwolscine (Bylund et al., 1994). However, a survey of the literature reveals a remarkable variation in the potency of prazosin in antagonizing α_1-adrenoceptor mediated effects (Agrawal et al., 1984; Medgett & Langer, 1984; Flavahan & Vanhoutte, 1986). Such variations spanning over approximately two orders of magnitude (i. e. 100 fold variation) would possibly indicate heterogeneity of α_1-adrenoceptors. In a study on the rat caudal artery preparation, Medgett & Langer (1984) observed that prazosin was more effective against methoxamine (K_B=0.2nM) than against NA (K_B=1.3nM). They also observed that corynanthine, another selective α_1-adrenoceptor antagonist was equipotent against methoxamine and NA. It was therefore, suggested that methoxamine selectively activated the α_1-adrenoceptor sub-type having high affinity for prazosin. NA presumably stimulated both high and low affinity prazosin binding sites. Holck et al (1983) also noted that prazosin was more potent against clonidine- than methoxamine-induced contractions of the rabbit pulmonary artery. The variation in prazosin affinity is not only observed between species, it could also occur within the same species. For example, the pK_B value for prazosin against NA in the rat aorta reported in the literature varies from 9.5-10.6 whereas in the tail artery, the pK_B is less than 9 (Agrawal et al., 1984). It also occurs between different agonists on the same tissue.

1.2.2.1 Concept of α_{1H}-, α_{1L}-and α_{1N}-adrenoceptor subtypes

Based on the various affinities of prazosin and yohimbine in the literature, Flavahan and Vanhoutte (1986) proposed a subdivision of α_1-adrenoceptors into α_{1H} (high affinity for prazosin and yohimbine) and α_{1L} (low affinity for prazosin and yohimbine). This type of subdivision would however not accommodate the rat tail

Table 1 Adrenoceptor agonists and antagonists commonly used in the subclassification of α_1- and α_2-adrenoceptors. (cited from Ruffolo et al., 1991)

α_1-Adrenoceptor selective	Nonselective	α_2-Adrenoceptor selective
Agonists		
Phenylephrine	Norepinephrine	Clonidine
Methoxamine	Epinephrine	α-Methylnorepinephrine
Cirazoline		B-HT 933
Amidephrine		B-HT 920
Sgd 101/75		UK-14,304
Antagonists		
Prazosin	Phentolamine	Rauwolscine
WB-4101	Tolazoline	Yohimbine
Corynanthine		Idazoxan

artery. Like other rat vessels, the potency of yohimbine against noradrenaline was high in the tail artery (K_B=100 nM). However, prazosin was much less potent (K_B < 1nM) as compared with other tissues of the rat resulting in a lower prazosin/yohimbine ratio in this preparation.

The functional classification of α_1-adrenoceptors into α_{1H}- and α_{1L}-subtypes by Flavahan and Vanhoutte (1986), has recently been modified to include a third group, α_{1N}, which have a relatively low affinity for prazosin and a higher than expected affinity for yohimbine (Muramatsu et al., 1990).

1.2.2.2 α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes

Morrow et al (1985) observed that, in the rat brain, WB-4101 and phentolamine produced a biphasic displacement of [^{3}H]-prazosin consistent with a two-receptor system. This was later confirmed by Morrow and Creese (1986) using rat cerebral cortex, who designated these sites as α_{1A}-(high affinity for WB-4101) and α_{1B}- (low affinity for WB-4101) adrenoceptors. Similar observations were made in different parts of the rat brain by Han et al (1987) who also showed that these receptor subtypes could be differentially affected by drugs. Thus WB-4101 was more effective against α_{1A}-(about 20 fold more potent) than α_{1B}-adrenoceptors. On the other hand, CEC, a reactive analogue of clonidine (LeClerk et al., 1980), inactivated α_{1B}- but not α_{1A}-adrenoceptor subtype. The site inactivated by CEC in the rat brain corresponded to the low affinity WB-4101 binding site, thus indicating an inverse sensitivity of α_{1A}- and α_{1B}-adrenoceptor subtypes to WB-4101 and CEC. Other compounds which differentially interact with the receptor subtypes include phentolamine, 5-methyl-urapidil, (+)-niguldipine, benoxathian and ipsapirone ($\alpha_{1A} > \alpha_{1B}$) (Morrow & Creese, 1986; Han et al., 1987; Boer et al., 1989; Gross et al., 1988; Eltze et al.,1991), or spiperone ($\alpha_{1B} > \alpha_{1A}$) (Michel et al., 1989a). The existence of α_{1A}- and α_{1B}-adrenoceptor subtypes is also supported by data from cloning studies (Schwinn et al., 1990; Lomasney et al., 1990 & Cotecchia et al., 1990) . The α_{1B}-adrenoceptor subtype was the first of the α_1-adrenoceptor family to be cloned. This clone was isolated from the hamster vas deferens and encoded for a protein having a sequence consistent with a G-protein-coupled receptor (Cotecchia et al., 1988). Expression of this cDNA resulted in a protein with radioligand-binding properties consistent with an α_{1B}-adrenoceptor, with a high affinity for prazosin and a low affinity for phentolamine, 5-

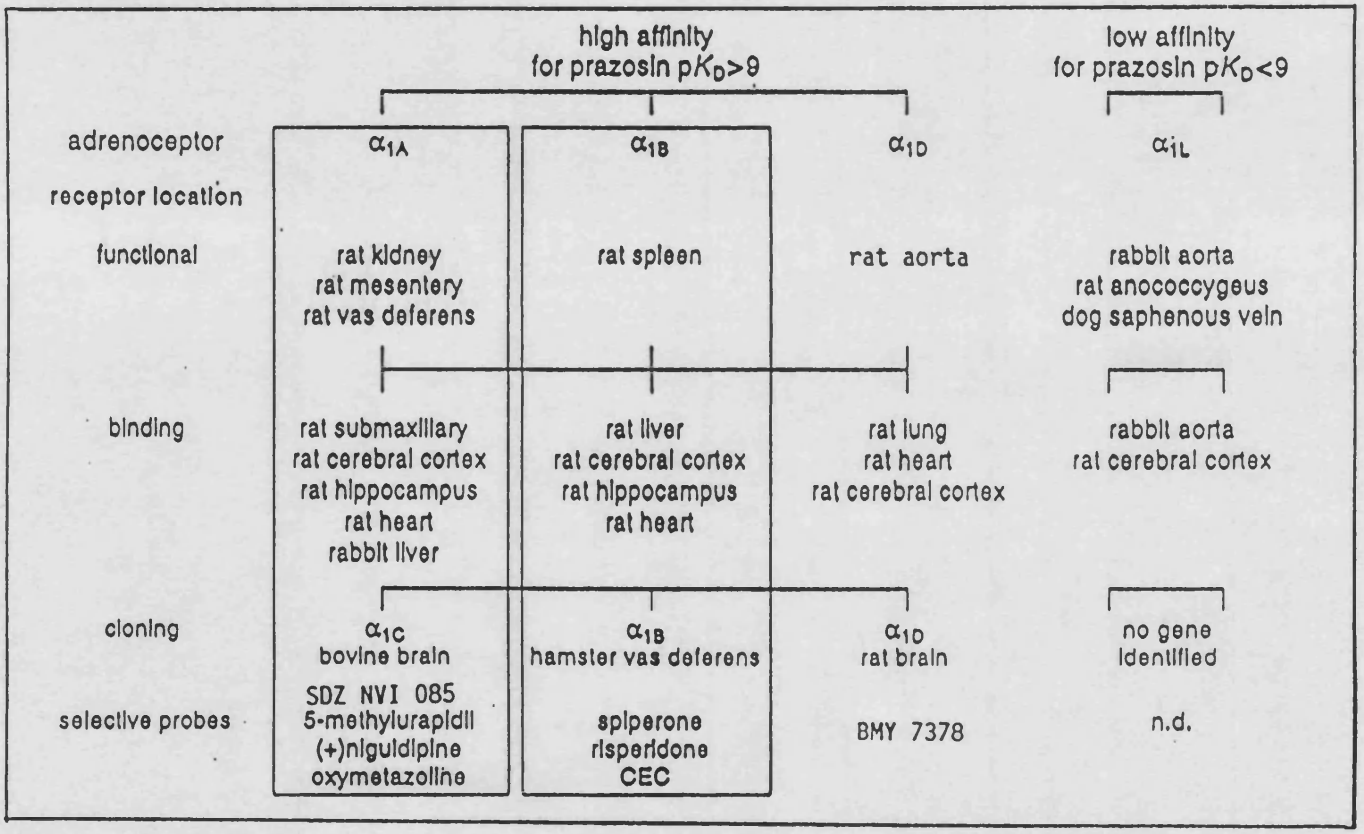
methylurapidil and yohimbine. Functionally, α_{1A}-adrenoceptors predominate in the vas deferens (Han et al.,1990) and intrarenal vascular bed of the rat (Clarke et al., 1990; Eltze et al., 1991; Munavvar & Johns, 1991; Elhawary et al., 1992).

Lomasney et al., (1991) have shown that screening of a rat brain library with a cDNA probe prepared from the hamster α_{1B}-adrenoceptor revealed a cDNA clone for another α_1-adrenoceptor subtype. The amino acid sequence of the protein expressed by this clone is consistent with a seven transmembrane spanning, G-protein linked receptor. The tissue distribution of mRNA transcribed by this clone suggested a similar distribution to that of the α_{1A}-subtype, and the expressed receptor had a high affinity for WB 4101. This led to the conclusion that this clone represented the pharmacologically defined α_{1A}-adrenoceptor (Lomasney et al., 1991). However, Perez et al., (1991), studying an almost identical clone also isolated from rat brain, found low affinity for the more selective α_{1A}-adrenoceptor antagonists, 5-methylurapidil and (+)-niguldipine. They concluded that a clone for a novel α_1-adrenoceptor subtype had been isolated and denoted it as α_{1D}. The clones isolated by Lomasney et al. (1991) and Perez et al. (1991) encode for 560 amino acid proteins differing in sequence at only two sites (99.8% amino acid identity), therefore, it seems likely that they represent the same subtype. This receptor subtype was designated $\alpha_{1A/D}$-adrenoceptor. Since the expressed cDNA has pharmacological properties substantially different from those found for the α_{1A}-adrenoceptor in native tissues, this recombinant receptor should be referred to as the $\alpha_{1A/D}$-adrenoceptor until these discrepancies can be explained and a native receptor having similar pharmacology identified (Bylund et al., 1994). The human homolog of this α_1-adrenoceptor subtype has been cloned recently (Forray et al., 1993) (Table 2).

1.2.2.3 α_{1C}-adrenoceptors

An additional cDNA clone has been found based on homology screening of a bovine cerebral cortex with a probe derived from the hamster α_{1B}-adrenoceptor. The protein expressed by this clone was found to be distinct from the α_{1B}-adrenoceptor, with 65% amino acid homology in the membrane-spanning domains. The pharmacological profile was also distinct from either the α_{1B}- or $\alpha_{1A/D}$-adrenoceptors, with a relatively high affinity for 5-methylurapidil and a high affinity for WB-4101. In view of its distinct pharmacological profile, this receptor was designated the α_{1C}-adrenoceptor.

Table 2 A modified putative classification scheme for α_1-adrenoceptors. (n.d., not determined; cited from Ford et al., 1994)



The affinities of a series of competitive antagonists for displacement of [^{3}H] prazosin from expressed α_{1C}-adrenoceptors correlates very well with their affinities for binding sites in native tissues that are known to express α_{1A}-adrenoceptors (Testa et al., 1995; Ford et al., 1994; Saussy et al., 1994; Langer et al.,1994). It could therefore be that the recombinant α_{1C}-adrenoceptor actually represents the native α_{1A}-adrenoceptor (Ford et al., 1994). Further studies have shown that, like native α_{1A}-adrenoceptors, recombinant rat (Laz et al., 1994) or human (Forray et al., 1994; Schwinn et al., 1991) α_{1C}-adrenoceptor is relatively resistant to alkylation by CEC. Recently, mRNA for the α_{1C}-adrenoceptor has been identified in human prostate (Price et al., 1993), and the contractile response induced by α_1-adrenoceptor activation in this tissue appears to be mediated by an adrenoceptor having pharmacological characteristics corresponding to those of the expressed recombinant α_{1C}-adrenoceptor (Marshal et al., 1992 & Forray et al., 1993). Recent data suggest that the α_{1C} clone, at least its rat homolog, may correspond to the pharmacologically defined α_{1A}-adrenoceptor (Laz et al., 1993). Comparison of the recombinant rat α_{1C}-receptor to the other two recombinant rat α_1-adrenoceptors, revealed that the former has significantly lower sensitivity to irreversible inactivation by CEC and relative antagonist affinities correlating well with that of the native α_{1A}-adrenoceptor (Laz et al., 1993).

1.2.2.4 Atypical α_1-adrenoceptors

The atypical α_1-adrenoceptor described by Abel et al., (1995) has a number of similarities to the α_{1L}-adrenoceptor described by Muramatsu and co-workers (1990; 1991). The α_{1L}-adrenoceptor is found in vascular smooth muscle (e.g. thoracic aorta) of the guinea-pig and is insensitive to inactivation by CEC. α_{1L}-adrenoceptor has a low affinity for prazosin (> 1nM) and a relatively low affinity for WB-4101 (1-10 nM). The atypical α_1-adrenoceptor in the vascular smooth muscle of the guinea-pig mucosa, ileum and in the rat parotid gland is also insensitive to inactivation by CEC. In all three tissues, the atypical receptor has a low affinity (1-4 nM) for prazosin and low affinity (5-18 nM) for WB-4101. These similarities in pharmacological characteristics and the identification of both α_{1L}- and the atypical α_1-adrenoceptor in vascular tissue from the guinea-pig suggest that these two receptor types may actually represent the same α_1-adrenoceptor sub-type (Abel et al., 1995). This is the population of α_1-adrenoceptors that displays a relatively low affinity for prazosin and, therefore,

has been largely ignored in many radioligand binding studies. These receptors are termed α_{1L}-adrenoceptors. Whether novel cDNAs exist for this adrenoceptor sub-family or whether they represent variants of the cloned α_1-adrenoceptors, remains to be determined (Ford et al., 1994).

1.2.3 Subclassification of α_2-adrenoceptors

The presence of post-junctional, vascular α_2-adrenoceptors has been demonstrated, primarily by using in vivo preparations such as the pithed rat (Drew & Whiting, 1979; Timmermans et al., 1979; Ruffolo et al., 1983). These receptors have been also studied in vitro using isolated cutaneous veins from the dog (Flavahan & Vanhoutte, 1986), rabbit (Schumann & Lues, 1983), and human (Docherty & Hyland, 1984). All known α_2-adrenoceptor sub-types are activated by NA and adrenaline, and there is no evidence that these physiological catecholamines show significant selectivity between any of the known α_2-adrenoceptor sub-types. All subtypes can be blocked by yohimbine and rauwolscine (receptor dissocation constant < 40 nM) and labelled with [^{3}H] analogues of these antagonists, although the affinity can vary substantially between sub-types. The earliest subdivision of α_2-adrenoceptors was based on species heterogeneity. For example, Waterfall et al. (1985) reported that the affinities of rauwolscine, yohimbine and a series of substituted benzoquinoxolazines were roughly equivalent at pre-junctional α_2-adrenoceptors in the rat vas deferens, while the affinities of yohimbine and rauwolscine were approximately 100-fold greater than those for the benzoquinoxolazines for the pre-junctional α_2-adrenoceptor in the rabbit vas deferens. However, careful analysis of the ability of prazosin to inhibit [^{3}H]-yohimbine binding to various regions of both rat (Bylund, 1985), and human (Petrash & Bylund, 1986) brain, have revealed discrete regions of heterogeneity within each species. In some regions, the inhibition was characterized by prazosin competing with low affinity for only one site, and in others it was characterized by prazosin competing with two sites, one with a low affinity, identical to that seen in the human platelet, and the other with high affinity identical to that seen in the neonatal rat lung. Bylund (1985) proposed a sub-classification of α_2-adrenoceptors based on the relative potency of prazosin, with the receptor having the low affinity for prazosin, typified by the human platelet α_2-adrenoceptor, termed α_{2A}, and the receptor having a relatively higher affinity for prazosin, typified by the neonatal rat lung α_2-adrenoceptor, termed

α_{2B}. More recent studies have confirmed this sub-classification of α_2-adrenoceptors and have identified other tissues that possess one or the other of these α_2-adrenoceptor subtypes (Bylund et al., 1988). Moreover, several ligands have been shown to have some degree of selectivity for one or the other of these α_2-adrenoceptor subtypes. Benoxathian, oxymetazoline and WB 4101 are relatively selective for α_{2A}-adrenoceptors, while chlorpromazine, imiloxan and prazosin are relatively selective for α_{2B}-adrenoceptors (Michel et al., 1989b). Importantly, functional studies of the inhibition of α_2-adrenoceptor-mediated attenuation of cAMP production with sub-type selective antagonists in HT29 and NG108-15 cells have confirmed the existence and definition of the α_{2A}- and α_{2B}-adrenoceptor subt-ypes (Bylund & Ray-Prenger, 1989). A third α_2-adrenoceptor subtype, α_{2C}-adrenoceptor, has been proposed. This receptor was identified in opossum kidney-derived cell line (OK cells) (Murphy and Bylund, 1988), which has characteristics similar to that of α_{2B}-adrenoceptors (a relatively high affinity for prazosin), but the ratio of affinities of prazosin and yohimbine is intermediate between α_{2A}- and α_{2B}-adrenoceptors (Table 3) (Bylund & Ray-Prenger, 1989). By correlation of antagonist affinity for inhibition of [^{3}H]-rauwolscine binding in different cell and tissue preparations, two additional α_2-adrenoceptor subtypes have been proposed, designated α_{2C} and α_{2D} (Bylund et al., 1994).

Results obtained in molecular biology studies have confirmed the existence of α_2-adrenoceptor subtypes. Several clear correlations exist between the α_2-adrenoceptor sub-types identified in native tissues and cell lines and the receptor proteins expressed from cDNA clones. These correlations are supported by hybridization of the α_2-adrenoceptor clones with tissues known to contain a particular α_2-adrenoceptor subtype (Bylund et al., 1992; Lomasney et al., 1990; Harrison et al., 1991).

1.2.3.1 Pre- versus post-junctional α_2-adrenoceptors

The rank order of potency of a series of α_2-adrenoceptor antagonists appears to be similar at pre- and post-junctional α_2-adrenoceptors in vivo (Berridge et al., 1984; Docherty & Hyland, 1985). Determination of receptor dissociation constants for standard α_2-adrenoceptor antagonists in isolated preparations measuring pre- and post-junctional α_2-adrenoceptor affinity likewise does not suggest a pharmacologic difference in these two receptors (Hieble et al., 1986a). Such antagonists include imidazolines (e.g. phentolamine), 3-benzazepines (SK&F 86466), yohimbine alkaloids

Table 3 A putative scheme for α_2-adrenoceptors. (cited from Watson & Girdlestone,1996)

Nomenclature	α_{2A}	α_{2B}	α_{2C}
Previous names	α_{2D}	–	–Δ13
Potency order	adrenaline ≥ NA	adrenaline ≥ NA	adrenaline ≥ NA
Selective agonists	oxymetazoline	–	–
Selective antagonists	–	prazosin (7.5) ARC239 (8.0)	prazosin (7.5) ARC239 (8.0)
Effector	$G_{i/o}$	$G_{i/o}$	$G_{i/o}$
Gene	*α2A*; chr 10	*α2B*; chr 2	*α2C*; chr 4
Structural information	450 aa human P08913 7TM 450 aa mouse Q01338 7TM 450 aa rat P22909 7TM	450 aa human P18825 7TM 455 aa mouse P30545 7TM 453 aa rat P19328 7TM	461 aa human P19328 7TM 458 aa mouse Q01337 7TM 450 aa rat P18089 7TM

(rauwolscine and yohimbine) and benzodioxanes (piperoxan). Modification of the 3-benzazepine structure yielded compounds such as SK&F 104078 which exibited different affinities for pre- and post-junctional α_2-adrenoceptors (Hieble et al., 1988; Ruffolo et al., 1987; Hieble etal., 1986b). Thus SK&F 104078 antagonized UK14,304-induced contractions of the canine saphenous vein without affecting the twitch-inhibiting effect of α-methylnoradrenaline in the guinea pig ileum (Hieble et al., 1988) and vas deferens. Further studies revealed that SK&F 104078 could differentiate between sub-types of pre-junctional α_2-adrenoceptors. In the rat vas deferens, SK&F 104078 antagonized twitch-inhibiting effect of clonidine but not UK14,304 or B-HT 920 (Oriowo et al., 1991). The potency of SK&F 104078 in inhibiting clonidine was similar to its potency at post-junctional α_2-adrenoceptors. It was concluded that there is a heterogenous population of pre-junctional α_2-adrenoceptors in the rat vas deferens with one population identical to the post-junctional α_2-adrenoceptors. On the other hand, guinea-pig atrium contains a homogenous population of SK&F 104078-insensitive prejunctional α_2-adrenoceptors.

1.3 Signal transduction mechanisms

Calcium plays a pivotal role as an intracellular second messenger. Although cells contain a lot of Ca^{2+}, most of it is not free as the Ca^{2+} is either bound by a variety of proteins or sequestered by organelles such as the mitochondria and endoplasmic reticulum (Berridge, 1984). Furthermore, there are several pumps and exchange systems which remove free Ca^{2+} from the cytoplasm if its concentration begins to rise. All these factors maintain the resting $[Ca^{2+}]_i$ at very low levels ($<10^{-7}$M) (Miller, 1987). During excitation, the transient increases in $[Ca^{2+}]_i$, which act as important intracellular signals, can be initiated in two ways. Firstly, calcium can be released from intracellular storage sites associated with the endoplasmic reticulum. Secondly, the $[Ca^{2+}]_i$ could also rise due to an increase in the Ca^{2+} permeability of the plasma membrane. This is achieved by the opening of Ca^{2+} channels by the action of some agonists on receptors or in response to changes in membrane potential (Bolton 1979).

Earlier studies have consistently shown that α_2-adrenoceptor mediated vasoconstriction, in vivo or in vitro was highly dependent on an influx of extracellular Ca^{2+}. Thus the response was virtually abolished in a Ca^{2+}-free medium (Matthews et

al., 1984; Cooke et al., 1985) and significantly reduced by organic and inorganic Ca^{2+} channel inhibitors (van Meel et al., 1981; Matthews et al., 1984). These observations were supported by the observation that B-HT 920, an α_2-adrenoceptor agonist promoted influx of $^{45}Ca^{2+}$ which was inhibited by nifedipine and verapamil (Matthews et al., 1984). The situation was not as straight forward following α_1-adrenoceptor activation. Pressor responses in vivo mediated by post-junctional vascular α_1-adrenoceptors were found to be resistant to inhibition by calcium channel antagonists (van Meel et al., 1981 & Cavero et al., 1983), suggesting that, unlike α_2-adrenoceptors, α_1-adrenoceptors may not rely heavily upon extracellular calcium to produce vasoconstriction. However, not all selective α_1-adrenoceptor agonists produce vasoconstriction in vivo that is resistant to inhibition by calcium channel blockers. For example, Sgd 101/75 and (-)-dobutamine all produce α_1-adrenoceptor-mediated pressor responses in pithed rats that are inhibited by calcium channel blockers (Ruffolo et al., 1984). Therefore, it would appear that some α_1-adrenoceptor agonists can produce vasoconstriction via both influx of extracellular calcium and release of intracellular calcium. Further analysis has revealed that partial α_1-adrenegic agonists produce vasoconstrictions that are attenuated by Ca^{2+} channel inhibitors while similar responses induced by full agonists are insensitive to calcium channel inhibitors. It was therefore suggested that agonist efficacy determines whether α_1-adrenoceptor mediated vasoconstriction is dependent on extra- or intracellular Ca^{2+}. This concept is also supported by results obtained in functional studies. For example, in the pithed rat preparation, B-HT 933 and cirazoline induced dose-dependent pressor effects and relative to cirazoline, B-HT 933 was a partial agonist. Diltiazem significantly inhibited B-HT 933, but not cirazoline-induced pressor responses. After treatment with phenoxybenzamine to reduce efficacy, the sensitivity of cirazoline-induced pressor responses to diltiazem was significantly enhanced (Ruffolo et al., 1984). The dependence of α_1-adrenergic agonists on extra- and intracellular Ca^{2+} is supported by results obtained in studies in which Ca^{2+} flux was studied using inorganic calcium inhibitors (Deth & Lynch, 1981). In rabbit aorta, α_1-adrenoceptor activation produces an influx of extracellular calcium (Cauvin et al., 1982). Moreover, even α_1-adrenoceptor agonists that are relatively insensitive to inhibition by calcium channel antagonists (e.g. cirazoline), produce a significant influx of extracellular calcium of a

magnitude that is greater than that produced by agonists (e.g. St 587) that show a high degree of sensitivity to inhibition by calcium channel antagonists (Chiu et al., 1986). In addition, studies of the efflux of Ca^{2+} have shown that α_1-adrenoceptor agonists also induce a release of calcium from intracellular stores (Deth and Lynch, 1981 & Cauvin et al., 1982), although only those agonists with high intrinsic efficacies produced a significant release of intracellular Ca^{2+} (Chiu et al., 1986). This would perhaps explain why, in many vascular smooth muscle preparations, α_1-adrenoceptor-mediated contraction is made up of two components, an initial fast (phasic) component and a secondary (tonic) phase. These phases, according to efflux studies utilize different Ca^{2+} pools. It has been suggested that the initial fast phase of NA-induced tension development depends to a large extent on Ca^{2+} release; while, the tonic phase is dependent on Ca^{2+} entry (Van Breemen et al., 1986). Based on these observations, the following hypothesis was proposed; upon α_1-adrenoceptor activation, calcium from a small labile intracellular store on the inner surface of the plasma membrane is released. This calcium does not activate smooth muscle contraction directly, but rather triggers the release of calcium from the sarcoplasmic reticulum. Simultaneously, calcium bound on the outer surface of the plasma membrane is released and enters the cell through receptor-operated calcium channels. These two processes, which are responsible for the early phasic component of contraction, are followed by an influx of calcium from the free extracellular calcium pool that produces the secondary tonic contraction (Leijten et al., 1985).

1.3.1 α_1-Adrenoceptor subtypes and Ca^{2+} utilization

The differential sensitivity of full and partial agonists to Ca^{2+} channel inhibitors could suggest that different α_1-adrenoceptor subtypes mediate the response to full and partial agonists. With this assumption, the enhanced sensitivity of a vasoconstrictor effect of a full agonist to a Ca^{2+} channel inhibitor following treatment with phenoxybenzamine was interpreted to suggest that phenoxybenzamine inactivated only the subtype coupled to intracellular Ca^{2+} release (Chiu et al., 1986). However, Oriowo et al.(1992) have shown, using receptor protection studies that in the rat aorta, the same α_1-adrenoceptor subtype could be coupled to more than one source of activator Ca^{2+} (i.e. to extra- and intracellular Ca^{2+}). The fact that NA induced contractions of the rat

aorta were mediated only by CEC sensitive α_1-adrenoceptors (Oriowo & Bevan, 1990; Oriowo & Ruffolo, 1992) would support this hypothesis.

Sub-types of α_1-adrenoceptors have recently been identified using pharmacological (ligand binding and functional studies) and molecular biological techniques (see section 1.2.2). Thus, presently, three subtypes, α_{1A}-, α_{1B}- and α_{1D}-subtypes exist (Watson & Girdlestone, 1996). It was earlier suggested that α_{1A}- and α_{1B}-subtypes were coupled to extracellular and intracellular Ca^{2+} respectively (Han et al., 1987; Minneman, 1988). This was based on the fact that α_{1A}-mediated NA-induced contractions of the rat vas deferens (Han et al., 1987) were abolished by nifedipine indicating total dependence on influx of extracellular Ca^{2+}. In the same study (Han et al., 1987), NA-induced contractions of the splenic strip (α_{1B}-mediated) were not significantly affected by nifedipine. However, later studies have shown that both α_{1A}- and α_{1B}-adrenoceptors could mobilize Ca^{2+} from extracellular and intracellular sources. This has been observed in guinea-pig hepatocytes (Garcia-Sainz et al., 1992), and rat brain and renal cortex (Michel et al., 1993; Han et al., 1990; Wilson & Minneman, 1990).

In most systems, calcium translocation produced by agonist interaction with α_1-adrenoceptors is believed to be secondary to enhanced PtdIns turnover, due to activation of PLC (Fig. 3) (Michell, 1979). There is a correlation between α_1-adrenoceptor-mediated contraction of rabbit aorta and the increase in PtdIns turnover (Villalobos et al., 1982). This would tend to suggest a causative link between α_1-adrenoceptor-mediated activation of PLC leading to hydrolysis of PtdIns and subsequent smooth muscle contraction. It was later observed that α_1-adrenoceptor-stimulation of phospholipid turnover was the result of the hydrolysis of phospho-inositides (Berridge, 1983). The products of this hydrolysis, DAG and Ins(1,4,5)P_3, have been proposed to be the second messengers responsible for transduction of the α_1-adrenoceptor signal (Berridge, 1984). Ins(1,4,5)P_3 has been shown to release calcium ions from the endoplasmic reticulum in liver (Burgess et al., 1984; Joseph et al., 1984), whereas DAG activates cytosolic PKC (Kishimoto et al., 1980), which under certain conditions, may induce slowly developing vascular smooth muscle contraction (Danthuluri and Deth, 1984; Rasmussen et al., 1984) possibly via an interaction with MLCK (Nishikawa et al., 1984). Phosphorylation of DAG, yield PA,

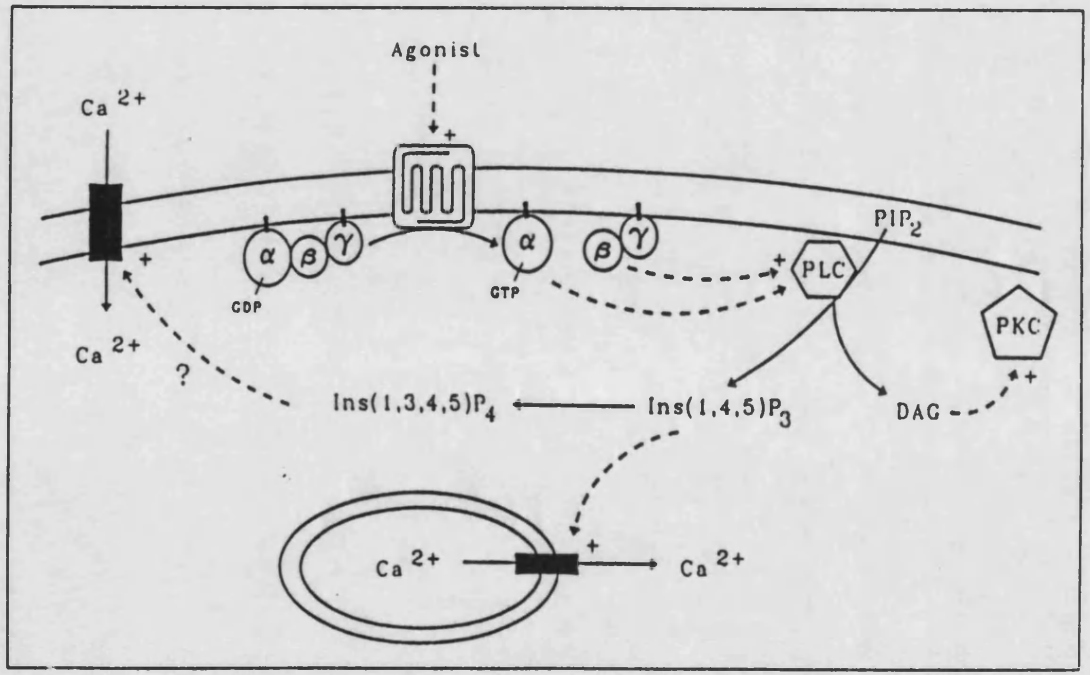


Fig. 3 Generation and action of inositol (1,4,5)-triphosphate. (cited from Boeynaems & Pirotton, 1994)

a calcium ionophore (Tyson et al., 1976), which causes calcium-dependent smooth muscle contraction (Salmon and Honeyman, 1980). There is the possibility that PA may also be involved in the transduction of α_1-adrenoceptor signal (Ruffolo and Hieble, 1994).

PKC is a phospholipid- and Ca^{2+}-dependent enzyme which has been shown to phosphorylate a number of substrates including myosin (Adelstein & Sellers, 1987). PKC plays a role in various biological responses including neurotransmitter release and neurotransmitter-induced contraction of vascular smooth muscle (Nishizuka, 1986; Rasmussen et al., 1987; Lee & Sverson, 1994). PKC is activated by DAG and is distributed in sympathetic nerves and other tissues (Malhotra et al., 1988; Kuo et al., 1980). Several isoforms of PKC have been identified in vascular smooth muscle (Liou & Morgan, 1994). In vascular smooth muscle, phorbol esters which activate PKC, increases Ca^{2+} influx (Sperti & Colucci, 1987; Jiang & Morgan, 1987) and have been reported to increase calcium channel currents in rat portal vein cells (Loirand et al., 1990) and rabbit saphenous artery cells (Oike et al., 1992). The ability of antiphosphoinositide antibodies to inhibit NA-induced increases in calcium channel currents in rat portal vein cells is consistent with this proposed role for PKC (Loirand et al., 1992).

The principal kinase involved in smooth muscle contraction is generally accepted to be MLCK. After binding the Ca^{2+}-CaM complex, MLCK phosphorylates the 20kDa light chain of myosin thus initiating smooth muscle contraction (Kamm & Stull, 1985). Although activation of MLCK is necessary for contractile force generation, activation of PKC may be required for force maintenance (Rasmussen et al., 1987). The activation of PKC is known to induce slowly developing but sustained cellular responses (Nishizuka, 1986). Jiang and Morgan (1987) have suggested that the activation of PKC increases the sensitivity of contractile elements to cytosolic calcium. Other structural and regulatory proteins of smooth muscle have been reported to become phosphorylated by PKC after phosphorylation of MLCK. Maintenance of these proteins in the phosphorylated state during the sustained phase of smooth muscle contraction after both dephosphorylation of myosin light chain and the return of intracellular Ca^{2+} to near basal level has been explained to suggest a role for PKC in the tonic phase of smooth muscle contraction (Rasmussen et al., 1987).

Pharmacological studies seem supportive of some role for PKC in smooth muscle contraction. Activators of PKC, such as PMA and some other phorbol esters, induced slow and sustained contractions of smooth muscle (Rasmussen et al., 1984; Forder et al., 1985). Moreover, inhibtors of PKC, such as polymyxin B, H-7 and HA-1077 (Asano et al., 1987; 1989) have been shown to produce relaxation of smooth muscle; consistent with a role for PKC in tension maintenance (Miller et al., 1986; Khalil & van Breemen, 1988). Contraction of smooth muscle by phorbol esters involves activation of PKC and is dependent on Ca^{2+} (Rasmussen et al., 1984; Miller et al., 1986). It has been hypothesized that PKC is involved in the maintenance rather than the initiation of smooth muscle contraction (Rasmussen et al., 1987), because the maintenance phase is associated with the phosphorylation of caldesmon and other structural and regulatory proteins by PKC, and then are dephosphorylated rapidly during relaxation (Park & Rasmussen, 1985; Adam et al., 1989).

Another intracellular second messenger is TK. Tyrosine-specific protein kinase activity is known to be associated with oncogene products of the retroviral src gene family (Hunter & Cooper, 1985). This kinase activity is strongly correlated with the ability of retroviruses to transform cells, since mutants with reduced kinase activity have lower transforming efficiency, and mutants which lack tyrosine kinase activity are transformation-defective (Bishop, 1983). Similar kinase activity is also associated with the cellular receptors for several growth factros such EGF (Ushiro & Cohen, 1980), PDGF (Ek et al.,1982), insulin (Kasuga et al., 1982) and insulin-like growth factor I (Jacobs et al., 1983). Therefore, it is possible that tyrosine phosphorylation plays an important role for cell proliferation and cell transformation (Akiyama et al., 1987).

Recent data obtained in cell culture systems pointed to a role for intracellular Ca^{2+} in modulating protein tyrosine phosphorylation (Huckle et al., 1990), and a role for tyrosine kinase activity in the regulation of peptide-stimulated Ca^{2+} influx (Lee et al., 1993). Some recent studies have suggested that tyrosine phosphorylation may participate in the contractile process and may also exert a modulatory influence on calcium channel activity in vascular smooth muscle (Di Salvo et al., 1993; Hollenberg, 1994; Toma et al., 1995). Several compounds have been reported to inhibit tyrosine kinase activity. One such is genistein, an isoflavone compound from fermentation

broth of pseudomonas sp. Genistein inhibits rather selectively TKs of c-src family, including the EGF-URO receptor kinase, without affecting other protein kinases, such as kinase C, cyclicAMP-dependent protein kinase, or phosphorylase kinase, at concentrations exceeding 100 μg/ml (or 370 μM) (Akiyama et al., 1987). The synthetic TK inhibitors called tyrphostins (TP), such as RG-50864, represent another class of enzyme inhibitors that are pseudo-substrate inhibitors of the enzyme (Gazit et al., 1989). Genistein has shown to be a highly specific inhibitor for tyrosine kinases but scarcely inhibits the activity of serine and threonine kinases and other ATP analogue related enzymes (Akiyama et al., 1987). Adam et al. (1994), found that genistein (100 μM) inhibited KCl-dependent contractions of murine aortas and porcine carotid arteries by 100% and 91% respectively. In carotid arteries, genistein inhibited mitogen-activated protein kinase (MAPK) activity by 40%. The results suggested that the effects of genistein are more complex than a simple inhibtion of cellular tyrosine kinases, however, the data demonstrate a potential utility for TK inhibitors as modulators of vascular tone.

1.4 Influence of the endothelium

1.4.1 Early observations of EDRF and its identification as nitric oxide

In 1980, Furchgott and Zawadzki provided the first experimental evidence demonstrating the participation of an endothelium-derived relaxing factor (EDRF) in vascular smooth muscle relaxation to acetylcholine. Since then, endothelium-dependent relaxation was found to occur in response to a number of vasodilators including 5-HT, substance P, adenine neucleotides, bradykinin, thrombin and calcium ionophore (A23187) (Furchgott, 1984). These compounds activate specific receptors at the endothelial cell surface leading to a rapid increase in the intracellular concentration of free Ca^{2+} and formation and release of NO. ß-Adrenergic agonists were originally believed to induce endothelium-independent vasodilation. However, recent reports tend to indicate that at least in some vascular smooth muscles such as rat aorta (Gray & Marshall, 1992), ß-adrenoceptor-mediated isoprenaline-induced relaxation is endothelium-dependent. In many instances, receptor-mediated agonists activate the Na^{+}/H^{+} exchanger leading to an increase in intracellular pH and it would appear that a sustained

increase in NO formation can be observed following intracellular alkalinization (Fleming et al., 1994). Some agents, however, such as the nitrovasodilators, atrial natriuretic factor and prostacyclin induce vascular relaxation by endothelium-independent mechanisms (Furchgott, 1984). Nitric oxide also functions as a powerful toxin released by white blood cells to kill bacteria and tumour cells and a messenger molecule in several areas of the brain to transmit nerve signals (Guyton & Hall, 1996). Nitric oxide is associated with other structures apart from the vascular endothelium. Nitric oxide occurs especially in areas of the brain that are responsible for long-term behaviour and for memory. EDRF has a very short half life of only seconds in oxygenated physiological salt solutions (Griffith et al., 1984; Cocks et al., 1985).

The effects of EDRF were found to be inhibited by hemoglobin and methylene blue (Martin et al., 1985) and to be mediated by stimulation of the soluble guanylate cyclase with the consequent elevation of intracellular cyclicGMP levels (Rapoport & Murad, 1983). The nature of EDRF was not known for long time. A comparison of the biological actions of EDRF and NO on vascular strips and on platelets showed that the two compounds were indistinguishable (Moncada et al., 1988). Both EDRF and NO caused relaxation of vascular smooth muscle strips (Palmer et al., 1987), inhibited platelet aggregation (Radomski et al., 1987a) and induced disaggregation of aggregated platelets (Radomski et al., 1987b). The actions of EDRF and NO on vascular strips and on platelets were similarly potentiated by SOD and cytochrome C and inhibited by Fe^{2+} and some redox compounds (Palmer et al., 1987; Hutchinson et al., 1987).

In mammalian cells, NO is formed from a terminal guanidino-nitrogen of L-arginine (Palmer et al., 1988). The reaction is catalysed by the enzyme, nitric oxide synthase (NOS) yielding L-citrulline as a coproduct of this reaction. Four cofactors (heme, FMN, FAD and H_4 biopterin) and two cosubstrates (O_2 and NADPH) participate in enzymatic NO formation (Fig. 4). Several isoforms of the NOS have been isolated and purified (Table 4) (Schmidt et al., 1991). These include constitutive (cNOS) and inducible (iNOS) forms of the enzyme. Inducible form is expressed in response to pathological stimuli while the constitutive enzyme is present under physiological conditions. The constitutive form is

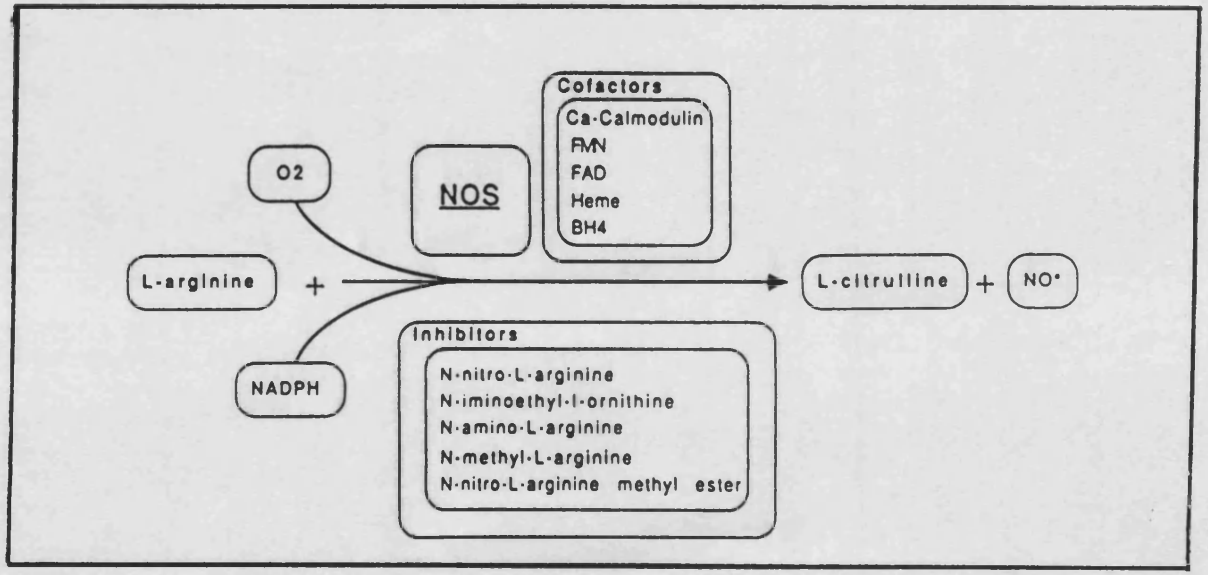


Fig. 4 Nitric oxide synthesis. (cited from Feletou et al., 1994)

Table 4 Isoforms of nitric oxide synthase. (cited from Yui et al., 1994)

	Type I Neuronal	Type II Inducible	Type III Endothelial
Cell type	Brain, NANC nerves, etc.	Macrophage, leukocyte, VSMC, etc.	Endothelium only
MW	160 kDa	130 kDa	135 kDa
Calcium	Dependent	Independent	Dependent
Inducibility	Constitutive	Inducible	Constitutive
Solubility	Soluble	Soluble	Particulate
Human chromosome	12	17	7

cytosolic, Ca^{2+}/calmodulin dependent and releases NO for short periods in response to receptor or physical stimulation. NO released by this enzyme acts as a transduction mechanism underlying several physiological responses (Moncada et al., 1991). The other form of the enzyme, is induced after activation of macrophages or endothelial cells and synthesizes NO for long periods (4-6hr) (Moncada et al., 1991; Corbett et al., 1993; Marczin et al., 1993). Like the cNOS, inducible NO synthase is also a predominantly cytosolic enzyme with essentially the same cofactor requirements as the constitutive enzyme (Cho et al., 1992).

1.4.2 Inhibition of NO synthesis

A number of compounds have been shown to inhibit the activity of NO synthase. These include L-NMMA (Palmer & Moncada, 1989; Moore et al., 1989; Mayer et al., 1989; Ress et al., 1989); L-NOARG ; L-NIO (Rees et al., 1990a,b) and 7-NI (Babbedge et al., 1993; Moore et al., 1993). The different potencies shown by these compounds in vascular tissue in vitro and vivo may be due to differences in uptake, distribution or metabolism of compounds. There is also a regional variation in the sensitivity of constitutive NOS to the inhibitors. Specifically, while 7-NI was potent against brain NOS, it was inactive against NOS in peripheral tissues (Babbedge et al., 1993; Moore et al., 1993). Perhaps this would suggest that the constitutive NOS is not homogenous. This selectivity could be advantageous when brain and not peripheral NOS is to be inhibited.

Aminoguanidine, a bifunctional molecule containing the guanido group of L-arginine linked to hydrazine, is equipotent to L-NMA as an inhibitor of cytokine-induced NO formation (Corbett et al., 1992), but is about 100-fold less potent as an inhibitor of the constitutive form (Misko et al.,1993).

The glucocorticoids, (dexamethasone, hydrocortisone and cortisol) inhibit the inducible but not constitutive form of NOS in vitro in vascular endothelial cells (Radomski et al., 1990). The finding that the glucocorticoids inhibit the induction of NOS is of special significance since it may explain , at least in part, the therapeutic and toxic action of these compounds (Moncada et al.,1991).

1.4.3 Endothelium-dependent NO-independent relaxation

As well as relaxing vascular smooth muscle in an endothelium-dependent fashion (by releasing EDRF), acetylcholine and other muscarinic agonists also stimulate

endothelium-dependent smooth muscle hyperpolarization (Bolton et al., 1984; Chen et al., 1988; Vanhoutte, 1988; Garland & McPherson, 1992). Smooth muscle hyperpolarization to acetylcholine, like the relaxation, is mediated, at least in part, by the release of a diffusible factor (Feletou & Vanhoutte, 1988; Chen et al., 1991). In contrast to the relaxation induced by acetylcholine, hyperpolarization was not blocked by either oxyhaemoglobin or methylene blue, leading to the suggestion that a separate hyperpolarizing factor (EDHF) was released simultaneously with EDRF (Chen et al., 1988; Taylor & Weston, 1988). Furthermore, it was suggested that EDHF might have a predominant role in small arterioles (Nilsson et al., 1994).

1.4.4 Endothelium-dependent vasoconstriction

Besides EDRF, the endothelium can release substances that induce or augment contraction of the underlying smooth muscle. This suggestion was made first on the basis of experiments in canine femoral arteries where the removal of the endothelium reduced the contractions of potassium ions (De Mey & Vanhoutte, 1981). It was later found that in isolated arteries and veins, rapid endothelium-dependent increase in tension can be obtained which are explained best by the production of mediators that activate the underlying vascular smooth muscle. These substances have been called endothelium-derived contracting factor(s) (EDCF) (Fig. 5) (Vanhoutte, 1987; Vanhoutte et al., 1991; Thulesius et al., 1988). It has been found that in certain blood vessels (peripheral veins and large cerebral arteries), the endothelium releases vasoconstrictor substances among which are superoxide anion and thromboxane A_2. The release of these endothelium-derived vasoconstrictors may contribute to the autoregulatory processes. Other stimuli which induce the release of vasoactive substances from the endothelium include arachidonic acid, thrombin and anoxia (De Mey & Vanhoutte, 1982; Rubanyi & Vanhoutte, 1985). Moreover, acetylcholine and serotonin induced the release of EDCF in vessels from spontaneously hypertensive rats (Luscher & Vanhoutte, 1986). Rapid stretch was found to evoke a transient contraction in canine basilar arteries with, but not in those without endothelium. These observations suggest that endothelial cells can act as sensors of changes in distension (Vanhoutte, 1987).

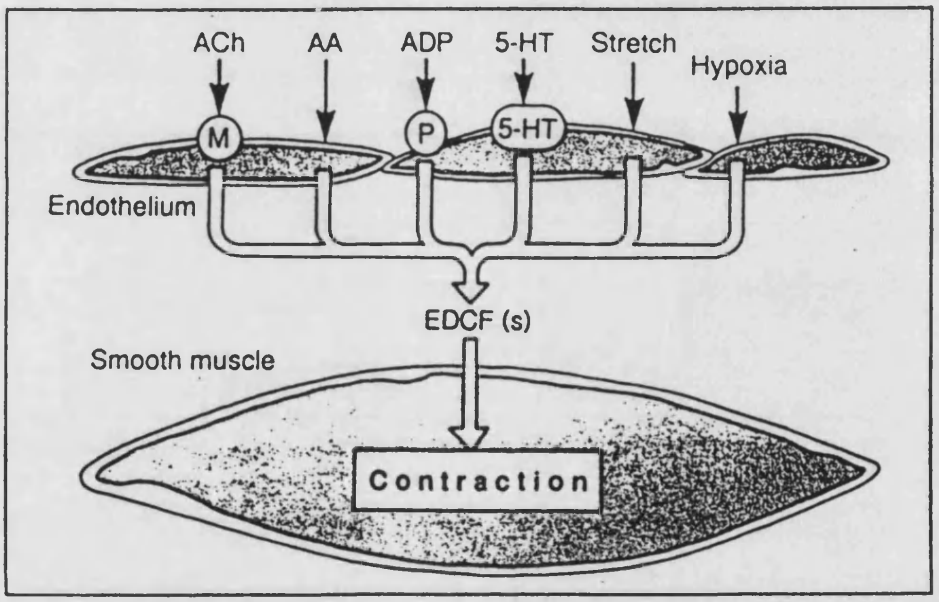


Fig. 5 A number of physicochemical stimuli, neurohumoral mediators [Ach, adenine diphosphate (ADP), 5-HT] and arachidonic acid (AA) can evoke endothelium-dependent contractions in certain blood vessels, presumably by the release of EDCF(s). (Vanhoutte et al., 1991)

1.5 Objectives of the study

It has been suggested that ovum maturation is a direct function of blood flow to the ovary (Virutamasen et al., 1971). It is expected that changes in blood flow would alter the delivery of hormones (FSH and LH) to the ovary. This in turn would influence the availability of hormones to receptors in the granulosa (which has no direct nerve supply) and, as a consequence, influence the onset of changes leading to follicular maturation. Since the major part of the adrenergic nerves in the ovary are perivascular, it seems logical to assume that a noradrenergic process would regulate tone of the ovarian vascular bed. Modulation of the adrenergic mechanism in the ovarian vascular bed would therefore be expected to interfere with tone of the vessels and hence blood flow to the ovary.

It has been reported that blood flow to the ovary is decreased by NA (Wurtman, 1964). This is consistent with an in vitro demonstration of NA-induced vasoconstriction in the perfused rabbit (Graham and Sani, 1971), ewe (Kuhl et al., 1974), and human (Varga et al., 1979) ovarian arteries. These studies did not elucidate the subtype(s) of α-adrenoceptors involved in NA-induced responses. Oriowo & Bevan (1986) have shown that α_1-adrenoceptors mediated NA-induced contraction of ring segments of the ovarian artery. However, reactivity of the main (muscular artery) may not always represent the pattern of reactivity in the whole vascular bed. For example, Graham & Sani (1971) showed that the perfused rabbit ovarian vascular bed was less sensitive to NA (compared with the ovarian artery). In the vascular bed, the arterioles are usually assumed to be the main site of agonist action. It has been shown that the arterioles differ from the muscular arteries in their Ca^{2+} handling mechanisms and in the distribution of receptors (Cauvin et al., 1984; Cauvin & Malik, 1984).

The objectives of this study are therefore to:

1. identify the adrenergic receptor sub-type(s) in the ovarian vascular bed,
2. study the influence of the vascular endothelium on responses to agonists in control and hormonally-treated rabbits,
3. study the signal transduction mechanism(s) mediating adrenergic responses,
4. study the effect of hormonal changes (including pregnancy) on α-adrenergic responses in the ovarian vascular bed.

CHAPTER TWO

MATERIALS & METHODS

2.1 Materials used in the study

2.1.1 Drugs

(-)Noradrenaline hydrochloride, phenylephrine hydrochloride, yohimbine hydrochloride, verapamil hydrochloride, polymyxin B sulfate, oestradiol-17ß, histamine hydrochloride, (±)propranolol hydrochloride, DOCA (deoxycorticosterone acetate), sodium nitroprusside (SNP), 6-hydroxy dopamine (6-OHDA), CHAPS (3-[(3-cholamidopropyl) dimethyl-ammonia] 1-propanesulfonate) and EGTA (ethyleneglycol-bis-(ß-amino ethyl ether) N,N,N',N'-tetra acetic acid) were obtained from Sigma chemicals, (St Louis, MO, USA).

LY-83,583 (6-phenylamino)-5,8-quinolinedione, phentolamine mesylate, genistein, nifedipine, oxymetazoline hydrochloride, ryanodine, HA-1077 (1-(5-isoquinolinylsulfonyl)-homopiperazine dihydrochloride), WB-4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-denzodioxane hydrochloride), SZL-49 (prazobind), CEC (chloroethylclonidine dihydrochloride), prazosin hydrochloride, UK14,304 (5-bromo-N-(4,5-dihydro-1H-imidazo;-2-yl)-6-quinoxalinamine), clonidine hydrochloride, (±)isoprenaline bitartrate, carbachol, aminoguanidine hemisulfate, N^G-nitro-L-arginine (L-NOARG), , N^G-nitro-D-arginine (D-NOARG),, methylene blue and S-(+)-niguldipine hydrochloride were obtained from Research Biochemicals International (RBI), Natick, MA, USA. L-ascorbic acid was obtained from BDH Chemicals Ltd. Tizanidine and SDZ NVI 085 were gifts from Sandoz pharma, Basle, Switzerland ; and methoxamine hydrochloride was from Borough Wellcome Company. Cocaine hydrochloride was a gift from Professor C. Pilcher, Pharmacology dept., Faculty of medicine, Kuwait University. U46619 was obtained from Cayman, B-HT 920 (5,6,7,8-tetrahydro-6-(2-propenyl)-4H-thiazolo[4,5-d]azepin-2-amine dihydrochloride) from Beecham and HCG (human chorionic gonadotrophin) from local pharmacy.

Stock solutions of SZL-49, prazosin, deoxycorticosterone acetate, nifedipine, U46619 and indomethacin were made in absolute ethanol and diluted fresh with Krebs' Henseleit solution (KH-solution) each day of the experiment, while all other drugs were dissolved in 0.1 N hydrochloric acid or distilled water.

2.1.2 Physiological solutions

In all experiments, KH-solution was used. The composition was as follows (mM): NaCl (118.3), KCl (4.7), $CaCl_2$ (2.5), $MgSO_4$ (1.2), $NaHCO_3$ (25), KH_2PO_4 (1.2) and glucose (11.2). The pH was 7.4. The solution was gassed with a mixture of 5% CO_2 and 95% O_2.

Different concentrations of KCl solution (20, 40, 60, 80 and 100 mmoles) were prepared by substituting KCl in the normal KH-solution.

Composition of 80mM KCl depolarized solution:

NaCl (118.3), KCl (80), $MgSO_4$ (1.2), $NaHCO_3$ (25), KH_2PO_4 (1.2) and glucose (11.2).

2.1.3 Choice of animal species

The investigations were carried out using the rabbit ovarian vascular bed. The choice of this vascular bed as the model was dictated by the following:

1. The rabbit is a non-cycling animal and therefore the influence of circulating hormones and hence variations in responses arising from varying levels of circulating hormones on agonist responses would be minimal.
2. Following coitus or HCG injection, ovulation takes place in the rabbit in about 12 hrs and therefore reactivity of the ovarian vascular bed during the periovulatory period can easily be studied.
3. The perfused rabbit ovary has been used extensively to study the ovulatory process in vitro (Wallach et al., 1978; Hamada et al., 1979; Kobayashi et al., 1981; 1983a; 1983b).
4. Finally, it is a convenient laboratory animal to handle.

2.1.4 Isolation of the ovarian vascular bed

Sexually mature female New Zealand White (NZW) rabbits, weighing 3.0-4.0 kg, were used in these experiments. The rabbits were anaesthetized with i.v. pentobarbitone sodium (50 mg/Kg) given i.v. via the marginal ear vein. Thereafter, the animal was exsanguinated by cutting the carotid artery. The abdominal cavity was opened up and the ovarian artery was identified (Fig. 6). The fatty tissue surrounding the ovarian bed was removed by pressing gently on both sides with the fingers. Then the vessel was cleared of as much fat as possible without damaging the artery. Branches of the ovarian artery were tied using surgical silk ligature (branches were

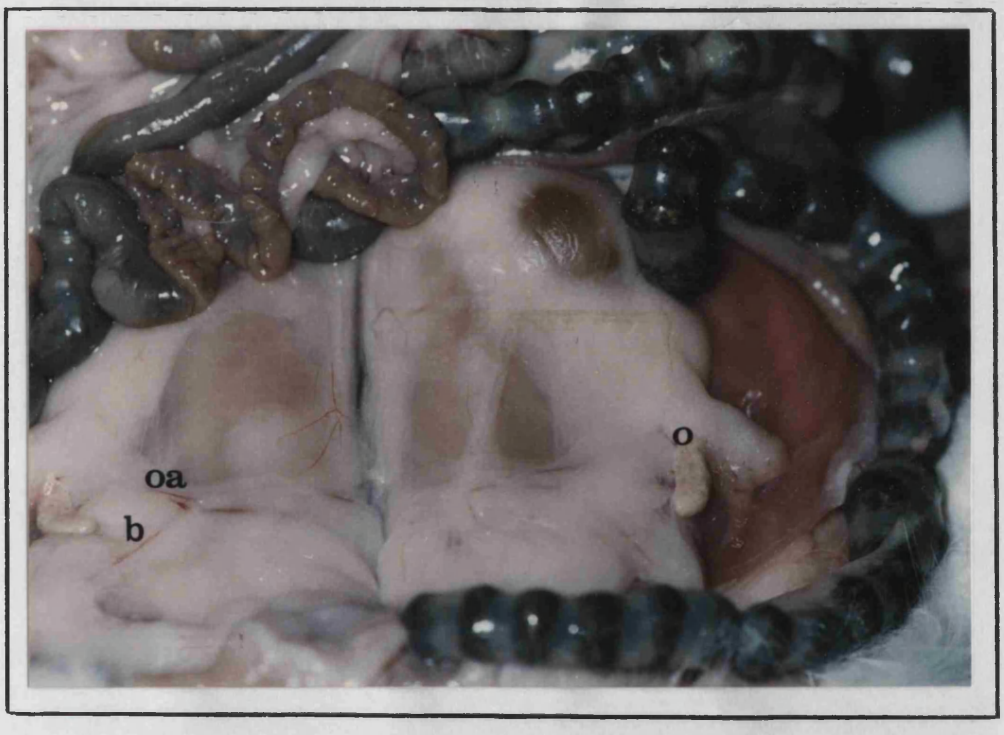


Fig.6 A photograph showing the rabbit ovarian vascular bed in situ; ovary (o), ovarian artery (oa) and branching of ovarian artery (b).

previously identified by injecting methylene blue dye into the ovarian artery through the abdominal aorta). The ovary was then separated from the adjacent tissue and made ready for isolation.

The ovarian artery was handled carefully, its origin was identified at its junction with the abdominal aorta, and the aorta was tied on both sides of the vessel. Finally the abdominal aorta was cut at both sides and the ovarian bed was isolated and transferred into a Petri dish containing KH-solution (Fig. 7). The ovarian bed was weighed before and after each experiment.

The ovarian bed was placed on a warm chamber filled with water circulating at 37°C and the ovarian artery was cannulated, using a polyethylene cannula (Fig. 8). The preparation was then perfused with KH solution (at 37°C), aerated with 95% oxygen and 5% carbon dioxide, delivered at a flow rate of 6 ml/min using a multichannel masterflex pump (7519-00). The perfusion pressure was recorded via a pressure transducer (SensoNor 840) connected to a Lectromed (2 channel recorder) (Fig. 9). The preparation was always left for at least 30 minutes to stabilize. A bolus injection of NA was usually given at the begining of the experiment as a test dose. Thereafter, successive doses of the agonists were given at regular intervals to generate a dose-response curve. This was repeated at least twice to ascertain reproducibility.

2.2 Experimental procedure

The reactivity of isolated vascular smooth muscle to NA is influenced by a number of factors including, uptake mechanisms (neuronal and extraneuronal); simultaneous activation of ß-adrenoceptors which by a process of physiological antagonism reduce the vasoconstrictor response; release of prostanoids which could result in either an enhancement or reduction of NA response depending on the relative proportions of vasoconstrictor and dilator prostanoids. Finally, reactivity is also modulated by endothelium-derived relaxing factor(s).

The primary objective of these preliminary studies was to examine the effect of blocking uptake processes, ß-adrenoceptors and generation of prostanoids on the vasoconstrictor responses to NA in an attempt to define the optimum conditions to be used in subsequent experiments.

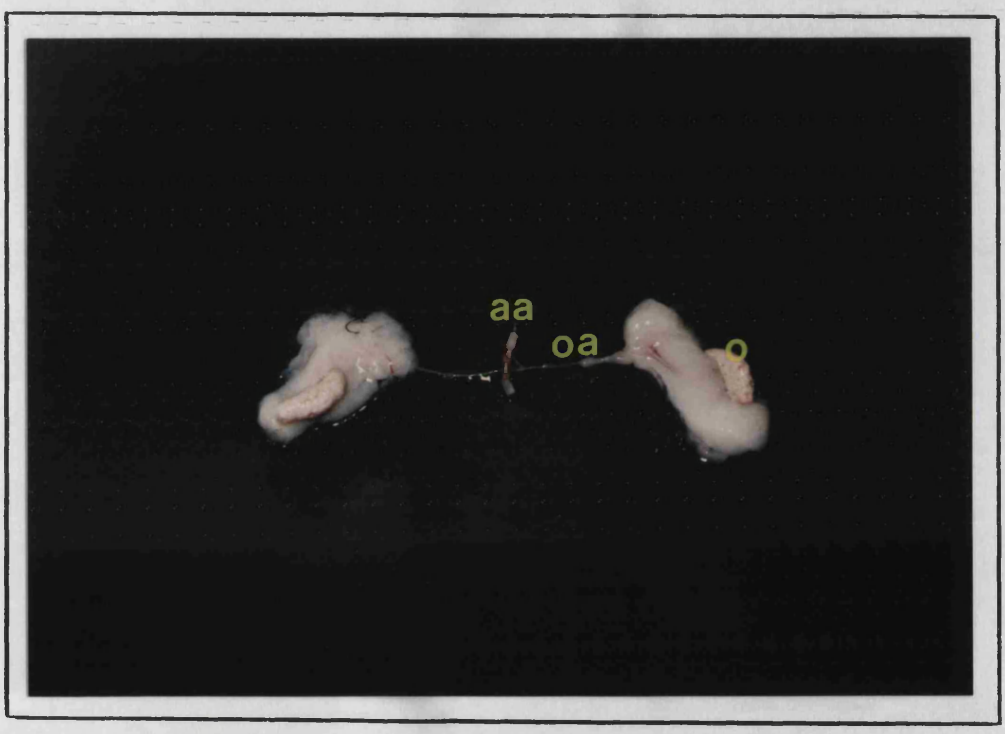


Fig.7 A photograph of an isolated rabbit ovarian vascular bed; ovary (o), ovarian artery (oa) and abdominal aorta (aa).

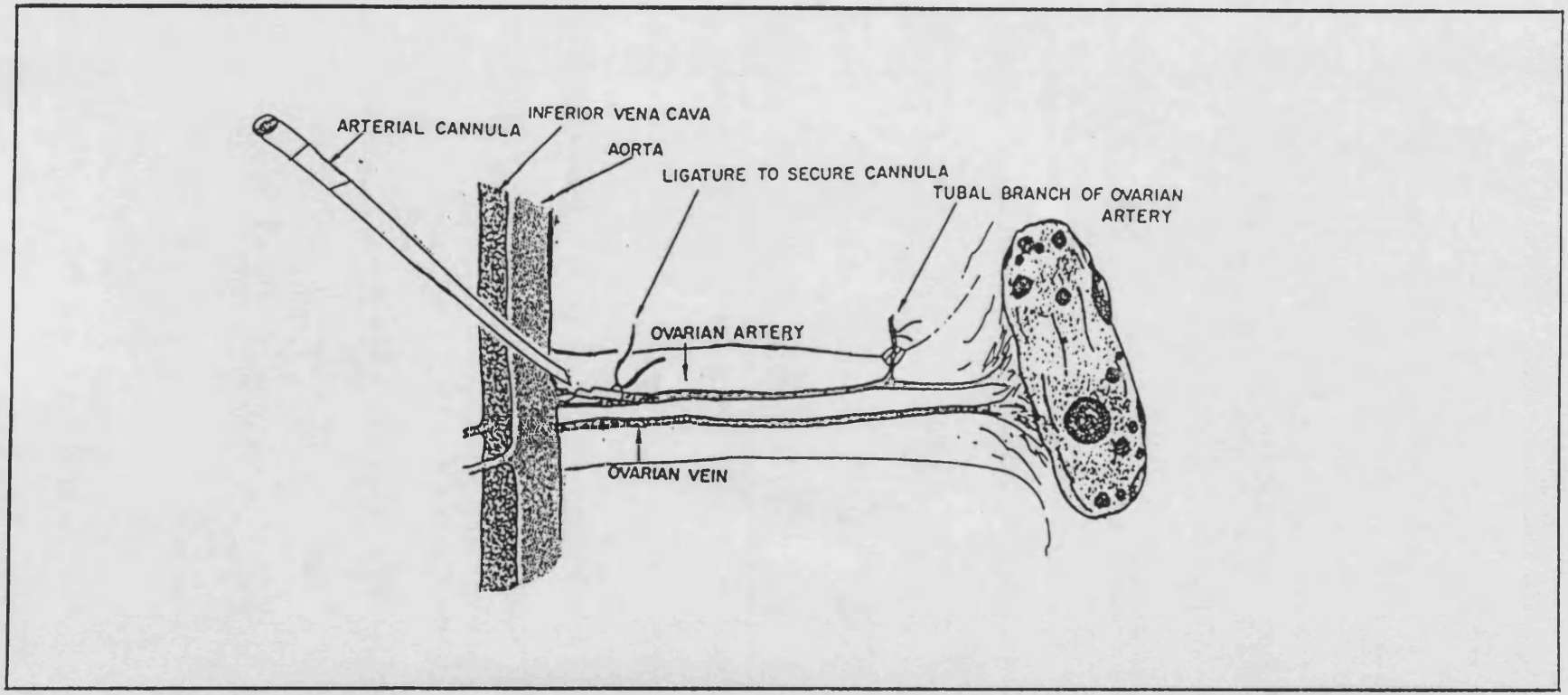


Fig.8 Rabbit ovarian vascular bed. The ovarian artery was cannulated at the junction of the abdominal aorta. (modified from Lambertsen et al., 1976)

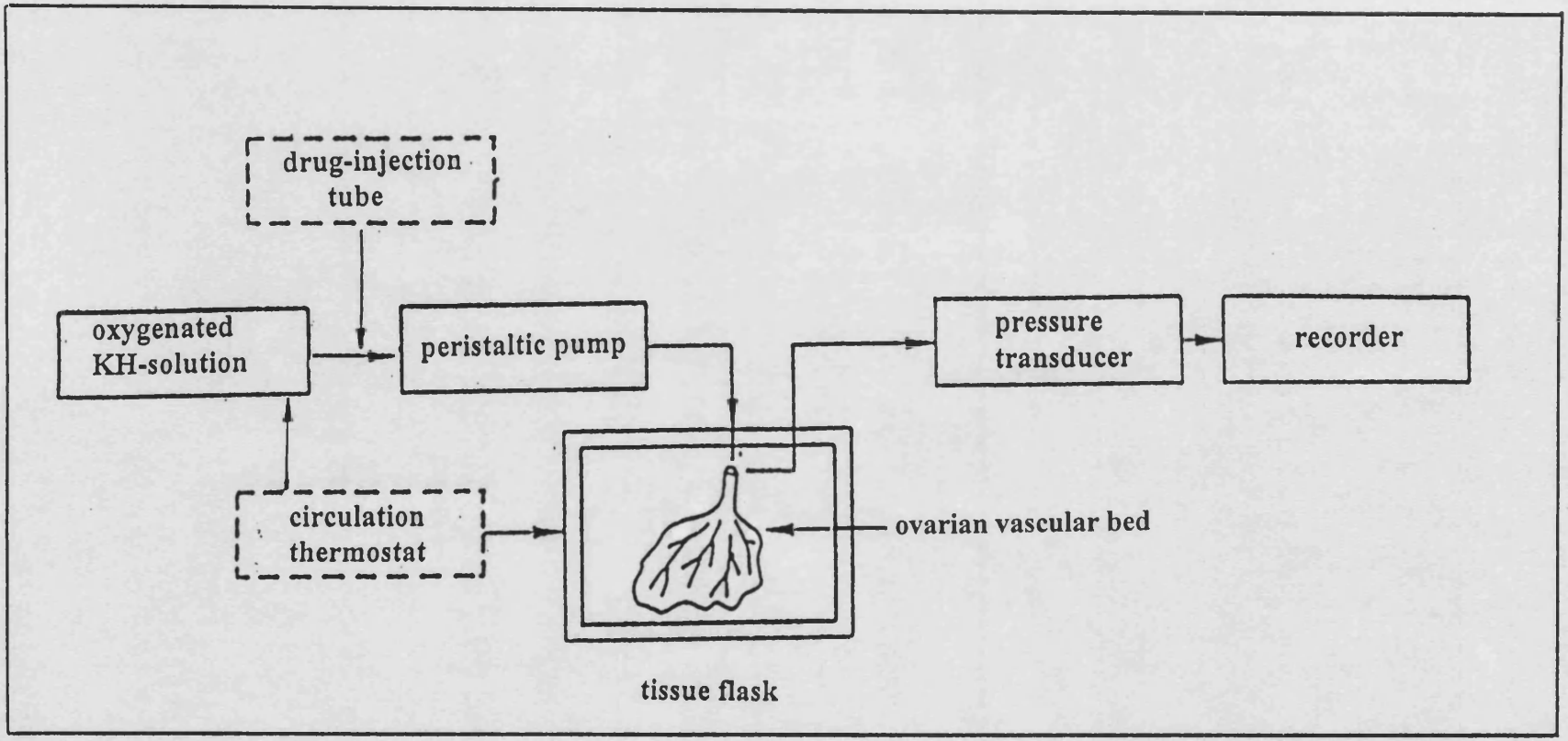


Fig.9 A schematic representation of the in vitro perfusion system used in this study.

2.2.1 Preliminary experiments

2.2.1.1 Influence of time on the responses to NA

One disadvantage of the perfused whole vascular bed is that with time, the tissue becomes oedematous and this could affect either the basal perfusion pressure or agonist response or both. Experiments were therefore designed to assess the responsiveness of the ovarian vascular bed to NA over a period of time. In this series of experiments, after the period of equilibriation up to 4 consecutive NA-dose response curves, at 45 min intervals, were obtained. In addition, the ovarian vascular bed was weighed before perfusion and at the end of the experiments. The difference in weight was taken as an index of oedema formation.

2.2.1.2 Effects of uptake and ß-adrenoceptor blockade on responsiveness to NA

After obtaining control NA dose-response curves, propranolol (10^{-6}M) was added to the perfusion fluid and allowed to equilibriate with the tissue for 30 min before re-establishing the NA dose-response curve. The same procedure was adopted in testing the influence of uptake blockers; cocaine ($uptake_1$) and DOCA ($uptake_2$) on NA-evoked vasoconstrictions. Only one inhibitor was tested on each preparation. However, in one series of experiments, the effect of a combined blockade of uptake and ß-adrenoceptors on noradrenaline-evoked vasoconstriction was tested by adding all the inhibitors, i.e. propranolol (10^{-6}M), cocaine ($3x10^{-6}$M) and DOCA (10^{-5}M) to the perfusion fluid and allowing a 30 min. contact time before repeating the NA dose-response curve.

2.2.1.3 Effects of indomethacin on NA-induced vasoconstriction

The influence of endogenously generated prostanoids in modulating the vasoconstrictor response to NA was tested. After obtaining a control NA-dose response curve, indomethacin ($5x10^{-6}$M) was added to the perfusion fluid and allowed to perfuse for 30 min before repeating NA-doses.

2.2.1.4 Effects of other agonists on NA-induced vasoconstriction

The effect of agonists other than adrenergic agonists on the ovarian vasculature was also studied. Dose-response curves were established for histamine, angiotensin II and bradykinin using a procedure to that used for NA.

2.2.1.5 Electrical stimulation of the perfused ovarian vascular bed

For electrical stimulation, the ovarian artery was passed through a platinum ring electrode. The electrode was placed close to the bifurcation of the artery into smaller branches. This is because there is evidence that adrenergic innervation increases as the vessel diameter decreases (Burnstock, 1975).

Preliminary experiments were also performed to determine the optimum conditions (voltage and pulse duration) for electrical stimulation. Briefly, electrically-induced vasoconstrictor responses (at a fixed frequency) were obtained at various voltages ranging from 30-70V in order to determine the maximum voltage for stimulation. This voltage was then used to determine the optimum pulse duration at which there will be no direct muscle stimulation. In these experiments, the preparation was stimulated at 70V, 40Hz (for 30 sec.) using pulse durations ranging from 0.5-3.0 msec.

2.2.2 Influence of the vascular endothelium

2.2.2.1 Effects of endothelial cell removal on agonist-induced responses

In this series of experiments, the ovarian vascular bed was perfused with KH-solution containing CHAPS 4.7 mg/ml for 30 seconds (Tesfamariam & Halpern, 1987; Moore etal., 1990), after which the tissue was perfused with normal KH-solution for 30 min before the NA dose-response curve was repeated. Loss of endothelial function was assumed when carbachol (1000 nmoles) failed to dilate the NA-preconstricted vascular bed.

2.2.2.2 Effects of NOS inhibitors on NA-induced vasoconstriction

2.2.2.2.1 Effects of L-NOARG and D-NOARG on NA-induced vasoconstriction

L-NOARG, an inhibitor of cNOS, was used to study the effect of the endothelium-derived NO on reactivity of the ovarian vascular bed to NA. Control dose response curves for NA were established as in the previous sets of experiments. Then, the tissue was perfused with KH-solution containing L-NOARG (10^{-6}M-10^{-4}M) for 30 min. Thereafter, and still in the presence of L-NOARG, a dose response curve for NA was re-established. In another series of experiments, D-NOARG at concentrations of 10^{-5}M and 10^{-4}M, was used.

Experiments were also performed to determine whether or not the effect of L-NOARG could be reversed by L-Arginine. Dose-response curves were obtained for NA in the

absence and in the presence of L-NOARG (10^{-5}M). Thereafter, L-arginine ($5x10^{-4}$M) was added to the KH-solution (containing L-NOARG, 10^{-5}M) and allowed to perfuse the ovarian bed for 30min before repeating NA dose-response curve.

2.2.2.2.2 Effects of aminoguanidine on NA-induced vasoconstriction

Control NA dose-response curve was established as usual. The ovarian vascular bed was then perfused for 30 min with aminoguanidine (10^{-5}M-10^{-4}M) before the NA dose-response curve was re-established. A similar set of experiments, was performed after removing the endothelium with CHAPS 4.7mg/ml, using aminoguanidine at a concentration of 10^{-4}M.

2.2.2.3 Effects of NOS inhibitors on agonist-induced vasodilator responses

In order to obtain dilator responses, the perfusion pressure was raised with NA (10^{-5}M) infusion. When a steady perfusion pressure was obtained, bolus injections of the agonists carbachol, isoprenaline and SNP were given and vasodilator responses were recorded. After obtaining control responses, L-NOARG (10^{-5}M) was added to the KH solution and was allowed to equilibriate with the tissue for 30 min before repeating agonist responses.

2.2.2.4 Effects of cyclicGMP inhibition on agonist-induced vasodilator responses

In order to determine whether or not vasodilator responses involved accumulation of cyclicGMP, the effect of cyclicGMP inhibitor, LY-83,583 was tested on carbachol-, isoprenaline- and SNP-induced vasodilator responses. Dose-response curves for carbachol were established after raising the basal perfusion pressure with NA (10^{-5}M). Thereafter, LY-83,583 (10^{-5}M) was added to the KH-solution and was allowed to perfuse the preparation for 10 min before repeating the carbachol dose-response curve. The cyclicGMP inhibitor was tested against only single doses of isoprenaline and SNP (100 nmoles). Another set of experiments was performed to verify the role of cyclicGMP in mediating the vasodilator responses of carbachol and SNP; using the cyclicGMP inhibitor, methylene blue. A similar protocol was followed as with LY 83583 after raising the basal perfusion pressure with NA (10^{-5} M). The preparation was perfused with methylene blue ($3x10^{-5}$ M) for 15 min before repeating agonsit-induced vasodilator responses.

2.2.3. Identification and characterization of adrenoceptors in the ovarian vascular bed

2.2.3.1 Identification of adrenergic receptors

A number of adrenergic agonists were used to identify the adrenergic receptors present in the ovarian vascular bed. The procedure consisted of establishing a control NA dose-response curve. This was to serve as the reference control curve against which other agonists would be compared. Thereafter, dose-response curves were established for the other agonists including methoxamine, oxymetazoline, phenylephrine, SDZ NVI 085, tizanidine, clonidine, B-HT 920 and UK 14, 304. It was not possible to test all the compounds on the same preparation, but in any experiment three or four compounds were tested in addition to NA which was the reference agonist. In all experiments, two consecutive dose-response curves were separated by 45-60 min intervals.

Isoprenaline, a non-selective ß-adrenergic agonist, was used to test for the presence of ß-adrenoceptors in the ovarian vascular bed. The procedure consisted of establishing a dose-response curve for isoprenaline in the "low tone preparation". Thereafter, perfusion pressure of the ovarian bed was raised by perfusing the preparation with KH-solution containing 10^{-5}M methoxamine or NA. After achieving a stable increase in perfusion pressure, increasing concentrations of isoprenaline were injected to establish a dose-response curve. To further confirm the presence of ß-adrenoceptors, the preparation was perfused with KH-solution (with 10^{-5}M methoxamine or NA) containing propranolol (10^{-6}M-10^{-5}M) for 30 min before re-establishing the isoprenaline dose-response curve.

2.2.3.1.1 Effects of prazosin and yohimbine on agonist-induced contractions

The effects of α -adrenoceptor subtype-selective antagonists on agonist-induced vasoconstriction were examined in order to characterize α-adrenergic receptor subtype(s) in the ovarian vascular bed. Antagonist potencies were expressed as pA_2 values determined according to the method described by Arunlakshana and Schild (1959), (pA_2 is the negative logarithm to base 10 of the concentration of an antagonist that makes it necessary to double the concentration of agonist needed to elicit a given submaximal response).

Briefly, after obtaining control responses to NA, the KH solution was changed to one containing various concentrations of prazosin (10^{-8}M-10^{-5}M). The antagonist was allowed to equilibriate with the tissue for 30 mins before re-establishing the NA dose-response curve. From the curves, ED_{50} values were obtained and these were used to calculate dose-ratios (DR). The concentration ratio is the ratio of the concentrations of NA giving an equal response (50%) in the presence or in the absence of the antagonist. A plot of log (DR-1) against antagonist concentration (M) was then made. The X-intercept of the regression line as well as the slope were obtained. The X-intercept is the pA_2 value corresponding to the antagonist concentration (M), which requires a doubling of the agonist concentration to produce the same response as in the absence of the antagonist. Antagonism was assumed to be competitive when the Schild regression line had a slope not significantly different from 1.0.

Other agonists were also tested in the presence of various concentrations of prazosin. One concentration of prazosin ($3x10^{-7}$M) was used against methoxamine and SDZ NVI 085 because a large suppression in the maximal agonist response was noticed after incubation with antagonist concentrations greater than or equal to $3x10^{-7}$M. In another series of experiments, yohimbine (10^{-7}M-10^{-5}M) was tested against NA and phenylephrine.

The effects of prazosin and yohimbine on vasoconstrictor responses evoked by electrical stimulation were also studied. In this series of experiments, responses were obtained in the absence, and in the presence of varying concentrations of the antagonist. Each antagonist concentration was allowed to perfuse the preparation for 30 min before stimulation.

2.2.3.2 Characterization of α_1-adrenoceptor subtypes

The α_1-adrenoceptor subtype(s) mediating agonist-induced vasoconstriction in the perfused ovarian vascular bed were investigated. This involved examining the effect of subtype-selective antagonists (competitive and alkylating) on agonist-induced vasoconstriction in the vascular bed.

2.2.3.2.1 Competitive antagonists

Following the period of equilibriation, two to three consecutive NA dose-response curves were obtained. The last curve was routinely used as the reference control curve after which, ascending concentrations of WB-4101 (10^{-8}M-10^{-5}M) were added to the

KH-solution perfusing the ovarian vascular bed. The antagonist was allowed to equilibriate with the tissue for 30 min before re-establishing the dose-response curve for NA. Antagonist potency was expressed as the pA_2 value obtained as described for prazosin. A similar experimental procedure was used to study the effect of other adrenoceptor agonists including phenylephrine, methoxamine, oxymetazoline and SDZ-NVI 085. Different concentrations of WB- 4101 were added to the KH-solution perfusing the ovarian vascular bed. The antagonist was allowed to perfuse the tissue for 30 min before re-establishing the agonists dose-response curves. Four concentrations of the antagonist were used against phenylephrine and oxymetazoline. Antagonist potency was expressed as pA_2 value obtained as described for prazosin. However only one concentration of WB-4101 ($3x10^{-7}M$) was used against methoxamine and SDZ-NVI 085 because concentrations more than $3x10^{-7}M$ significantly suppressed the maximum response to these agonists. In this case, antagonist potency was expressed as (pK_B) values determined according to the equation of Furchgott (1972):

$$K_B = \frac{[\text{antagonist}]\ M}{DR - 1}$$

The effect of WB-4101 on vasoconstrictor responses evoked by electrical stimulation was also investigated. The response to stimulation was obtained in the absence and also after incubation with varying concentrations of the antagonist. Each antagonist concentration was allowed to perfuse the preparation for 30 min before stimulation was applied.

2.2.3.2.2 Irreversible antagonists

Control dose-response curves for NA, phenylephrine, oxymetazoline, methoxamine and SDZ NVI 085 were established as usual. The ovarian vascular bed was then perfused with KH-solution containing a concentration of $5x10^{-6}M$ of the irreversible alkylating antagonist SZL-49 for 30 min after which the vascular bed was perfused with drug free KH-solution for at least another 30 min before repeating agonists dose-response curve. A similar procedure was also used to test the effect of CEC ($3x10^{-5}M$-$10^{-4}M$) on NA and oxymetazoline induced contractions of the ovarian vascular bed.

The effects of CEC and SZL-49 on vasoconstrictor responses evoked by electrical stimulation were studied. In this series of experiments, responses were obtained before

and after perfusing the preparation with SZL-49 ($5x10^{-6}$M) or CEC (10^{-4}M) as described above.

2.2.4 Signal transduction mechanisms

The objective of this set of experiments was to study the signal transduction mechanisms involved in the vasoconstrictor response of the ovarian vascular bed to several agonists.

2.2.4.1 Calcium-free solution

A control dose-response curve for NA was established as usual. The preparation was then incubated in Ca^{2+}-free KH-solution (with or without EGTA 2mM) for 30 min before re-establishing the NA dose-response curve.

In another set of experiments, the time-course of the loss of vasoconstrictor effect to NA in Ca^{2+}-free solution (with or without EGTA) was determined. A vasoconstrictor response to a submaximal dose of NA was obtained (300 nmoles) and this was repeated until constant. The normal KH-solution was then replaced with Ca^{2+}free-KH-solution (with or without EGTA) followed by bolus injections of NA at 10-15 min intervals (the first administration was given 5 min after perfusion with Ca^{2+}-free solution).

2.2.4.2 Calcium channel blocking agents

A control NA dose-response curve was established as usual. The ovarian vascular bed was then perfused with KH-solution containing verapamil (10^{-7}M-10^{-5}M) or nifedipine (10^{-8}M-10^{-5}M) for 30 min before repeating the NA dose-response curve. Since it has been shown in different preparations, that partial agonists are more susceptible to blockade by Ca^{2+} channel antagonists (Ruffolo & Nichols, 1988), the effect of the calcium channel blockers, verapamil and nifedipine were tested against vasoconstrictor responses induced by SDZ NVI 085, which is a partial agonist, in the ovarian vascular bed.

In another set of experiments, responses to calcium channel antagonists were tested against KCl-induced vasoconstriction. The ovarian vascular bed was injected twice with 200 μmoles of KCl solution at 5 min intervals to establish steady control responses. Thereafter, the preparation was perfused with KH-solution containing different concentrations of verapamil and nifedipine for 30 min before repeating the injection of KCl solution.

2.2.4.3 Effects of inhibitors of intracellular calcium release

In order to determine whether a release of intracellular Ca^{2+} was involved in NA-induced vasoconstriction, the effect of ryanodine on NA-induced vasoconstriction was examined. The protocol was as follows. After establishing a NA dose-response curve, the ovarian vascular bed was then perfused with KH-solution containing ryanodine (10^{-5}M) for 30 min before repeating the NA dose-response curve.

2.2.4.4 Effects of protein kinase C inhibitors

After establishing control NA dose-response curves, the ovarian vascular bed was then perfused with KH-solution containing HA-1077 ($3x10^{-7}$M-$3x10^{-6}$M) for 30 min before repeating the NA dose-response curve. The same procedure was used to study the effects of polymyxin B (10^{-6}M-10^{-4}M). Another set of experiments involved depolarization of the ovarian vascular bed with 80 mM KCl depolarized KH-solution. A single dose of $CaCl_2$ solution (100 μM) was given at 15 min interval antil a steady and reproducible response was established. Thereafter, the preparation was perfused with the PKC inhibitors, HA-1077 ($3x10^{-7}$ M -3 $x10^{-6}$ M) and polymyxin B (10^{-6}M - 10^{-4}M). Perfusion with the PKC inhibitors was allowed for 30 min at each concentration, before repeating the injection with $CaCl_2$.

2.2.4.5 Effects of tyrosine kinase inhibitor

In this series of experiments, control dose-response curves were established for NA in the perfused ovarian vascular bed. Thereafter, the preparations were perfused with KH-solution containing genistein (10^{-6}M-10^{-5}M) for 30 min before repeating the NA dose-response curves.

2.2.5 Effect of hormonal changes on vascular reactivity

2.2.5.1 Effect of ovulation induction with HCG

A group of 6 NZW rabbits weighing 3.0-4.0 Kg were used for this study. Each rabbit was injected with 100 I. U. of HCG intravenously (Greenwald, 1963; Shmidt et al., 1985), 12 hours prior to sacrificing it for experiment.

2.2.5.2 Effect of 17-ß-oestradiol

A group of 5 NZW rabbits (3.0-4.0 Kg) were injected intramuscularly with 5mg/rabbit of oestradiol 17ß-propionate and used one week later.

In another sereis of experiments, a dose-response curve for NA was established as usual. Thereafter, 17-ß-oestradiol was added to the KH-solution perfusing the ovarian vascular bed at concentrations of $3x10^{-9}$M- $3x10^{-5}$M. The ovarian vascular bed was incubated for 30 min with these different concentrations before repeating the NA dose-response curve.

2.2.5.3 Effect of pregnancy

A group of 20 NZW rabbits (3.0-4.0 Kg) were mated and separated individually. After three weeks of gestation, the animals were sacrificed and the ovarian vascular bed was isolated in the usual manner.

2.2.5.4 Experimental procedure

The following procedure was used to study the rabbit ovarian vascular bed in these different groups of the rabbits. Vasoconstrictor responses to NA were recorded as usual. Following the NA control curve, dose-response curves were also established for oxymetazoline and histamine. The influence of the endothelium in the different groups of the treated and pregnant rabbits was studied, where the effect of L-NOARG (10^{-5}M) on NA-induced vasoconstriction was studied. After establishing a control NA-dose-response curve, the preparation was treated with L-NOARG (10^{-5}M) for 30 min before repeating the NA dose-response curve. In another set of experiments, the basal perfusion pressure was raised by NA (10^{-5}M) to study the relaxant responses to carbachol and SNP before and after incubation with L-NOARG (10^{-5}M). Regarding the pregnant rabbits, they were compared with the controls for the idetification of α-adrenoceptors. The ovarian vascular bed was incubated either with yohimbine (10^{-5}M) or prazosin (10^{-7}M) for 30 min before repeating the NA dose-response curve. Vasoconstrictor responses to NA were also tested after treating the preparations with

CEC ($3x10^{-5}$M) or SZL-49 ($5x10^{-6}$M). The influence of the endothelium on the partial agonist, oxymetazoline, was also studied in the pregnant rabbits, using L-NOARG (10^{-5} M).

2.3 Statistical analysis

Data are presented as mean $\pm$ s.e. of 'n' number of experiments. Where necessary, mean values were compared using Student's t-tests or paired as appropriate. The difference was considered to be significant when p value was less than 0.05. The degree of significance is indicated as follows:

$p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

CHAPTER THREE

RESULTS

3.1 Preliminary experiments

3.1.1 Influence of time on the responses to NA

NA (0.1 - 1000 nmoles) produced a dose dependent vasoconstriction in the perfused rabbit ovarian vascular bed. The vasoconstricting effect of NA was maintained over a 5hr period as concentration response curves established at 45min intervals were reproducible (Fig.10). Responsiveness of the preparation usually persisted for as long as 7-8 hr of perfusion. Reproducibility and stability of response of the preparation was found adequate for studying the the reactivity of ovarian vascular smooth muscle. The mean basal perfusion pressure was 41.7±3.3 mmHg (n=20). There was a significant increase in weight of the ovarian bed taken at the end of the experiment (1.1gm ± 0.2 in the beginning, compared to 1.7 gm ± 0.2 at the end of the experiment) indicating that there was some oedema formation. This was however, not accompanied by a change in the basal perfusion pressure or the response of the vascular bed to agonists

3.1.2 Effects of uptake inhibitors and ß-adrenoceptor blockade on responsiveness to NA

Neither DOCA (10^{-5}M) nor cocaine ($3x10^{-6}$M) or propranolol (10^{-6}M) altered the basal perfusion pressure of the ovarian vascular bed. A combination of them (the three inhibitors) did not significantly enhance NA response in the perfused ovarian vascular bed ($p > 0.05$) (Fig. 11).

3.1.3 Effects of indomethacin

A possible role for prostanoids in modulating vasoconstrictor response to NA was also examined. After obtaining a control NA dose-response curve, indomethacin ($5x10^{-6}$M) was included in the KH-solution and allowed to perfuse the preparation for 30 min before repeating NA dose-response curve. The results showed that indomethacin ($5x10^{-6}$) did not enhance NA-induced pressor responses (Fig. 12)

3.1.4 Effects of other agonists

Several agonists were tested for their vasoconstrictor responses on the perfused rabbit ovarian vascular bed, apart of NA. Different concentrations of KCl (20, 40, 60, 80 & 100 mmoles) were perfused through the ovarian vascular bed until a maximum constrictor level was established, before returning back to the normal KH-solution. A dose-dependent response was obtained for KCl (Fig. 13). The KCl response at 80

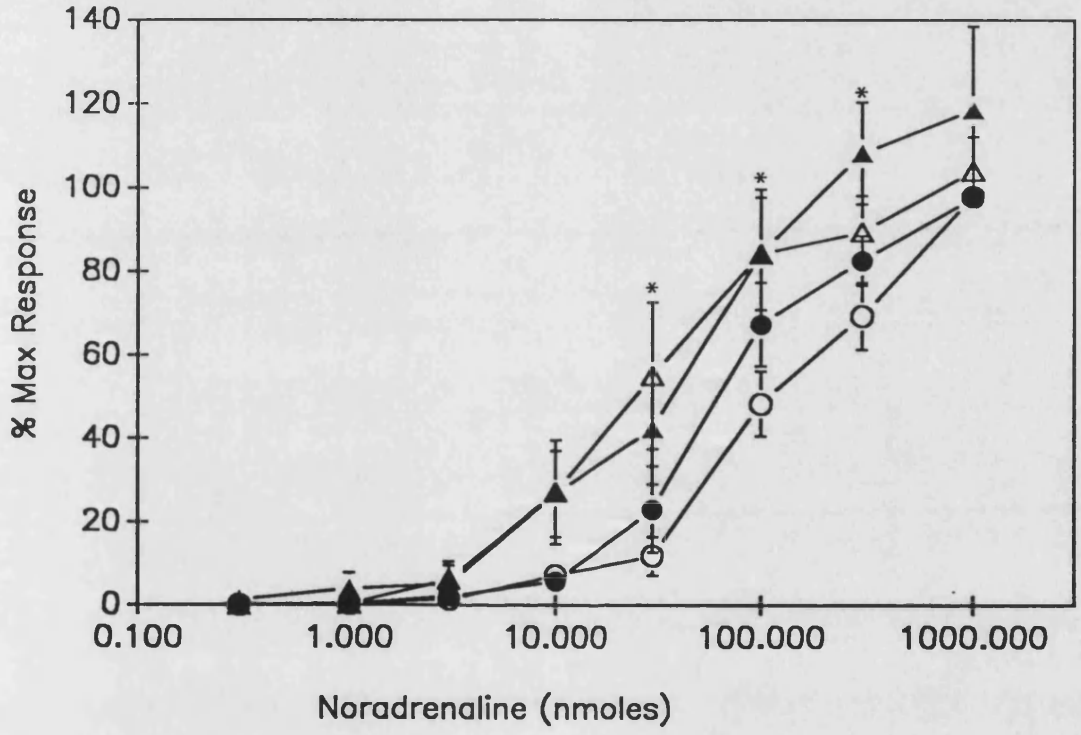


Fig.10 Consecutive dose-response curves to NA obtained at 45 min intervals in the perfused rabbit ovarian vascular bed (mean±se, n=5-7).
(O) first, (●) second, (Δ) third and (▲) fourth trials. (* $p < 0.05$)

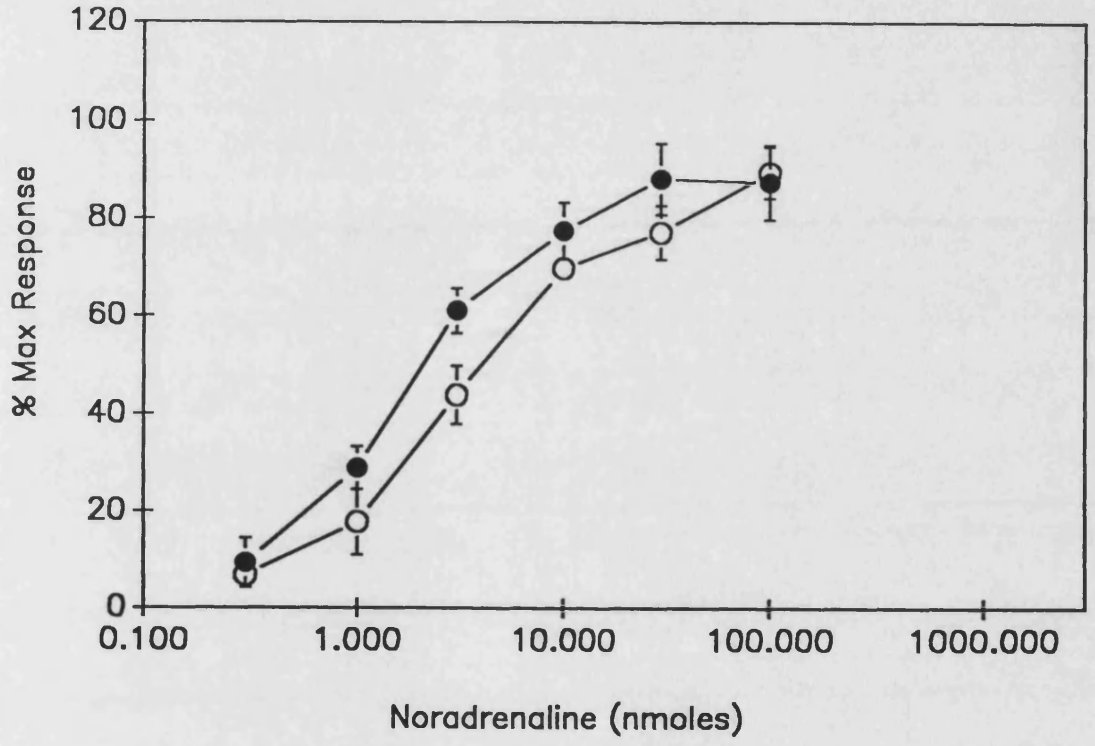


Fig.11 NA-induced vasoconstriction in the perfused rabbit ovarian vascular bed (O) is not affected by a combination of propranolol, DOCA and cocaine (●) (mean±se, n =3-4).

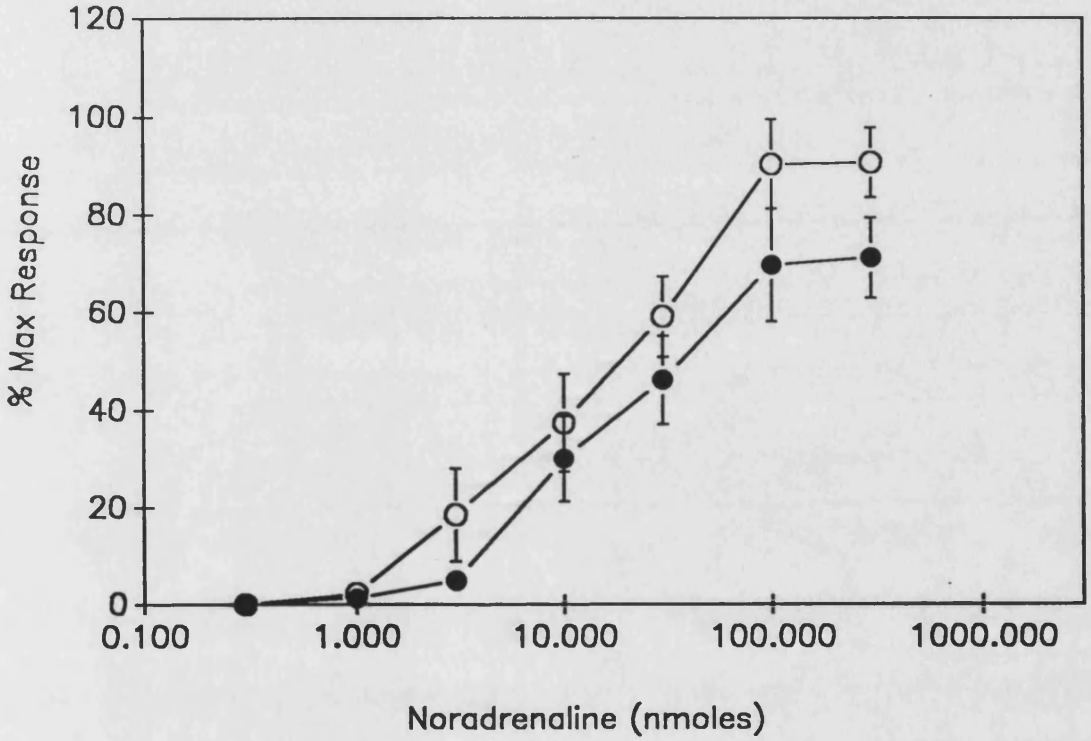


Fig.12 Effect of indomethacin, $5x10^{-6}$M (●) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).

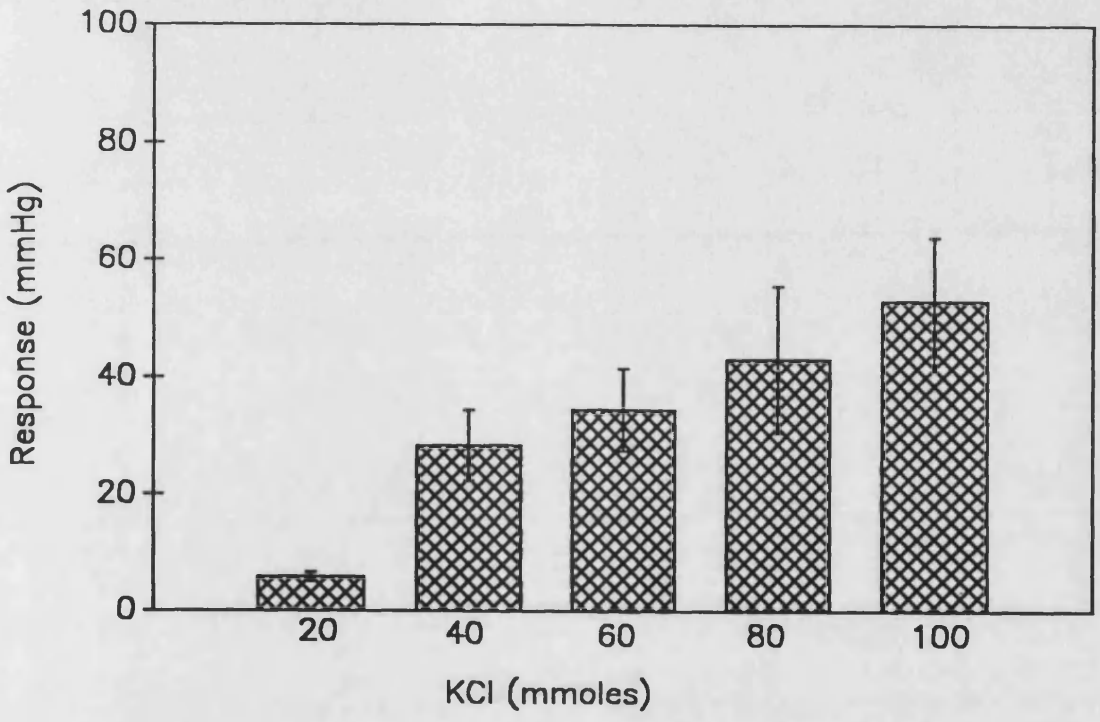


Fig.13 KCl-induced vasoconstrictor response in the perfused rabbit ovarian vascular bed (mean±se, n=4).

mmoles corresponded to 81.1% of maximal response and was chosen to be used in the subsequent experiments involving tests for the integrity of endothelium.

Vascular responses to bradykinin, angiotensin II and histamine were also investigated (Fig. 14). Bradykinin had no effect on the ovarian vascular bed unless the basal tone was raised with NA (10^{-5}M).

3.1.5 Effects of electrical stimulation

There was a voltage-dependent (at a fixed frequency and pulse duration) vasoconstrictor response to electrical stimulation. As shown in Figure 15a, the maximal vasoconstriction was produced at 60-70 volts. Therefore, supramaximal voltage (70V) was chosen and used in the subsequent experiments. A frequency-response relationship at 70 volts and 0.5 msec, revealed that a frequency of 40 HZ produced a submaximal response (Fig. 15b). In the second series of experiments, different pulse durations (0.5-3.0 msec) were tested. The results showed that electrical stimulation produced a reliable reproducible response at 0.5 msec pulse duration. The response increased with increasing pulse duration up to 3 msec which was the highest pulse duration tested. In order to confirm that the response to electrical stimulation was neurogenic, tetrodotoxin ($3x10^{-7}$M) was included in the perfusion fluid and allowed to perfuse the tissue for 30 min before repeating electrical stimulation. It was observed that only responses at 0.5 msec pulse duration were abolished by TTX (Fig. 16) while at other pulse durations, the responses were resistant to TTX, indicating direct muscle stimulation. Accordingly, the parameters chosen to produce optimum response to electrical stimulation were a voltage of 70V and a pulse duration of 0.5 msec. A typical trace of the vasoconstrictor response of the ovarian vascular bed to electrical stimulation (at 70 V, 0.5 msec and 40 HZ) is shown in Figure 17. The maximum increase in vasoconstrictor response to electrical stimulation at optimum conditions was 28.4 % of the maximum response to NA.

In order to determine whether or not the response to electrical stimulation was adrenergic in origin, the following experiments were performed. After obtaining control responses to electrical stimulation, the ovarian vascular bed was perfused with KH-solution containing 6-OHDA (10^{-4}M) (Karaki et al., 1988b) for 30 min and then perfusing with normal KH-solution (i.e. 6-OHDA free) for another 30 min before repeating electrical stimulation. Ascorbic acid was added to the 6-OHDA containing

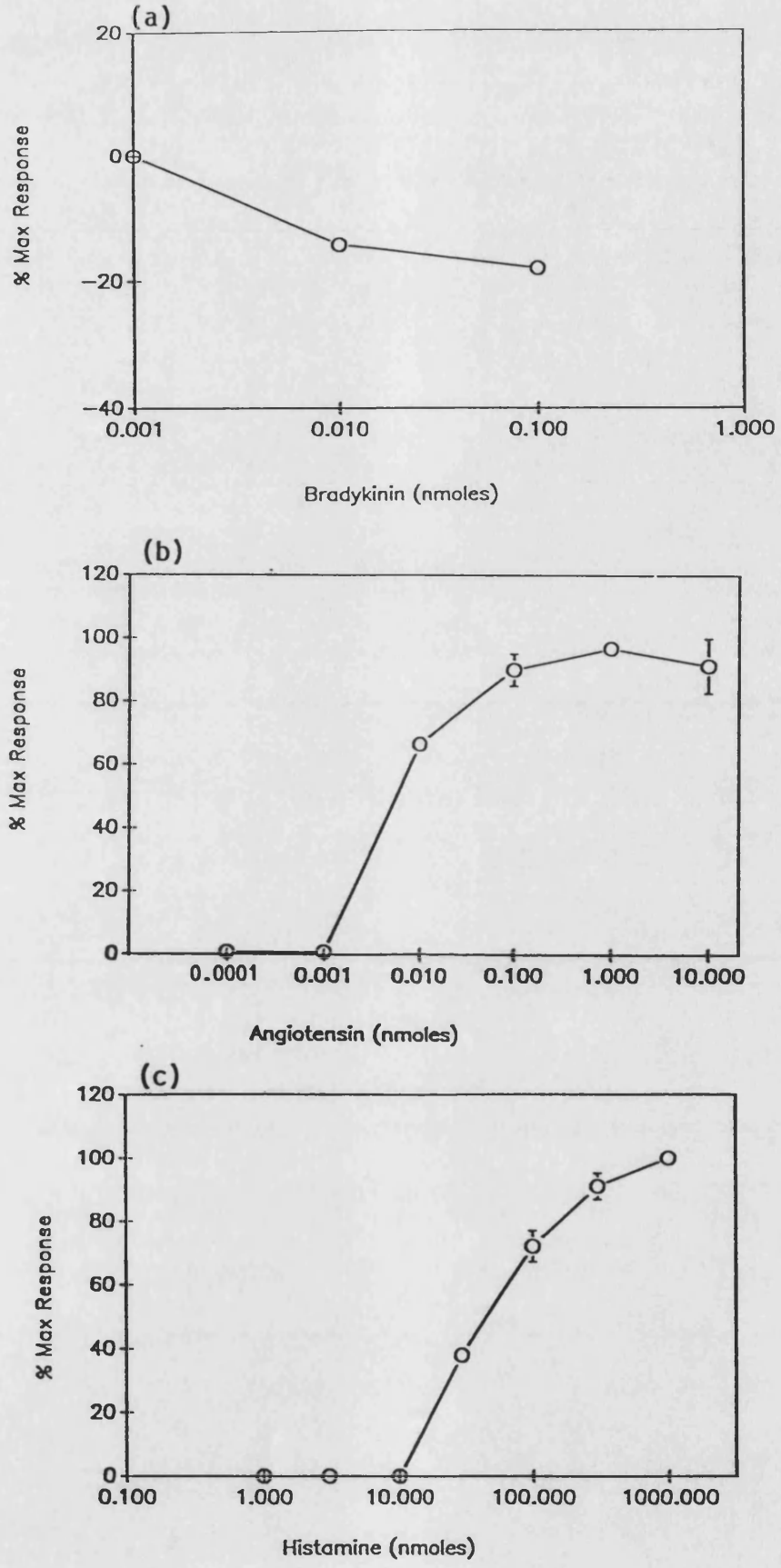


Fig.14 Vasodilator and vasoconstrictor responses of the perfused rabbit ovarian vascular bed to different agonists (mean±se, n=4).

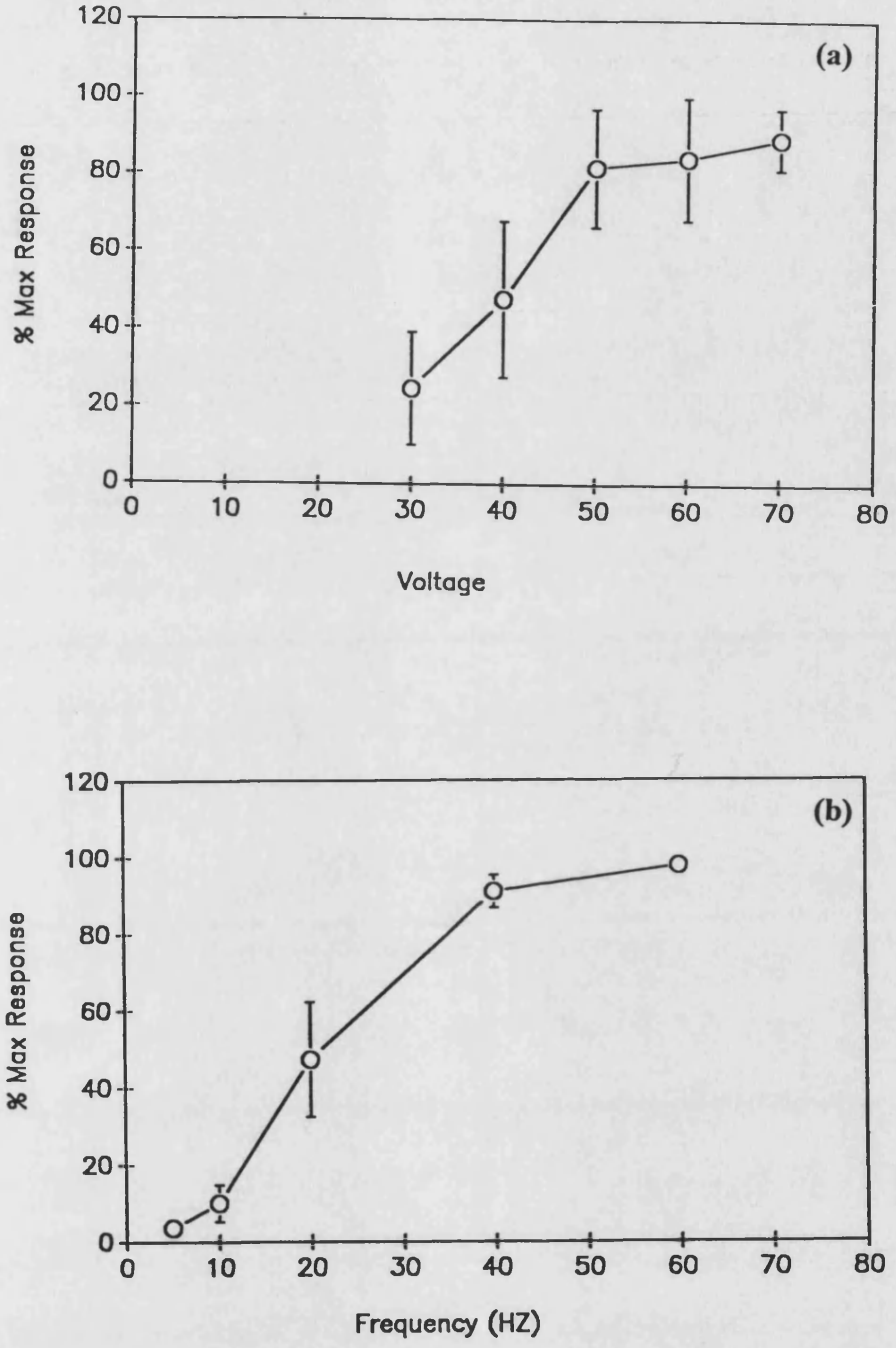


Fig.15 Vasoconstrictor response of the ovarian vascular bed to electrical stimulation, (a) voltage-response relationship (b) frequency-response relationship (mean±se, n=4).

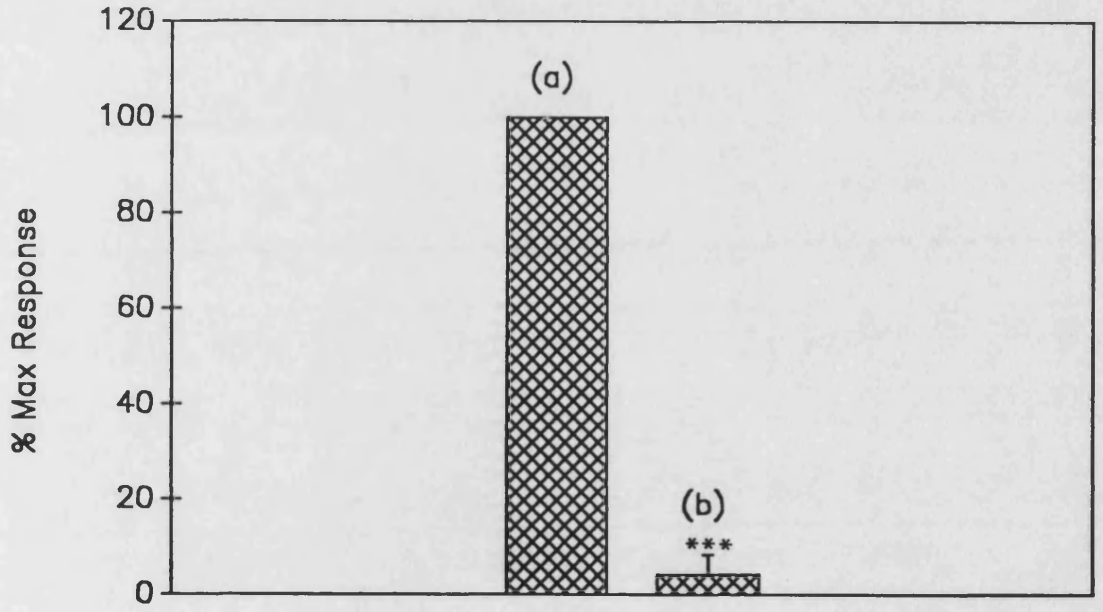


Fig.16 Vasoconstrictor response of the perfused rabbit ovarian vascular bed to electrical stimulation (70 V, 0.5 msec & 40 HZ) before (a) and after (b) TTX $3x10^{-7}$M (mean±se, n=4). (*** $p < 0.001$)

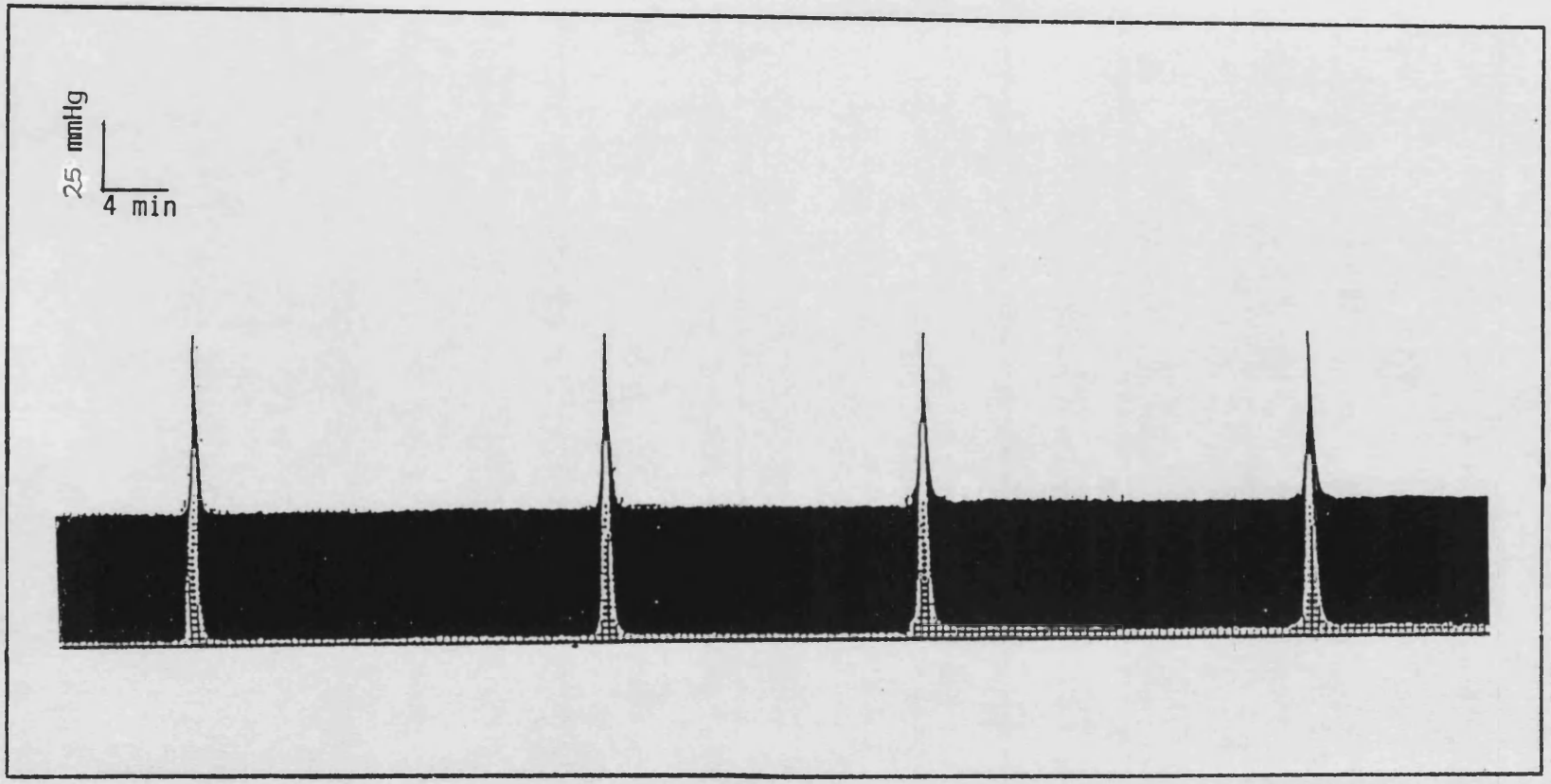


Fig.17 A typical trace of the electrically stimulated perfused rabbit ovarian vascular bed at optimum conditions.

KH-solution to prevent oxidation. As shown in Figure 18a, the response to electrical stimulation was abolished after treatment with 6-OHDA. Tyramine-induced vasoconstriction was also abolished (Fig. 18b).

3.2 Influence of the endothelium

3.2.1 Effects of endothelial cell removal on agonist-induced responses

In all cases, perfusion with CHAPS (4.7 mg/ml) produced a small elevation in the basal perfusion pressure. To confirm that perfusion with CHAPS did remove the endothelium, vasodilator responses to carbachol (on preparations pre-constricted with NA) were tested before and after treatment with CHAPS. There was an attenuation of carbachol-induced responses after CHAPS, confirming loss of the endothelium (Fig. 19). Endothelium removal with CHAPS had no effect on the vasoconstrictor responses to low doses of NA, but there was a significant depression ($p < 0.01$) of the responses to high doses of NA (ED_{50} = 17.5 nmoles before vs. 7.2 nmoles after treatment with CHAPS, maximum response was 100% & 54.6% respectively) (Fig. 20a). Because CHAPS (4.7 mg/ml) reduced the response to NA at higher concentrations, some experiments were carried out using a lower concentration of CHAPS (3.0 mg/ml). The results showed that this concentration of CHAPS did not affect NA-induced pressor effects, neither did attenuate carbachol-induced relaxation. A concentration of 4.7 mg/ml was therefore used in all experiments involving denudation of the endothelium.

In order to determine whether the reduced vasoconstrictor effect of NA, following treatment of the ovarian vascular bed with CHAPS, resulted from non-specific tissue damage, vasoconstriction to KCl before and after CHAPS treatment was also examined. The ovarian vascular bed was perfused with 80 mmoles KCl solution before and after CHAPS. The results summarized in Figure 20b, show that the vasoconstrictor effect of KCl was not affected by pretreatment with CHAPS.

3.2.2 Effects of NOS inhibitors on NA-induced vasoconstriction

3.2.2.1 Effects of L-NOARG and D-NOARG on NA-induced vasoconstriction

L-NOARG (10^{-6}M-10^{-4}M) alone did not alter the basal perfusion pressure. However, L-NOARG significantly ($p < 0.05$) potentiated the vasoconstricting effect of NA, which was only significant at 10^{-5}M (ED_{50} = 25.1±6.0 & 9.8±0.7 nmoles and

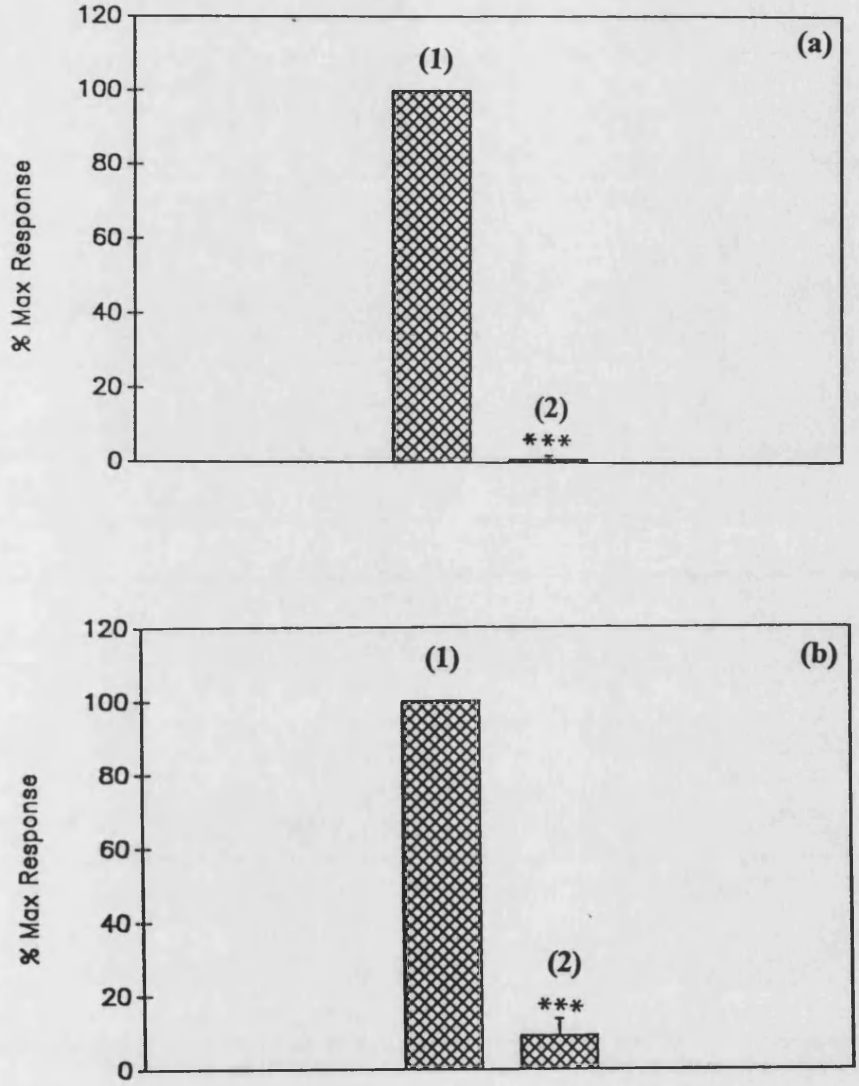


Fig.18 Vasoconstrictor response of the perfused ovarian vascular bed to (a) electrical stimulation and (b) tyramine; (1) before and (2) after 6-OHDA (10^{-4}M). Perfusion pressure was raised with NA (10^{-5} M). (*** $p < 0.001$)

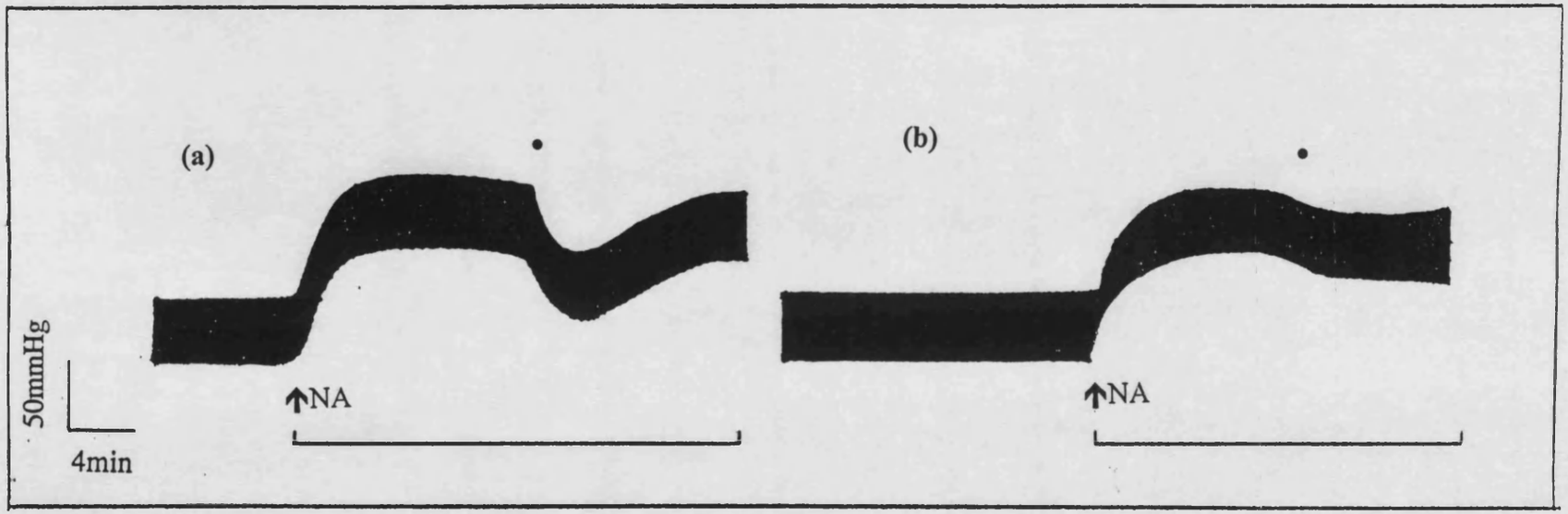
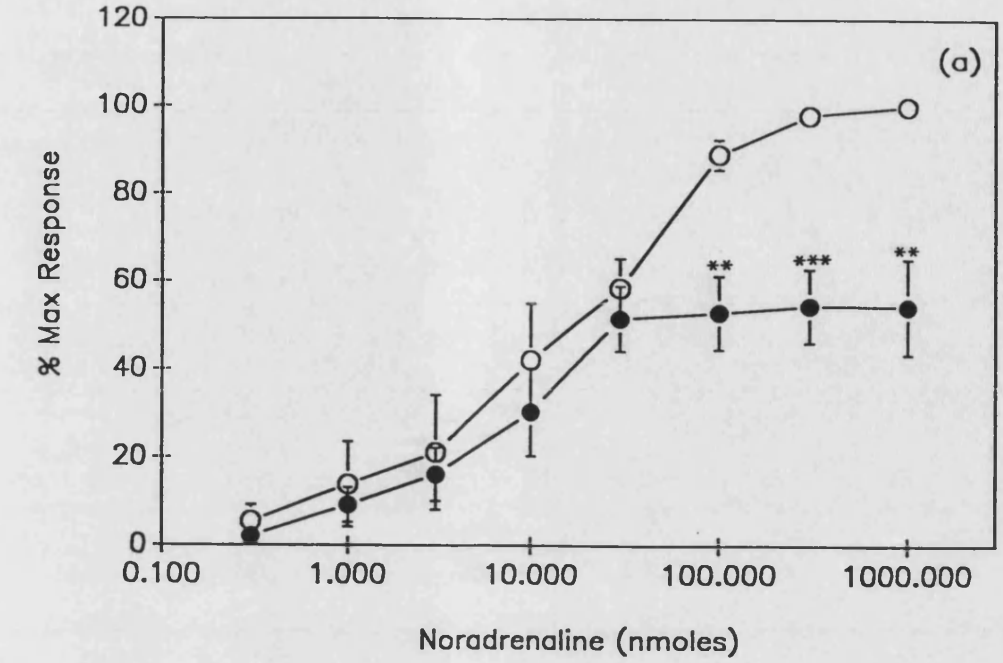


Fig.19 A typical trace of carbachol-induced vasodilatation (a) before and (b) after removal of the endothelium (with CHAPS) in the perfused rabbit ovarian vascular bed.
[• represents 1000 nmoles carbachol]



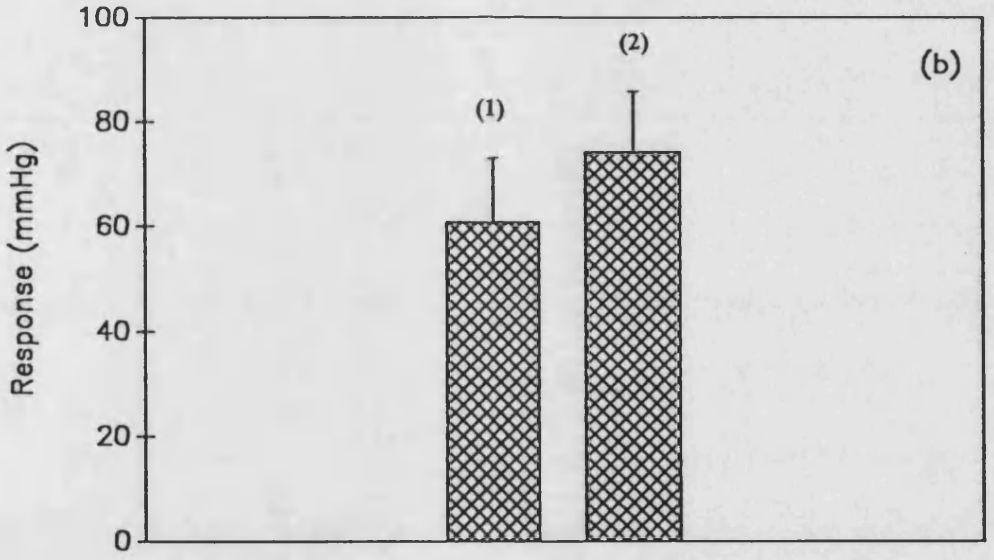


Fig. 20a NA-induced vasoconstriction in the perfused rabbit ovarian vascular bed before (O) and after (●) treatment with CHAPS, 4.7 mg/ml for 30 sec (mean±se, n=4).

Fig. 20b KCl-induced vasoconstriction in the perfused rabbit ovarian vascular bed (a) before and (b) after treatment with CHAPS 4.7 mg/ml for 30 sec (mean±se, n=5).

maximum response was 100% & 126.8% before and after treatment with 10^{-5}M L-NOARG, respectively). Maximum potentiation was observed at a concentration of 10^{-5}M (Fig. 21a). However, D-NOARG (10^{-5}M and 10^{-4}M) did not increase the vasoconstrictor response to NA, in fact it inhibited them. Both concentrations of D-NOARG were equi-effective (Fig. 21b).

The response to NA was not affected by incubation with L-NOARG (10^{-5}M) after endothelium removal with CHAPS (Fig. 22).

L-Arginine ($5x10^{-4}$M) partially reversed the potentiating effect of L-NOARG (10^{-5}M) on NA-induced vasoconstriction (Fig. 23).

3.2.2.2. Effects of aminoguanidine on NA-induced vasoconstriction

Aminoguanidine (10^{-5}M and 10^{-4}M) did not affect the basal perfusion pressure of the ovarian vascular bed on the NA-induced vasoconstrictor responses in endothelium-intact (Fig. 24) or endothelium-denuded preparations.

3.2.3 Effects of NOS inhibitors on agonist-induced vasodilator responses

Carbachol (1-1000nmoles), isoprenaline (1-1000nmoles) and SNP (1-1000nmoles) produced reproducible dose-dependent vasodilator responses in the perfused rabbit ovarian vascular bed in which perfusion pressure was raised with NA, 10^{-5}M. Addition of L-NOARG (10^{-5}M) further increased the perfusion pressure, consistent with inhibition of NO synthase activity. At this concentration (10^{-5}M) however, L-NOARG did not significantly affect the vasodilator responses of carbachol in about 60% of the experiments (Fig. 25), while in the remainder of experiments there was a reduction in the vasodilator response ($p < 0.05$). A dose response curve for the overall response to carbachol after L-NOARG (10^{-5}M) is shown in Figure 26. On the other hand, vasodilator responses to SNP and isoprenaline were maintained after treatment with L-NOARG (10^{-5}M).

3.2.4 Effects of cyclicGMP inhibition on agonist-induced vasodilator reponses

Relaxant responses to carbachol were significantly inhibited after perfusion with methylene blue ($3x10^{-5}$ M) ($p < 0.01$) or LY 83583 10^{-5}M ($p < 0.001$) (Fig. 27a). However, the relaxant responses to SNP and isoprenaline (1000 nmoles) were not significantly affected by LY 83583 (Fig. 27b). Methylene blue, another cyclicGMP inhibitor, produced a significant attenuation of carbachol-induced vasodilator response.

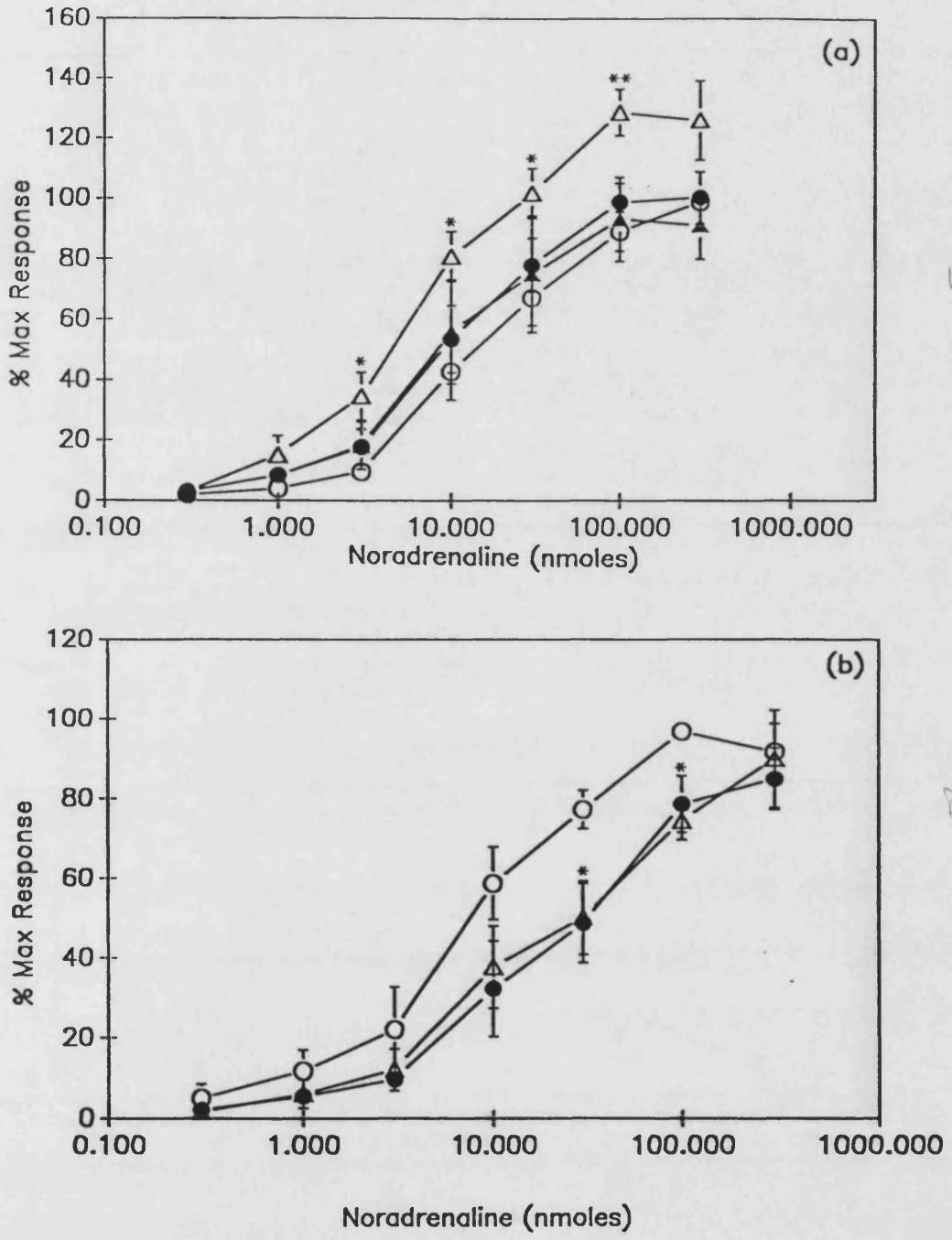


Fig.21 Effect of (a) L-NOARG and (b) D-NOARG on NA-induced vasoconstriction in the perfused rabbit ovarian vascular bed; control (O), 10^{-6}M (●), 10^{-5}M (Δ) and 10^{-4}M (▲) (mean±se, n=4-5). (* $p < 0.05$ & ** $p < 0.01$)

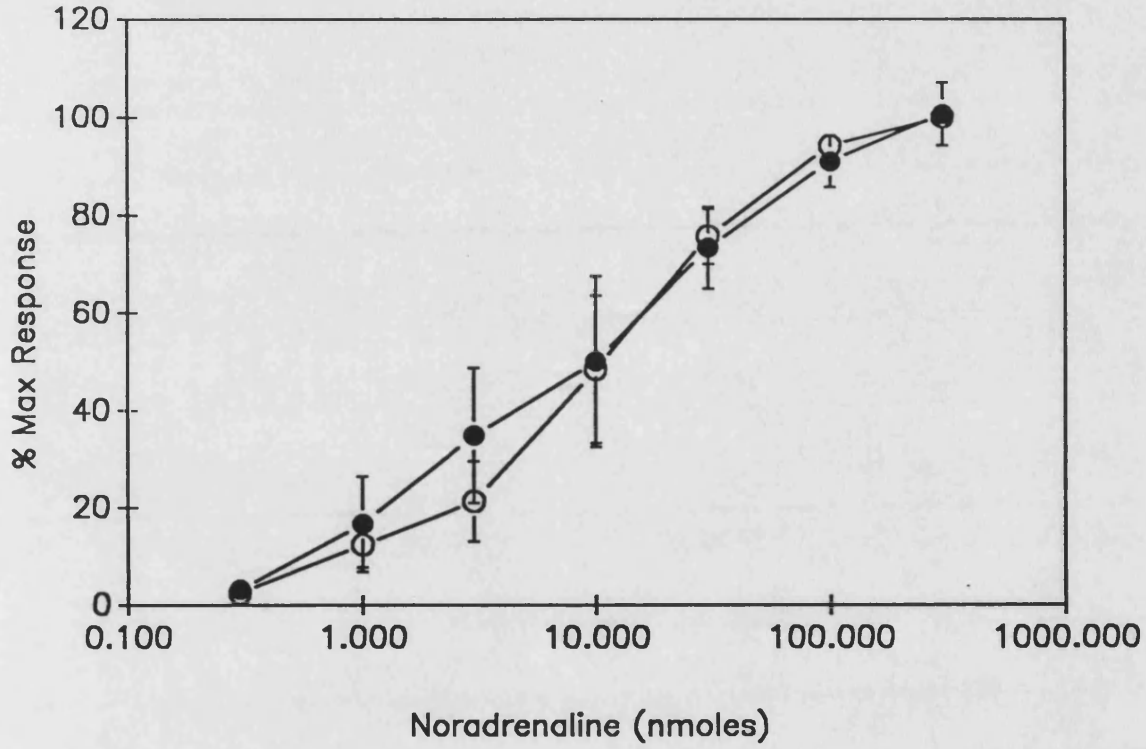


Fig.22 Effect of L-NOARG 10^{-5}M (●) on NA-induced vasoconstriction (O) in the CHAPS (4.7 mg/ml)-treated perfused rabbit ovarian vascular bed (mean±se, n=4).

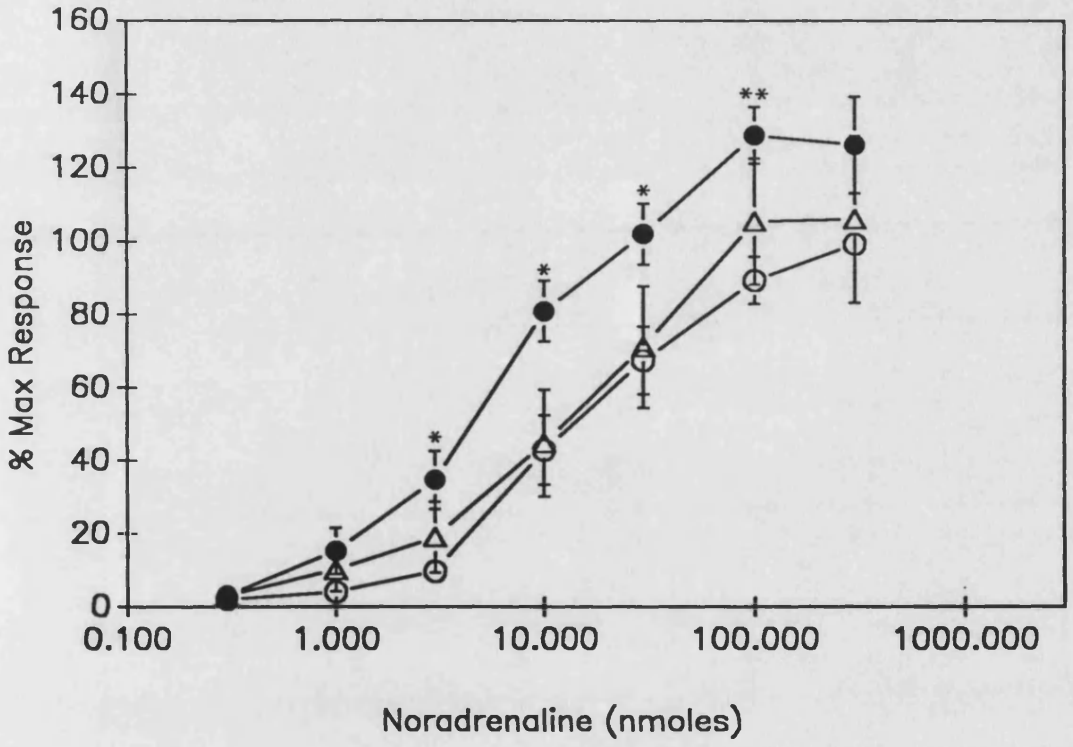


Fig. 23 Effect of L-arginine on L-NOARG-induced potentiation of vasoconstrictor responses to NA in the perfused rabbit ovarian vascular bed (mean±se, n=4-5).
(O) control, (●) with L-NOARG (10^{-5}M) and (Δ) with L-NOARG (10^{-5}M) & L-arginine ($5x10^{-4}$M). (* $p < 0.05$ & ** $p < 0.01$)

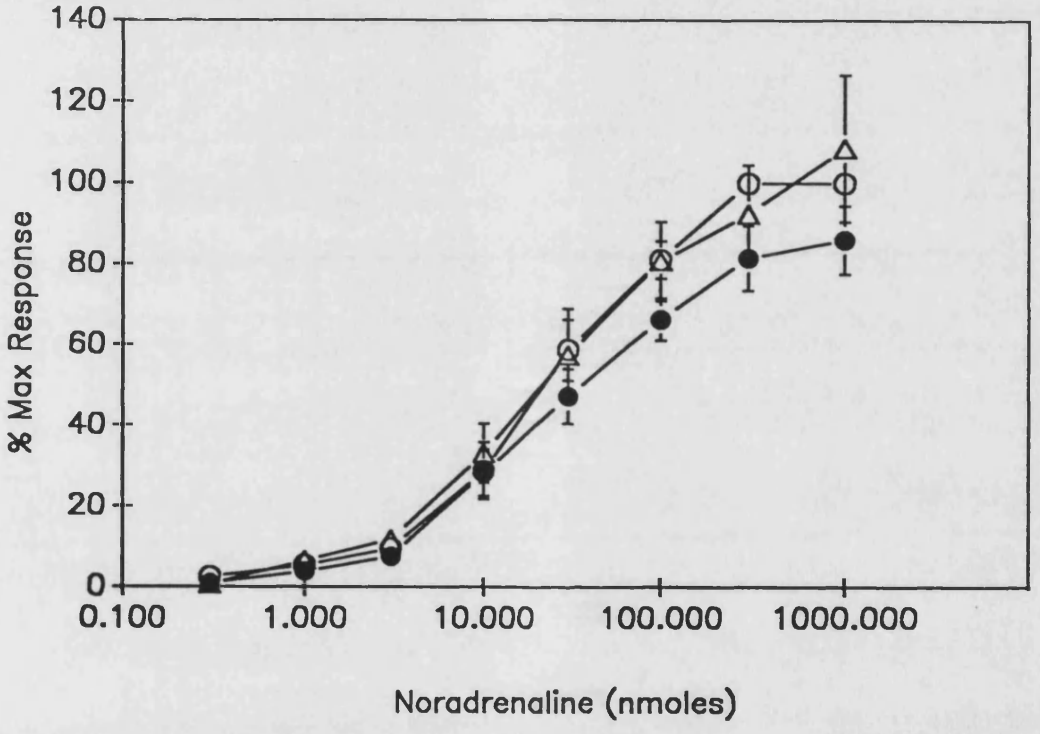


Fig. 24 Effect of aminoguanidine 10^{-5}M (●) and 10^{-4}M (Δ) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=5).

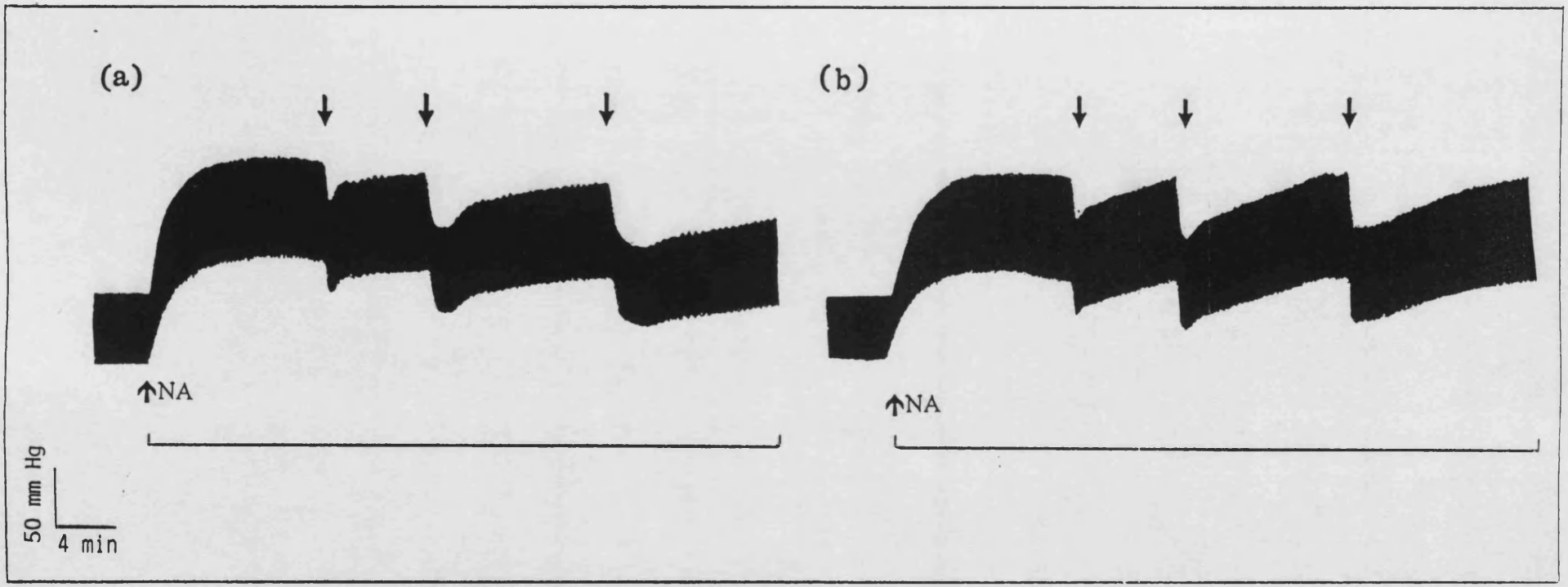
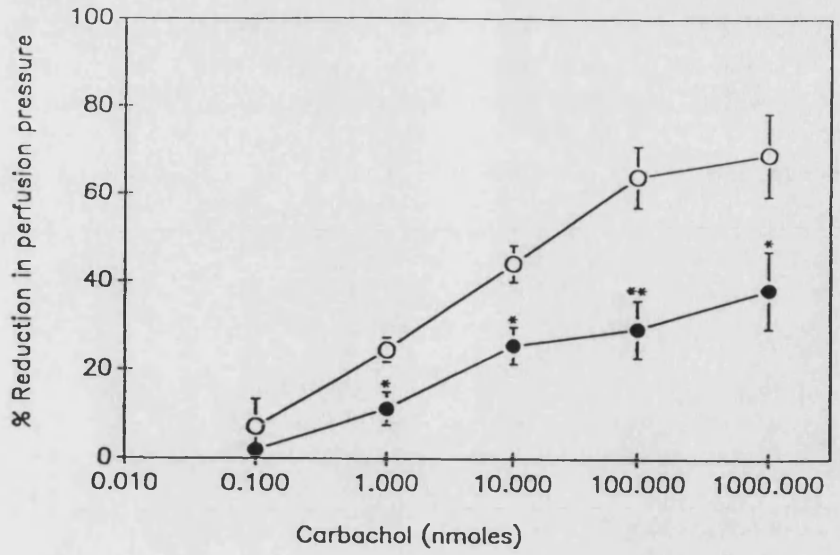
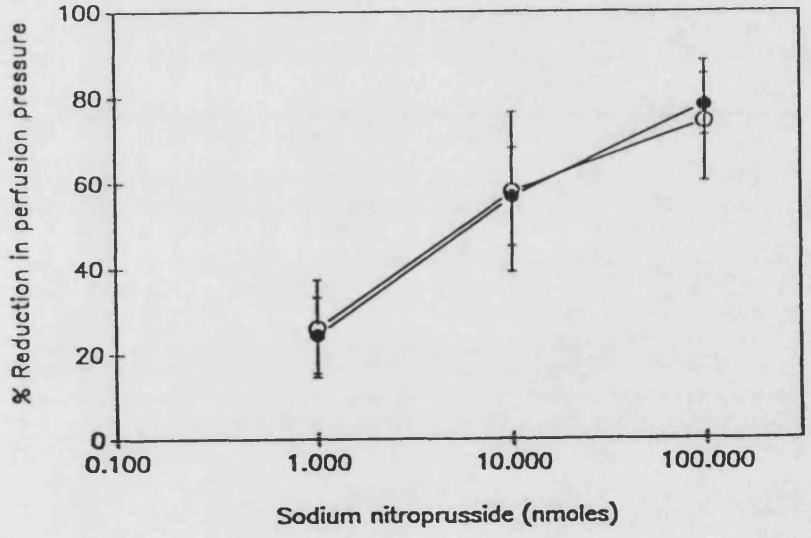


Fig. 25 A typical trace of carbachol-induced vasodilatation (a) before and (b) after L-NOARG (10^{-5} M). Perfusion pressure was raised with NA (10^{-5} M).

[↓ represents 1, 10 & 100 nmoles carbachol]





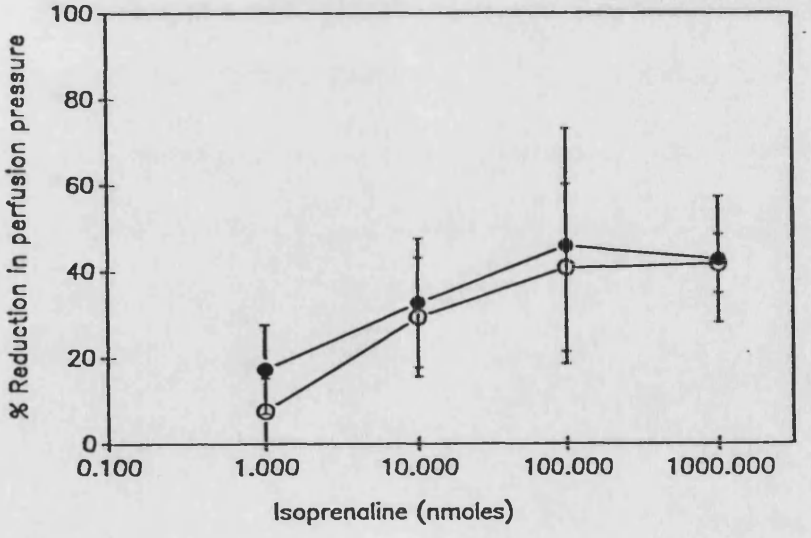


Fig. 26 Vasodilator responses to carbachol, sodium nitroprusside and isoprenaline before (O) and after (●) L-NOARG (10^{-5}M) in the perfused rabbit ovarian vascular bed (mean±se, n=4-5). (* $p < 0.05$ & ** $p < 0.01$)

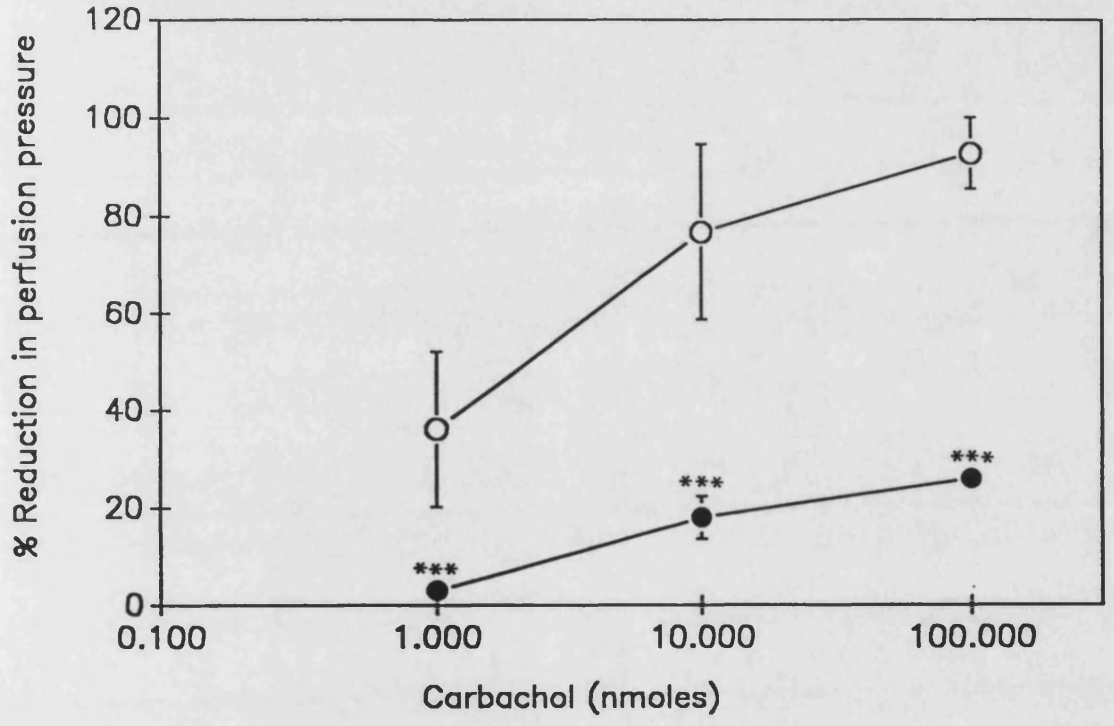


Fig. 27a Carbachol-induced vasodilatation before (O) and after (●) LY 83583 (10^{-5}M) in the perfused rabbit ovarian vascular bed (mean±se, n=3). (*** $p < 0.001$)

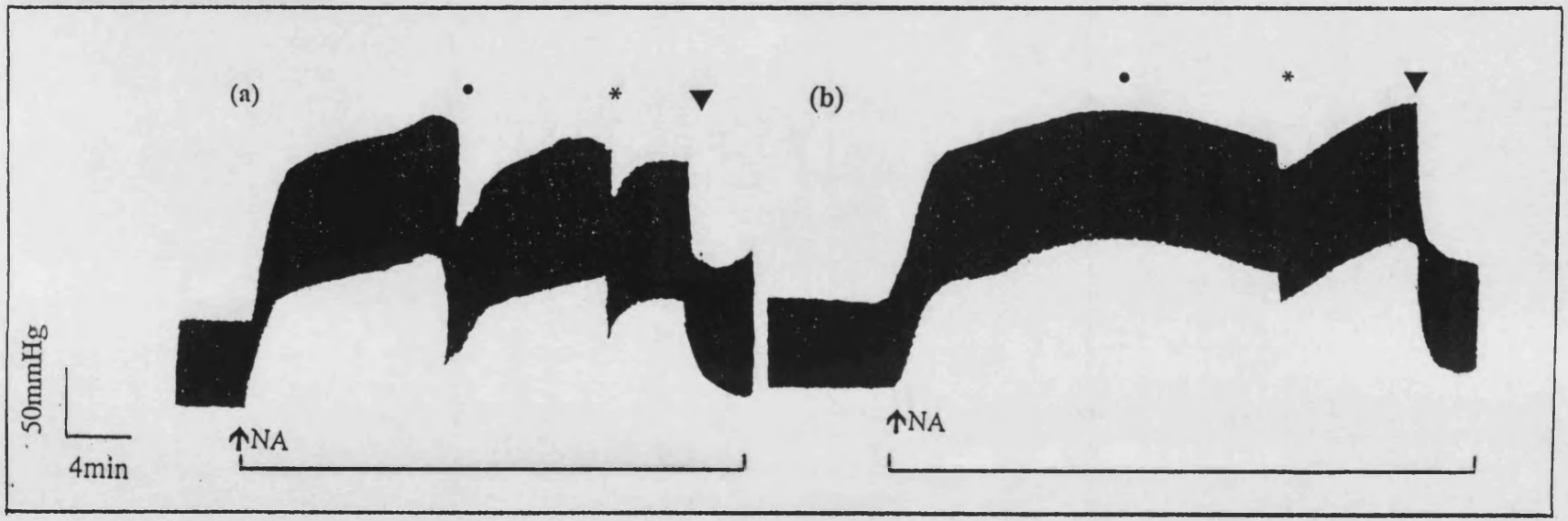


Fig. 27b A typical trace of vasodilator responses to 100 nmoles of (•) carbachol, (*) isoprenaline and (▼) sodium nitroprusside in the perfused rabbit ovarian vascular bed, (a) before and (b) after incubation with LY 83583 (10^{-5}M). Perfusion pressure was raised with NA 10^{-5}M.

However, SNP-induced vasodilatation was not inhibited, but rather potentiated after treatment with a similar concentration of methylene blue (Fig. 28).

3.3 Identification and characterization of adrenoceptors

3.3.1 Identification of adrenoceptors

Phentolamine (10^{-6}) did not affect the basal perfusion pressure of the ovarian vascular bed. However, at this concentration, phentolamine antagonized NA-induced vasoconstriction. There was a concentration-dependent right-ward shift of the NA dose-response curve ($p < 0.001$) (Fig. 29), with a pK_B value of 7.7 ± 0.08.

3.3.2 Effects of adrenergic agonists on perfusion pressure of the ovarian vascular bed

Since phentolamine is not selective for α_1- or α_2-adrenoceptors, experiments were carried out to characterize the subtype of α-adrenoceptors involved in NA-induced pressor responses. NA (1 - 1000 nmoles), phenylephrine (10 - 10000 nmoles), methoxamine (10 - 10000 nmoles), SDZ NVI 085 (1-1000 nmoles) and oxymetazoline (1 - 1000 nmoles) produced concentration-dependent increases in perfusion pressure of the ovarian vascular bed. Relative to NA; phenylephrine, methoxamine, SDZ NVI 085 and oxymetazoline were partial agonists. Tizanidine (10 - 10000 nmoles), clonidine (1 - 1000 nmoles), B-HT 920 (0.1-1000 nmoles) and UK 14, 304 (1 - 10000 nmoles) did not increase the perfusion pressure (Fig. 30), even after raising the basal perfusion pressure with NA (10^{-5}M).

Unlike the other agonists, oxymetazoline-induced vasoconstriction developed more slowly and was more persistent giving a 'spread out' type of response (Fig. 31). ED_{50} and intrinsic activity values are shown in Table 5. Isoprenaline (1-3000 nmoles) produced a little vasoconstrictor response in some of the preparations (2 out of 7). However, when the tone of the ovarian vascular bed was raised with 10^{-5}M methoxamine (or 10^{-5}M NA), isoprenaline produced a vasoconstrictor response (in all the preparations) at lower doses (3-30 nmoles), and a relaxant effect was observed at higher doses (> 30 nmoles) (Fig. 32). The maximum fall in perfusion pressure produced by isoprenaline was 52.4%. This relaxant effect was not inhibited by propranolol (10^{-6}M) (Fig. 33). The relaxant response to isoprenaline was found to be maintained even when the concentration of propranolol was increased to

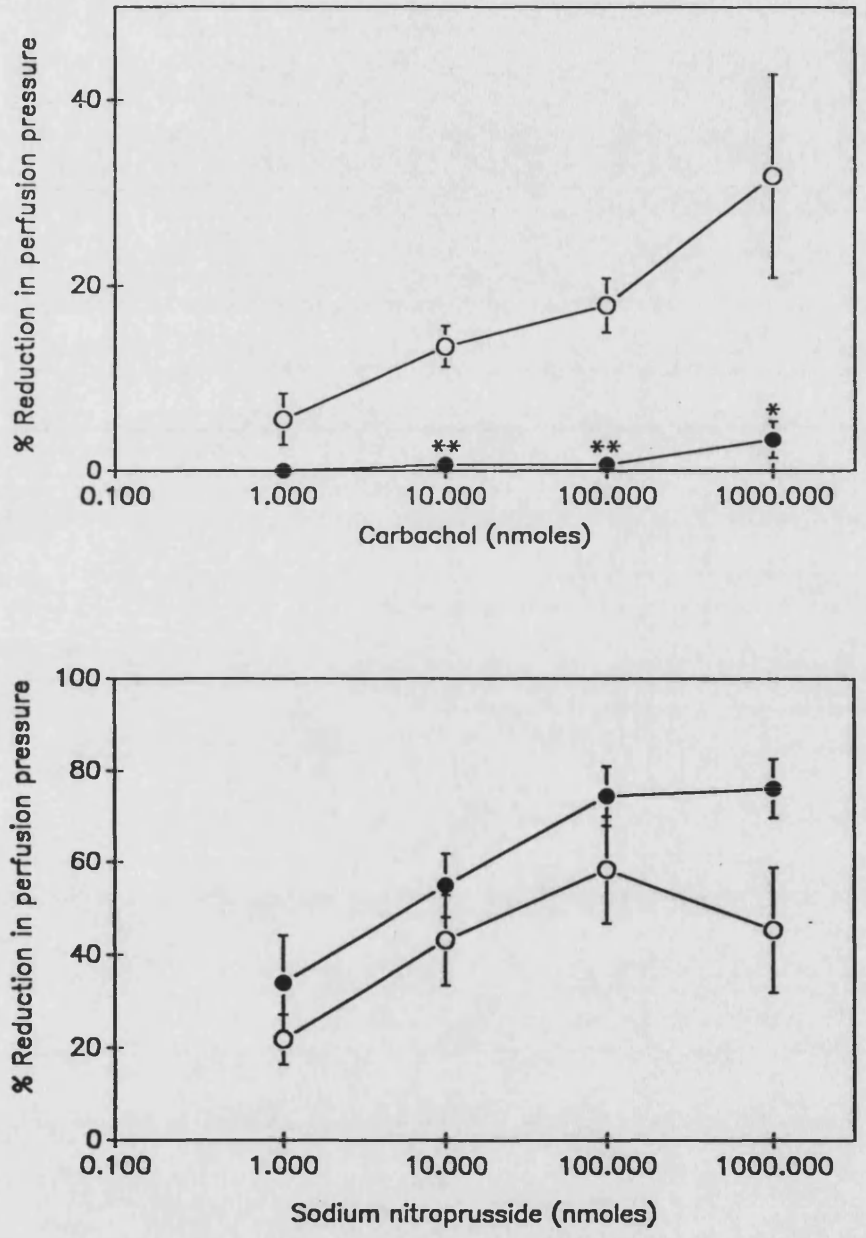


Fig. 28 Vasodilator responses to carbachol and sodium nitroprusside; (0) before and (●) after methylene blue ($3x10^{-5}M$) in the perfused rabbit ovarian vascular bed (mean±se, n=4). (* $p < 0.05$ & ** $p < 0.01$)

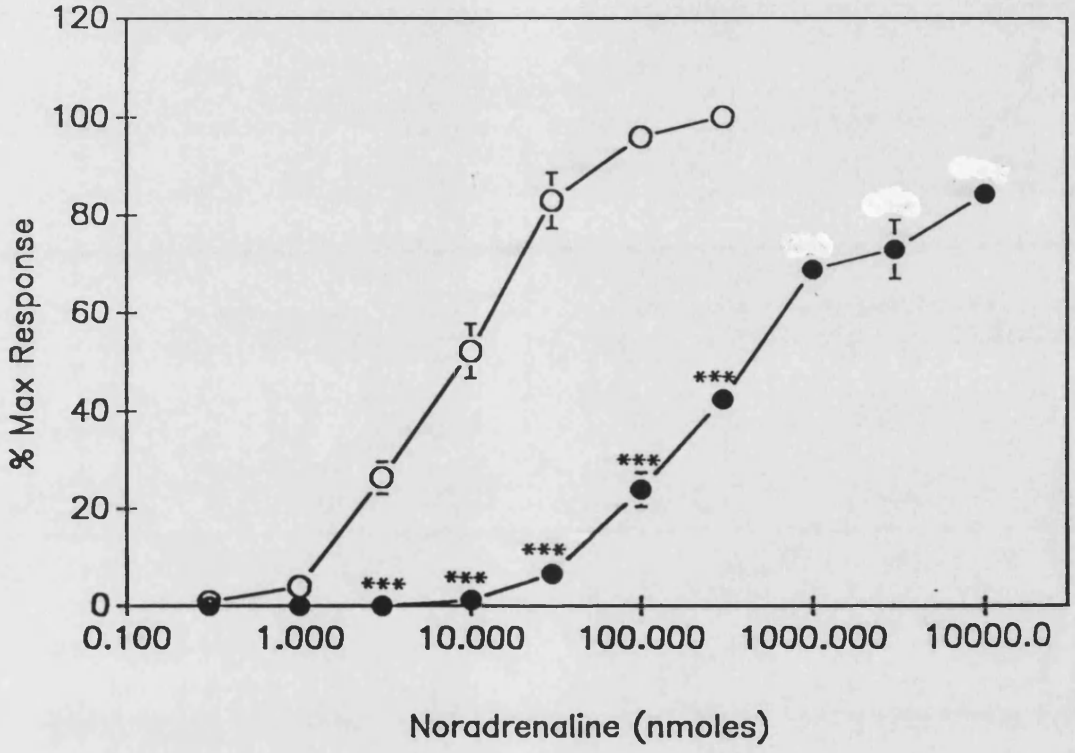


Fig. 29 Effect of phentolamine (10^{-6}M) on NA-induced vasoconstriction in the perfused rabbit ovarian vascular bed (mean±se, n=4). (O) control, and (●) after treatment with phentolamine. (*** $p < 0.001$)

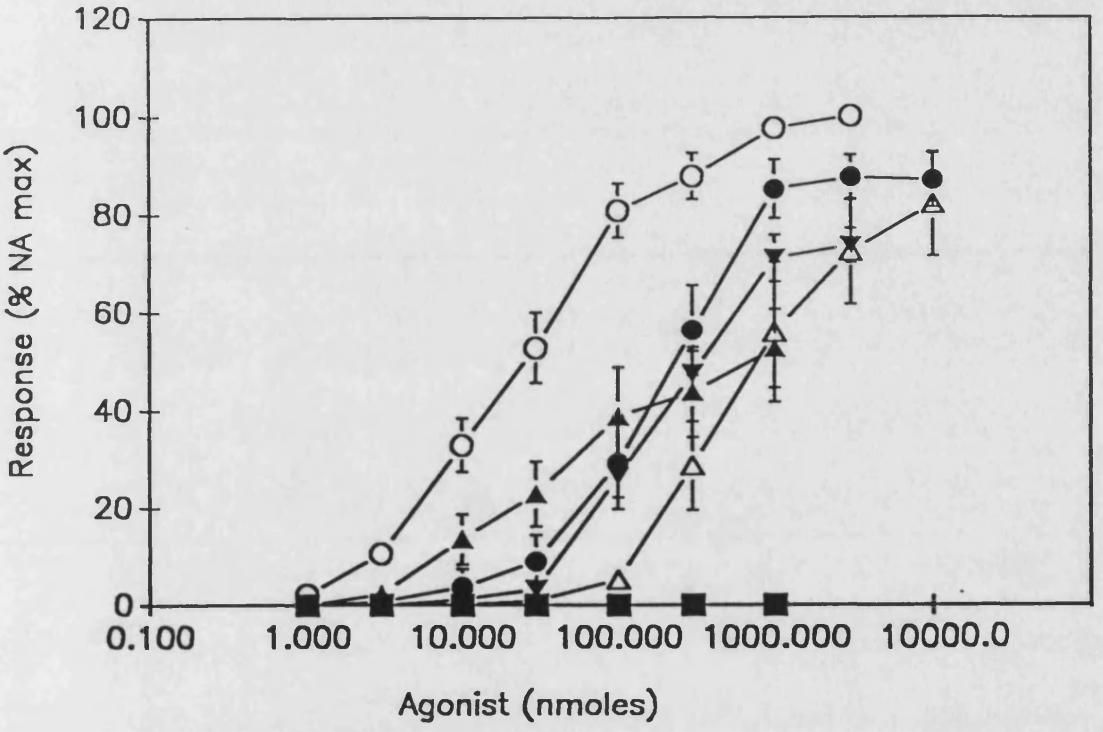


Fig. 30 Vasoconstrictor effects of some adrenergic agonists on the perfused rabbit ovarian vascular bed (mean±se, n=5).

(O) NA, (●) phenylephrine, (Δ) methoxamine, (▲) oxymetazoline, (▼) SDZ NVI 085, (■) clonidine, tizanidine, B-HT 920 & UK 14 304.

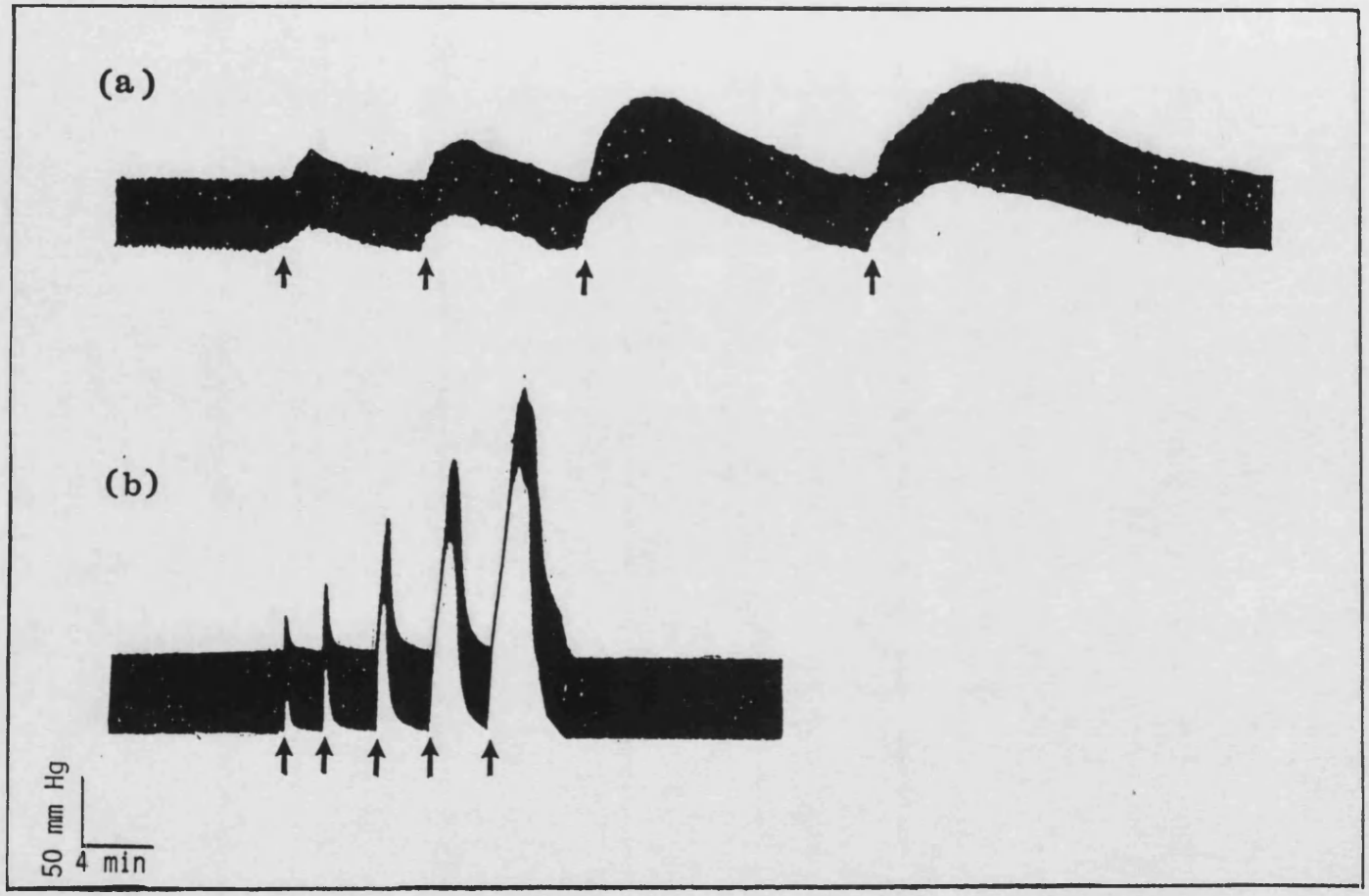


Fig. 31 A typical trace of (a) oxymetazoline- and (b) NA-induced vasoconstriction in the perfused rabbit ovarian vascular bed.

[↑ represents 3, 10, 30, 100 and 300 nmoles in ascending order]

Table 5 ED_{50} and Emax values of some adrenergic agonists in the perfused rabbit ovarian vascular bed, (mean±se, n=5).

a- no vasoconstriction.

b- expressed as a fraction of noradrenaline maximum.

Agonist	ED_{50}(nmoles)	Emax[b]
Noradrenaline	25.1±6.0	1.00
Phenylephrine	241.2±58.2	0.87
Methoxamine	777.6±262	0.82
Oxymetazoline	53.4±15.6	0.53
SDZ NVI 085	193.1±36.4	0.74
Isoprenaline	-[a]	-[a]
UK 14304	-[a]	-[a]
Clonidine	-[a]	-[a]
B-HT 920	-[a]	-[a]
Tizanidine	-[a]	-[a]

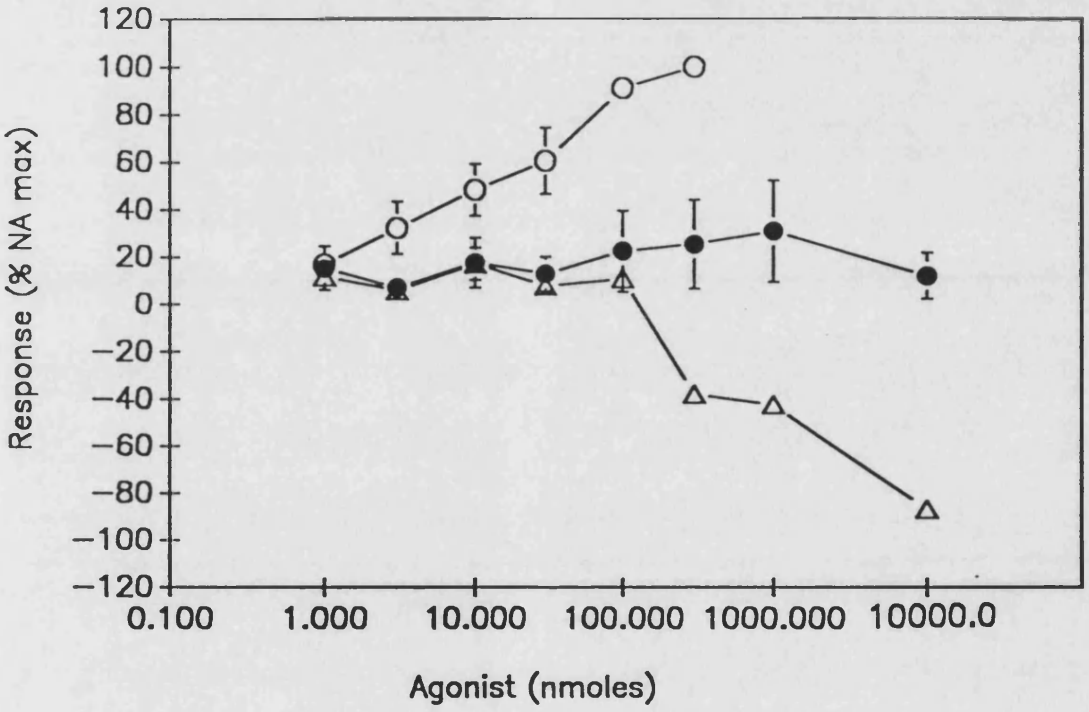


Fig. 32 Dose-response curves to NA (O) and isoprenaline (●) in the perfused rabbit ovarian vascular bed, (Δ) is response to isoprenaline after raising the perfusion pressure with methoxamine (10^{-5}M) (mean±se, n=4).

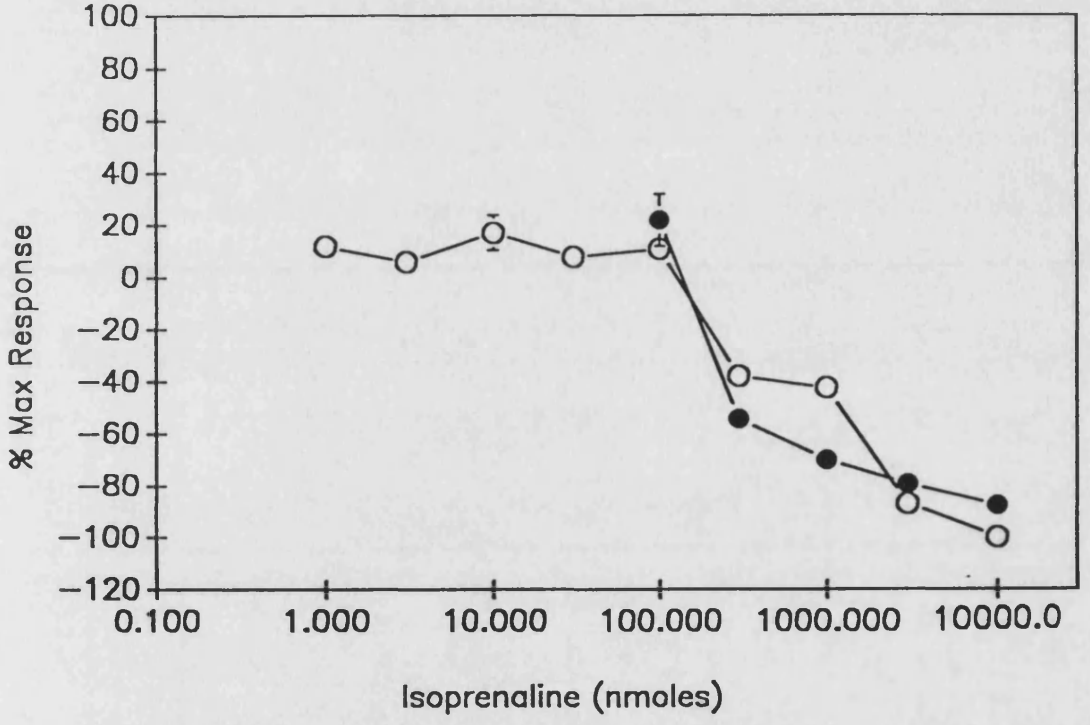


Fig. 33 Vasodilator effect of isoprenaline on the perfused rabbit ovarian vascular bed (O) before and (●) after treatment with propranolol (10^{-6}M); perfusion pressure was raised with methoxamine (10^{-5}M), (mean±se, n=4).

10^{-5} M. In some experiments, perfusion pressure was raised with U46619 ($3x10^{-7}$M) or histamine (10^{-5}M). Under these conditions, isoprenaline produced vasoconstrictor responses at all doses. These vasoconstrictor responses were attenuated by phentolamine (10^{-6}M) (Fig. 34).

3.3.3 Effects of prazosin and yohimbine on agonist-induced vasoconstriction

Prazosin (10^{-8} - 10^{-5}M) did not affect the basal perfusion pressure of the ovarian vascular bed. At these concentrations, however; prazosin antagonized NA-induced vasoconstriction. There was a concentration-dependent rightward shift of the NA dose-response curve with no suppression of the maximum response (Fig. 35). The Schild plot was monophasic with a slope of 1.0±0.1 indicating competitive antagonism. The pA_2 value was 7.35±0.3. Prazosin also produced a concentration-dependent rightward shift of phenylephrine and oxymetazoline dose-response curves (Fig. 35). The Schild plots for phenylephrine and oxymetazoline were monophasic with slopes of 1.1±0.1 and 0.96±0.04 respectively, indicating competitive antagonism. The pA_2 values calculated for prazosin against phenylephrine and oxymetazoline were 7.27±0.1 and 6.38±0.2 respectively.

Concentrations of prazosin greater than $3x10^{-7}$M significantly suppressed the maximum response to methoxamine and SDZ NVI 085. Therefore, K_B values were determined for prazosin using $3x10^{-7}$M as the antagonist concentration. Prazosin produced a significant shift ($p<0.01$) of the methoxamine and SDZ NVI 085 dose-response curves to the right (Fig. 36). The pK_B values were calculated to be 7.7±0.28 and 7.4±0.2 against methoxamine and SDZ NVI 085 respectively (Table 6).

Yohimbine at concentrations up to 10^{-5}M did not alter the basal perfusion pressure of the ovarian vascular bed. At the lower concentrations (10^{-7}M and 10^{-6}M), yohimbine did not antagonize NA-induced vasoconstriction, while at 10^{-5}M, it produced a parallel nonsignificant rightward shift of the NA dose-response curve with no reduction in the maximum response (Fig. 37). The pK_B was $7.1(\pm2.2)x10^{-6}$. Thus the prazosin / yohimbine ratio was 112.2.

Yohimbine at concentrations of 10^{-7}M-10^{-6}M caused a leftward shift of the phenylephrine dose-response curve (Fig. 37). The more significant shift was observed at yohimbine 10^{-5} M, where the pK_B value was found to be 5.7±0.2.

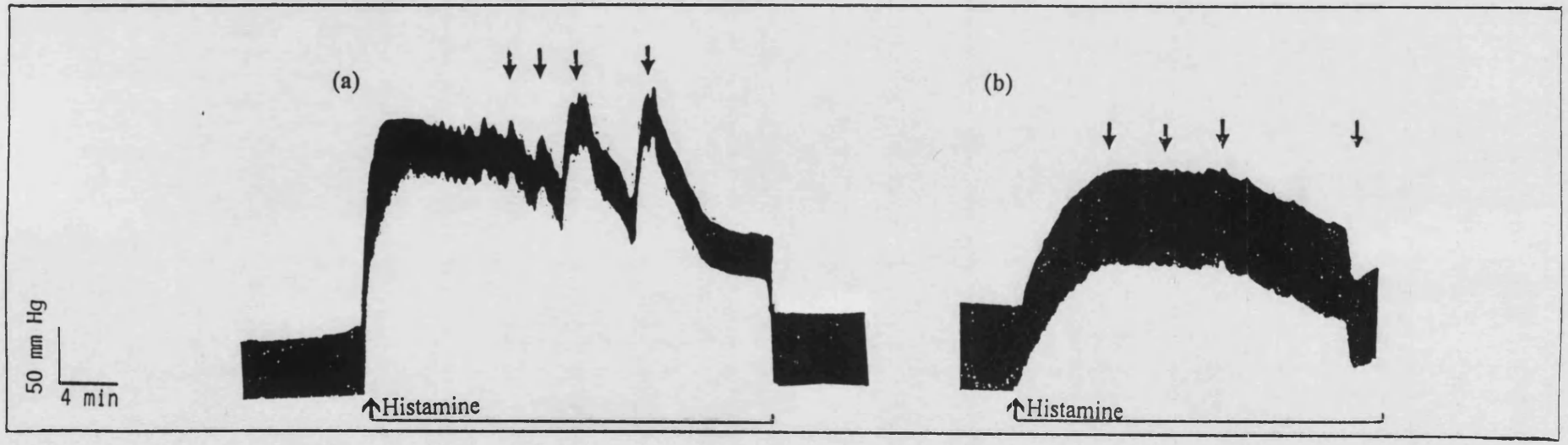


Fig. 34 A typical trace of isoprenaline-induced vasoconstriction after raising the basal perfusion pressure with histamine 10^{-5}M, (a) before and (b) after incubation with phentolamine 10^{-6}M.

[↓ represents 10, 100, 1000 and 10000 nmoles in ascending order]

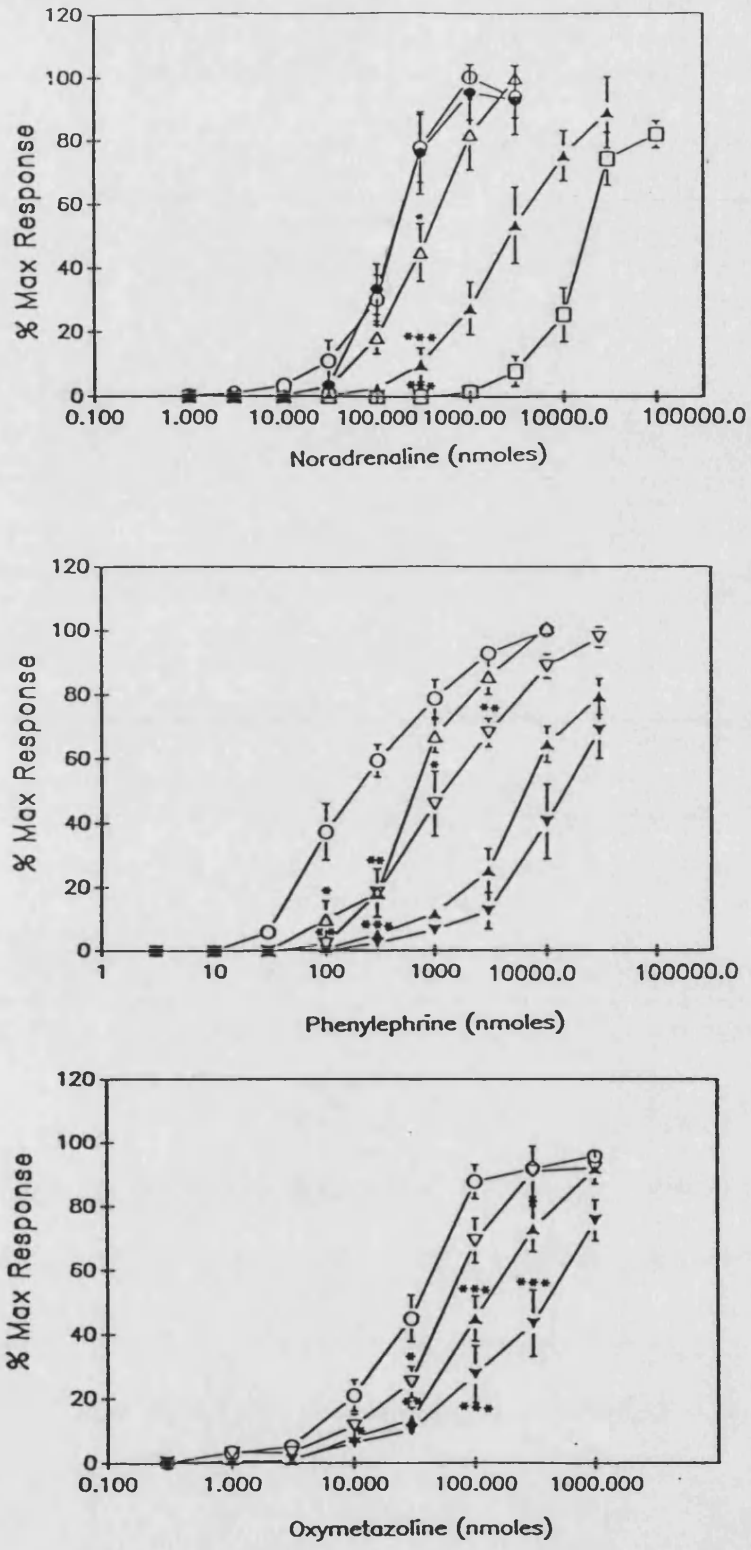


Fig. 35 Effect of prazosin on NA-, phenylephrine- and oxymetazoline-induced vasoconstriction in the perfused rabbit ovarian vascular bed (mean±se, n=5). (O) control, (●) 10^{-8} M, (Δ) 10^{-7} M, (▽) $3x10^{-7}$ M, (▲) 10^{-6} M, (▼) $3x10^{-6}$ M and 10^{-5}M (□) (mean±se, n=5-6). All values starting from 1000 nmoles are extremely significant at antagonist concentrations of 10^{-6} M, $3x10^{-6}$ M and 10^{-5} M.

(* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)

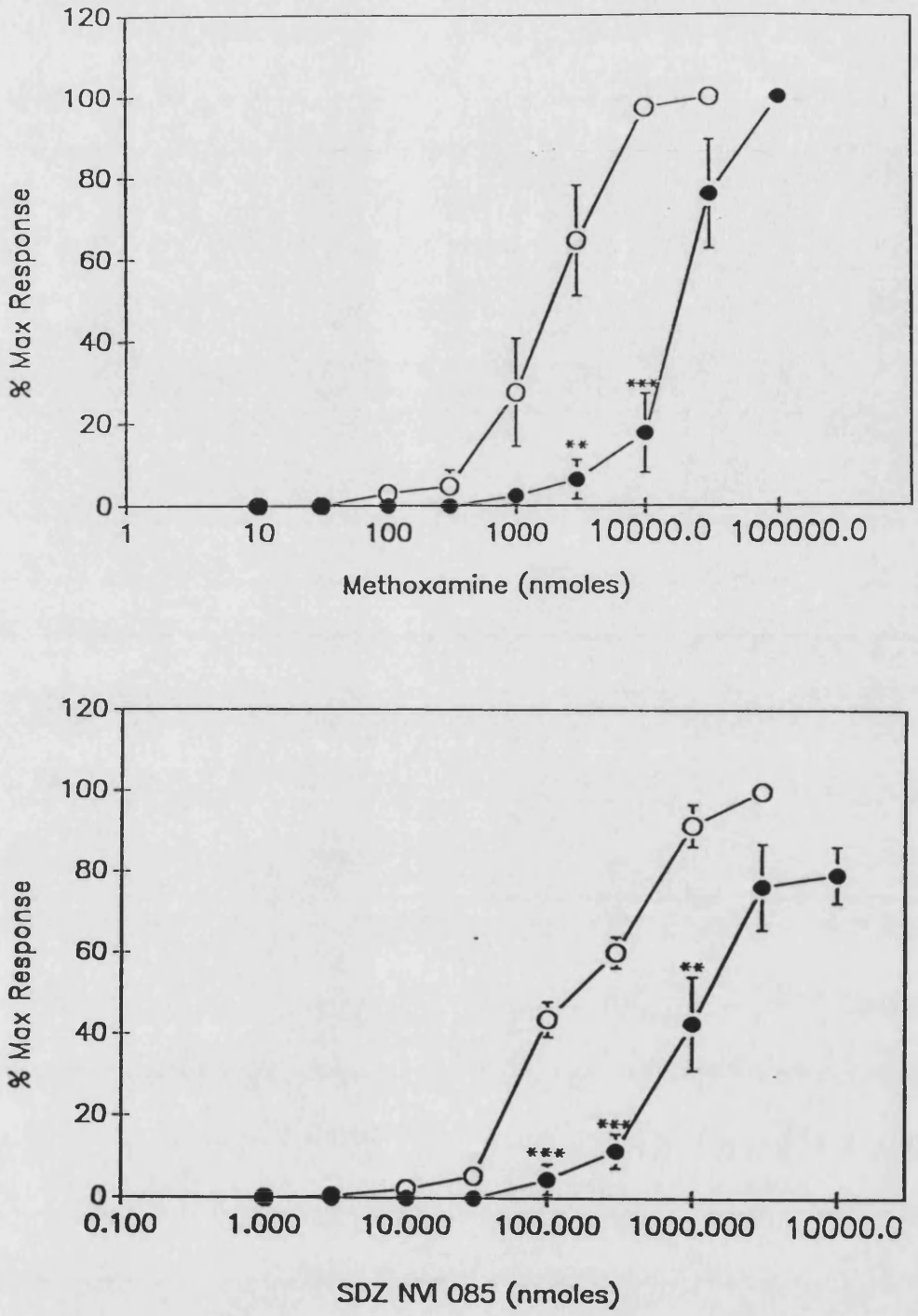


Fig. 36 Effect of prazosin $3x10^{-7}$M (●) on methoxamine- and SDZ NVI 085-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=5-6). (* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)

Table 6 Antagonist potencies of prazosin in the perfused rabbit ovarian vascular bed, (mean±se, n=5).

a- not calculated (* = $p<0.05$)

	Prazosin	
	pA_2 /pK_B	Slope
Noradrenaline	7.35±0.3	1.0±0.1
Phenylephrine	7.27±0.1	1.1±0.1
Oxymetazoline	6.38±0.2*	0.96±0.04
Methoxamine	7.7±0.28	--[a]
SDZ NVI 085	7.4±0.2	--[a]

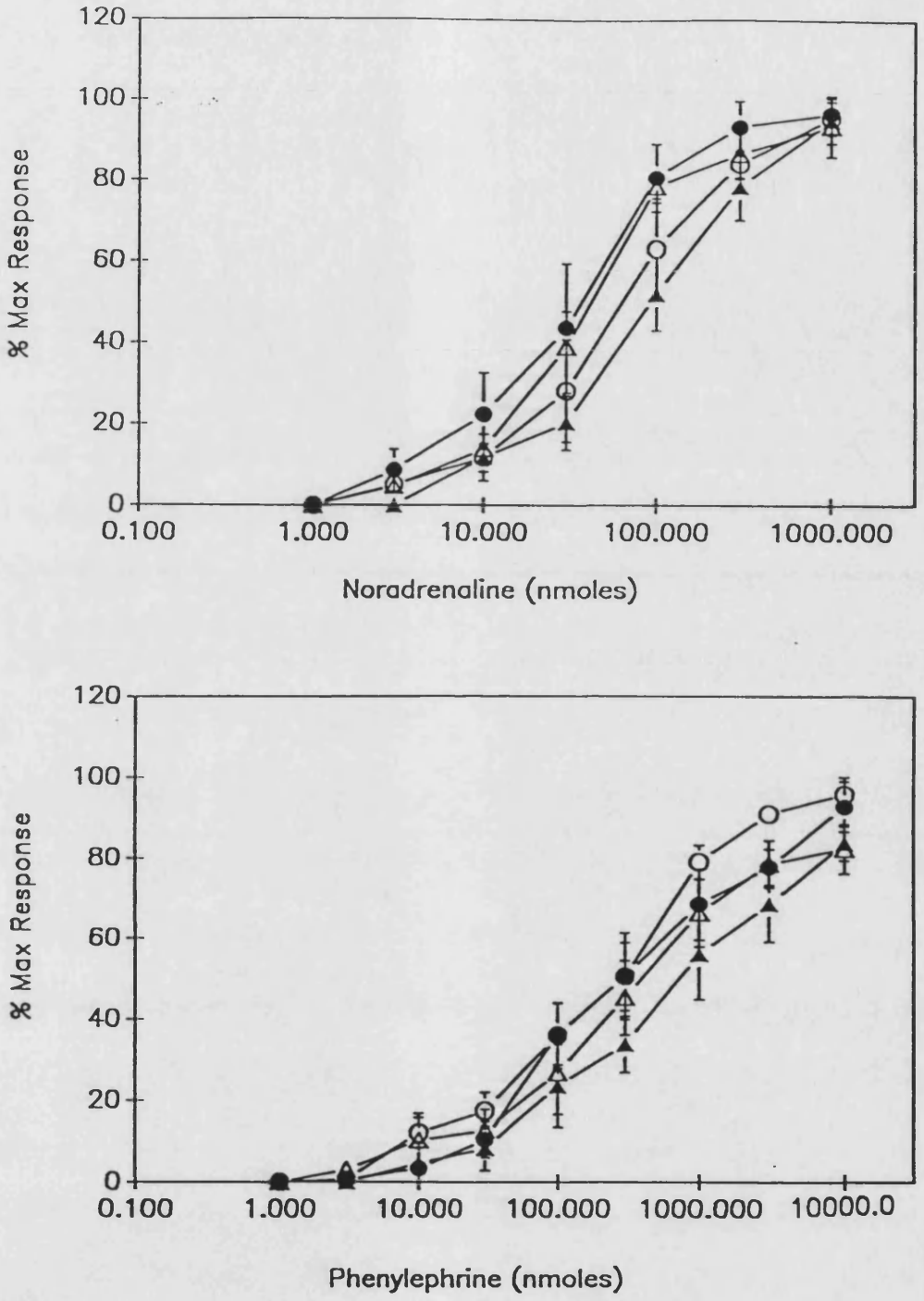


Fig. 37 Effect of yohimbine, 10^{-7}M (●), 10^{-6}M (Δ) and 10^{-5}M (▲) on NA- and phenylephrine-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).

3.3.4 Effects of α-adrenoceptor antagonists on electrically-induced vasoconstriction

Prazosin (10^{-7}M-10^{-5}M) produced a significant concentration-dependent reduction of the electrically-induced vasoconstrictor responses ($p<0.001$). However, the effect of yohimbine (10^{-7}M-10^{-5}M) was not significant ($p>0.05$) (Fig. 38) At the highest concentration used (10^{-5}M), yohimbine reduced the response to electrical stimulation by only 15%.

3.3.5. Effects of subtype-selective α_1-adrenergic antagonists on agonists-induced vasoconstiction

3.3.5.1 Competitive antagonists

None of the concentrations of WB-4101 used in this study altered the basal perfusion pressure of the ovarian vascular be. WB-4101 (10^{-8} - 10^{-5}M) produced dose-dependent parallel rightward displacement of NA dose-response curve. There was no reduction in the maximum response (Fig. 39). The Schild regression line was a straight line having a slope of 1.0±0.1, indicating competitive antagonism. The pA_2 value was 7.9±0.2 (Table 7). WB 4101 also produced concentration-dependent parallel rightward displacements of phenylephrine and oxymetazoline dose-response curves without a significant reduction in the maximum response ($p > 0.05$) (Fig. 39). The Schild plot for the two agonists were straight lines with slopes of 0.85±0.1 and 0.86±0.1 respectively. These values were not significantly different from 1.0, indicating competitive antagonism. The pA_2 values were 7.3±0.2 and 6.9±0.4 respectively. As with prazosin, concentrations of WB-4101 greater than $3x10^{-7}$M reduced the maximum response to methoxamine and SDZ NVI 085. Therefore, only one concentration of WB-4101 ($3x10^{-7}$M) was used to determine pK_B values. As shown in Figure 40, WB-4101 produced a rightward shift of the dose-response curves to methoxamine and SDZ NVI 085. The pK_B values were calculated to be 7.1±0.3 and 7.7±0.1 respectively. Niguldipine (10^{-9}M) produced a highly significant rightward displacement of the NA dose-response curve ($p < 0.001$) (Fig. 41).

WB-4101 ($3x10^{-8}$M-$3x10^{-7}$M) produced a significant concentration-dependent suppression of the electrically induced vasoconstrictor response ($p < 0.001$) (Fig. 42).

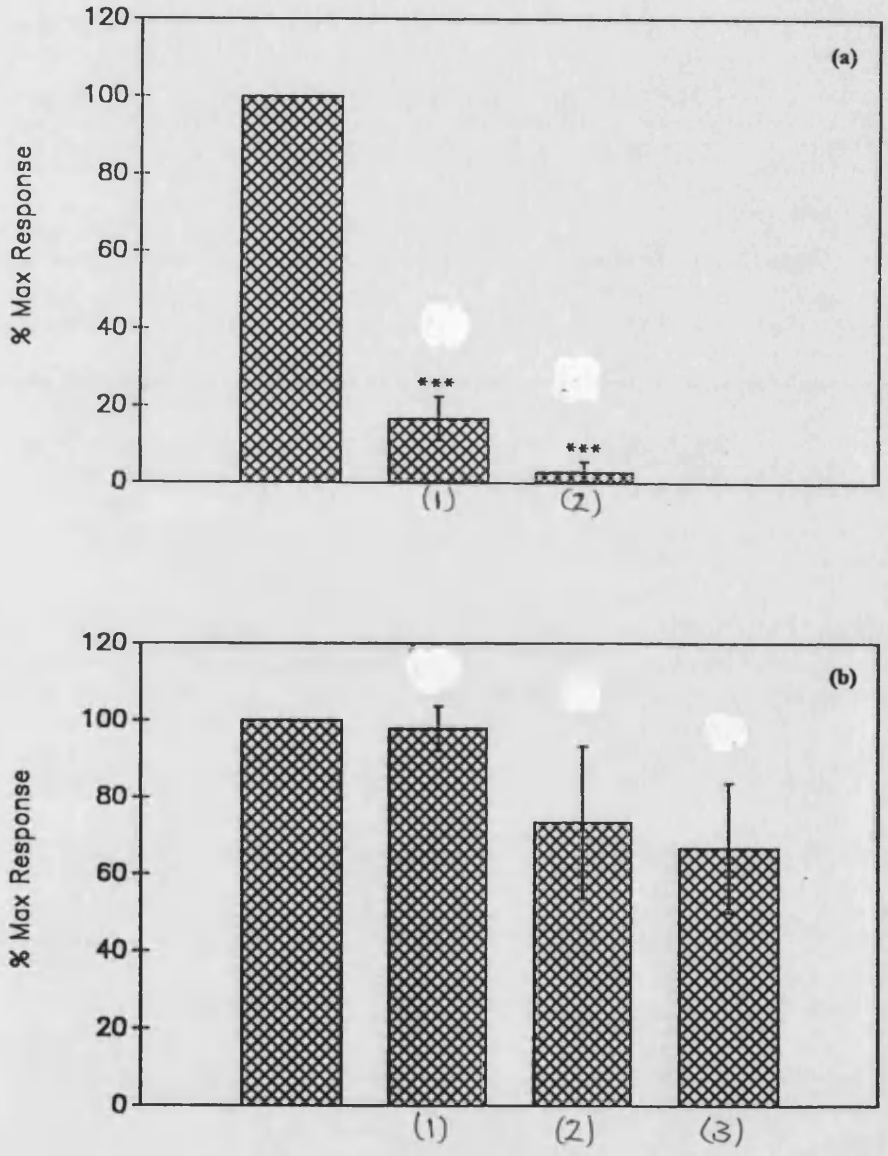
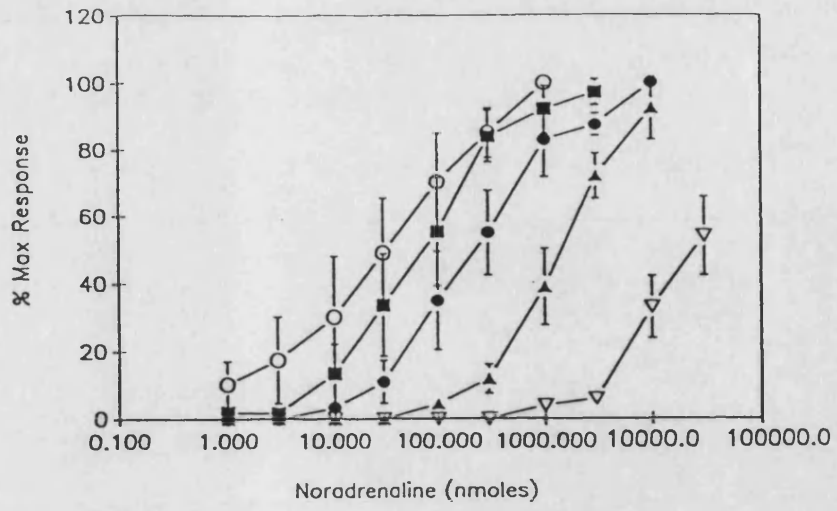
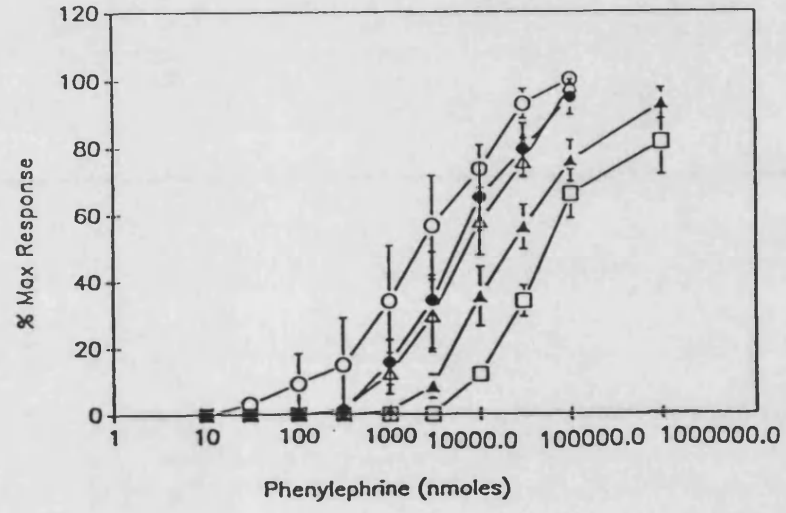


Fig. 38 Effect of (a) prazosin and (b) yohimbine on the response of the perfused rabbit ovarian vascular bed to electrical stimulation (mean±se, n=4).
[10^{-7}M (1), 10^{-6}M (2) and 10^{-5}M (3)] (*** $p < 0.001$)





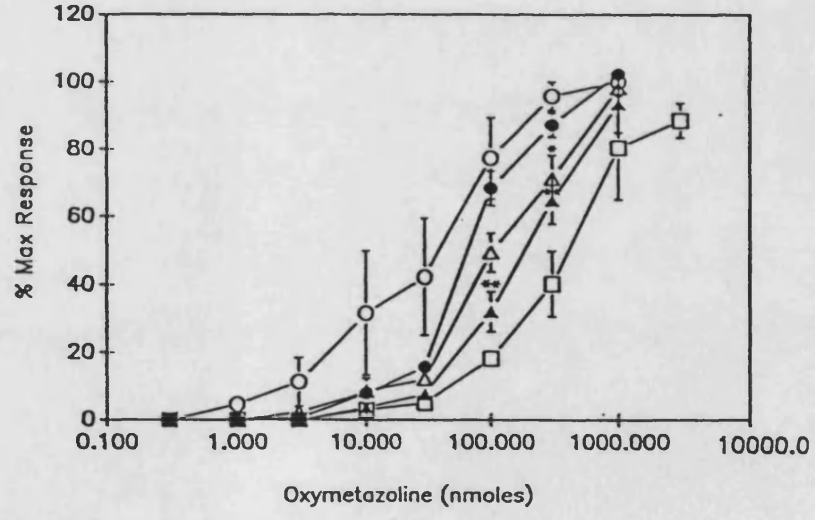


Fig.39 Effect of WB-4101 on NA-, phenylephrine- and oxymetazoline-induced vasoconstriction in the perfused rabbit ovarian vascular bed (mean±se, n=5).
(O) control, (■)10^{-8}M, (●) 10^{-7}M, (Δ) $3x10^{-7}$M, (▲) 10^{-6}M, (□) $3x10^{-6}$M and (▽) 10^{-5}M. [For NA, phenylephrine and oxymetazoline, all values starting from 10, 1000 and 100 nmoles respectively, are extremely significant starting at antagonist concentration of 10^{-6}M] (* $p < 0.05$ & ** $p < 0.01$)

Table 7 Antagonist potencies of WB-4101 in the perfused rabbit ovarian vascular bed, (mean±se, n=5)

a- not calculated

	WB 4101	
	pA_2 /pk_B	Slope
Noradrenaline	7.9±0.2	1.0±0.1
Phenylephrine	7.3±0.2	0.85±0.1
Oxymetazoline	6.9±0.4	0.86±0.1
Methoxamine	7.1±0.3	--[a]
SDZ NVI 085	7.7±0.1	--[a]

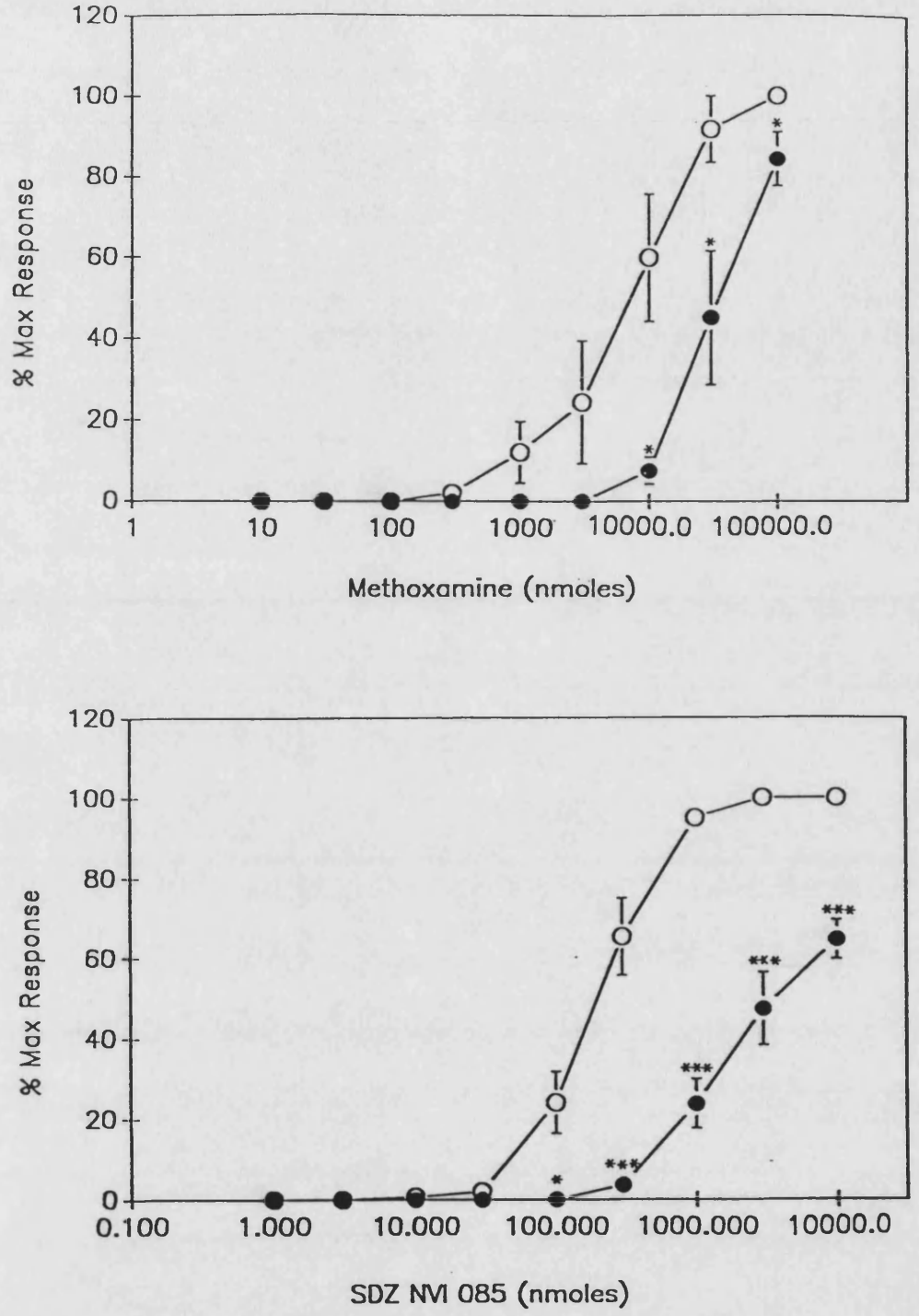


Fig. 40 Effect of WB 4101 $3x10^{-7}$M (●) on methoxamine- and SDZ NVI 085-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=5-6).
(* $p < 0.05$ & *** $p < 0.001$)

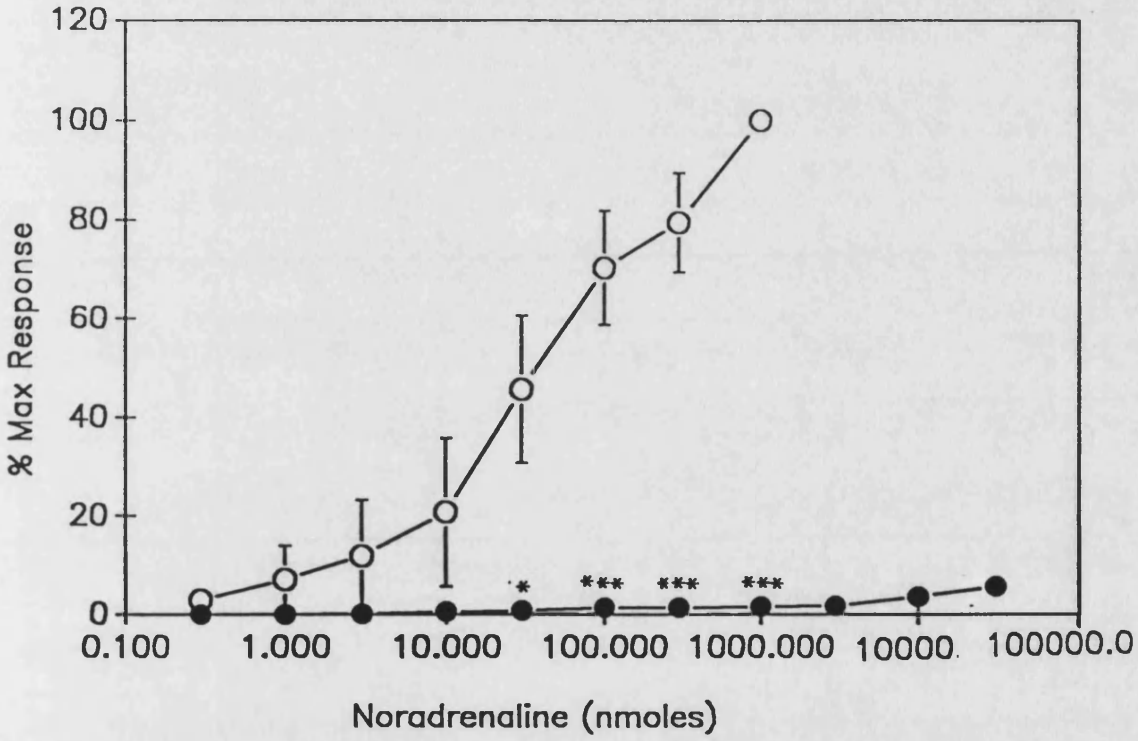


Fig. 41 Effect of niguldipine, 10^{-9}M (●) on NA-induced vasoconstriction (O) in the perfused ovarian vascular bed (mean±se, n=6). (* $p < 0.05$ & *** $p < 0.001$)

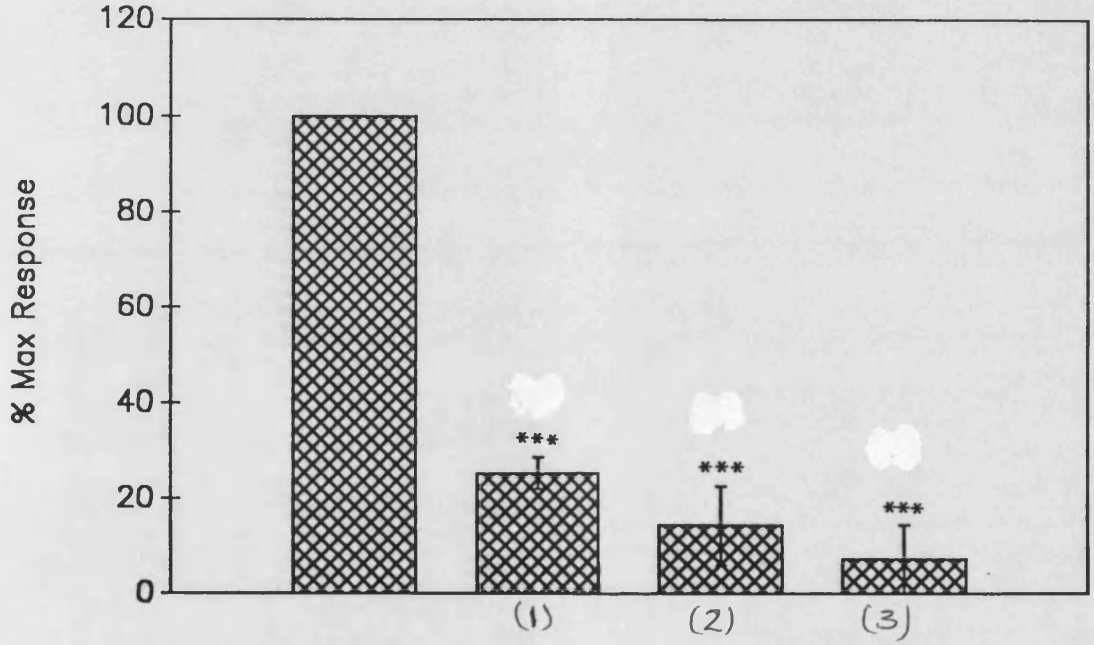


Fig. 42 Effect of WB_4101 on the response of the perfused rabbit ovarian vascular bed to electrical stimulation (mean±se, n=4). [$3x10^{-8}$M (1), 10^{-7}M (2) and $3x10^{-7}$M (3)] (*** $p < 0.001$)

3.3.5.2 Irreversible antagonists

CEC ($3x10^{-5}M$-$10^{-4}M$) did not alter basal perfusion pressure of the ovarian vascular bed. At these concentrations, CEC did not reduce NA-induced vasoconstriction. Instead, it produced a slight but not significant leftward shift of the NA dose response curve ($p > 0.05$). At a concentration of ($10^{4}M$), CEC did not reduce the oxymetazoline-induced vasoconstriction (Fig. 43).

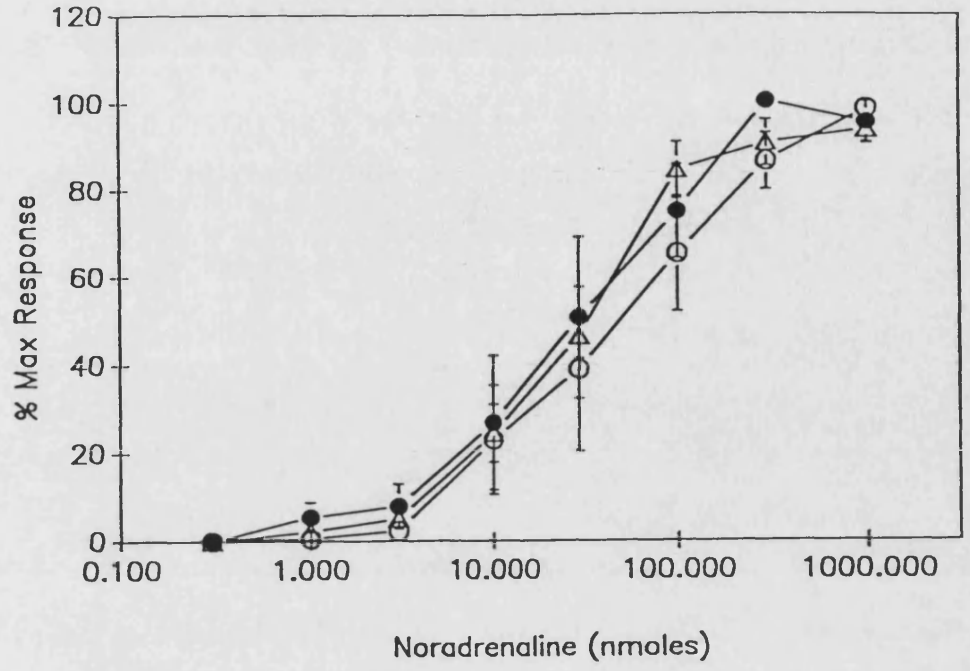
SZL-49 ($5x10^{-6}M$) did not alter the basal perfusion pressure of the ovarian vascular bed. But at this concentration, SZL-49 ($5x10^{-6}M$) displaced significantly ($p < 0.05$) the NA-dose response curve to the right (fig. 44). Moreover, SZL-49 ($5x10^{-6}M$) produced a rightward shift ($p < 0.05$) of the agonist dose-response curves, phenylephrine, oxymetazoline, methoxamine and SDZ NVI 085 (Figs. 44 & 45). There was no reduction in the maximum response to any of the agonists.

SZL-49 ($5x10^{-6}M$) significantly reduced the vasoconstrictor response to electrical stimulation ($p<0.001$), while CEC ($10^{-4}M$) had no significant effect ($p > 0.05$) (Fig. 46).

3.4 Signal transduction mechanisms

3.4.1 Calcium-free solution

Incubation of the ovarian vascular bed in Ca^{2+}-free KH solution did not alter the basal perfusion pressure. However, there was a highly significant reduction in the NA-induced vasoconstriction ($p < 0.001$). In this Ca^{2+}-free medium, the dose-response curve was shifted to the right and the maximum response to NA was reduced to approximately 15% of the control (Fig. 47). Addition of EGTA (2mM) to the solution abolished the residual response to NA. As shown in Figure 48, there was a time-dependent loss of NA-induced vasoconstriction when the ovarian vascular bed was perfused with Ca^{2+}-free KH-solution. The response to NA (300 nmoles) was reduced by approximately 60% within the first 5min of perfusion with KH-solution and less than 5% after 45min. The half life for the loss of responsiveness to NA was found to be 4 min. Addition of EGTA (2mM) to the Ca^{2+}-free KH-solution accelerated the loss of vasoconstrictor effect (Fig. 49). Under these conditions, vasoconstrictor effect of NA was reduced by approximately 90% within 5min and by 15min the response was abolished. The half life for the loss of



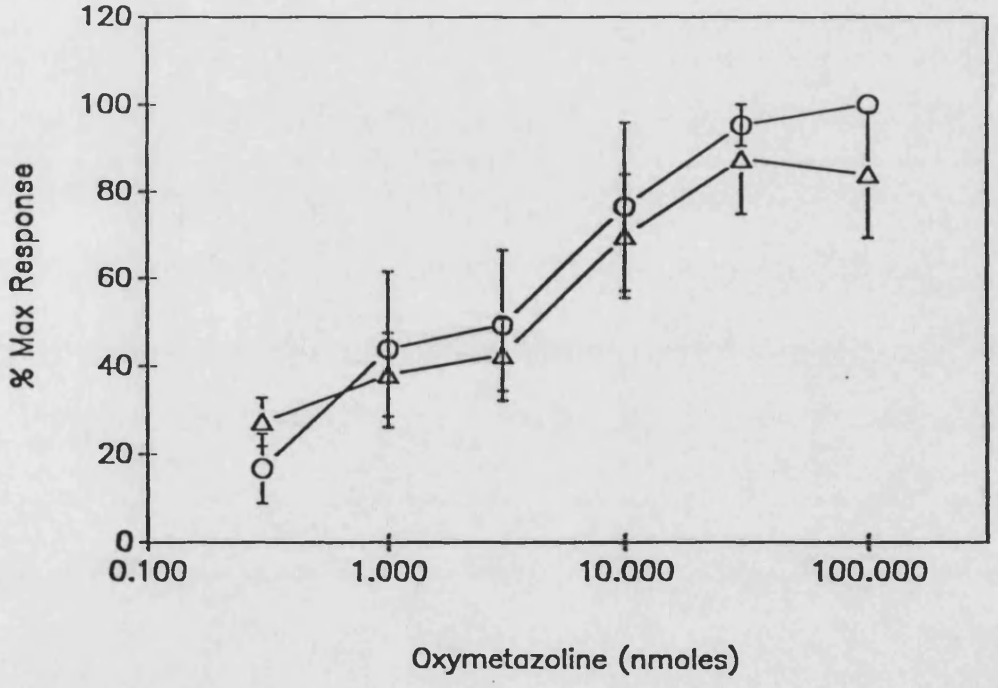
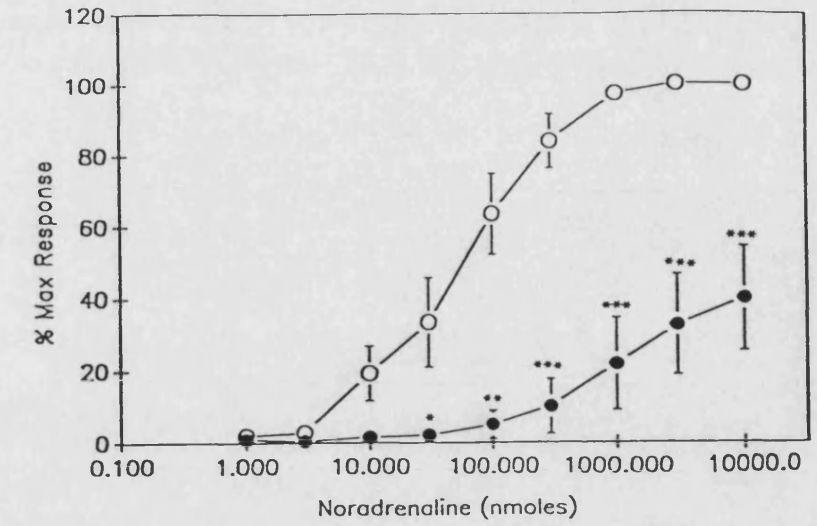
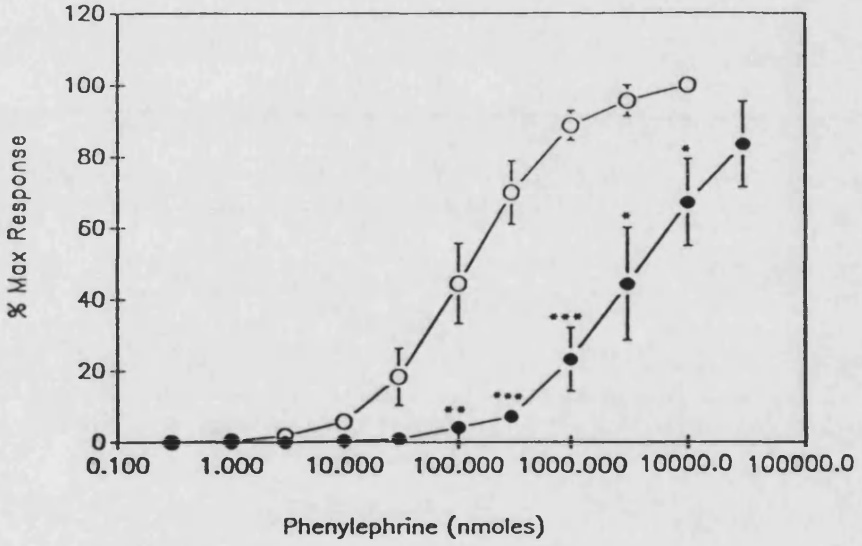


Fig. 43 Effect of CEC, $3x10^{-5}$M (●) and 10^{-4}M (Δ) on NA- and oxymetazoline-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).





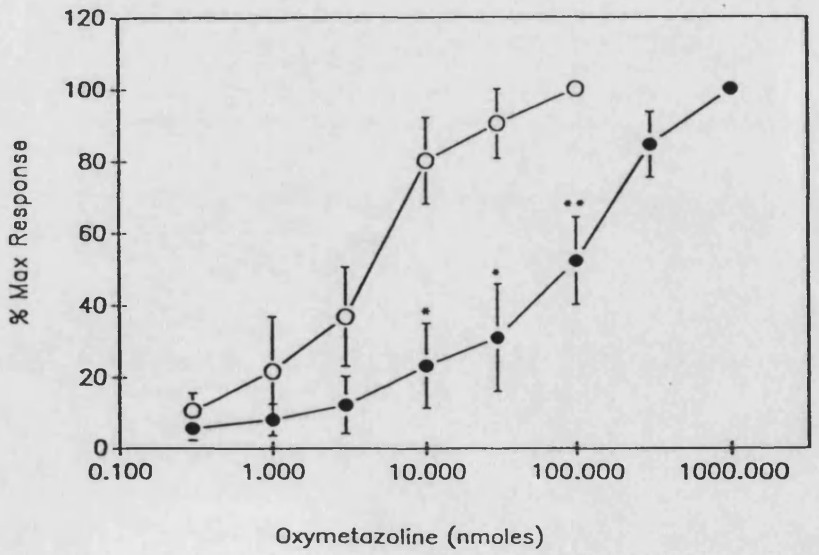
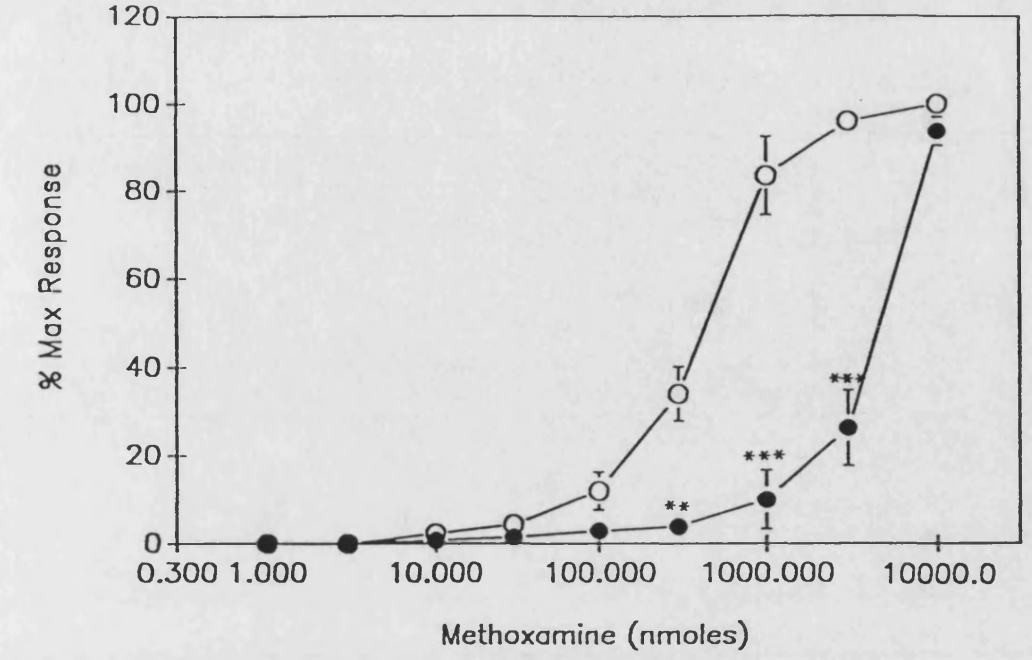


Fig. 44 Effect of SZL-49, $5x10^{-6}$M (●) on NA-, phenylephrine- and oxymetazoline-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4-5). (* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)



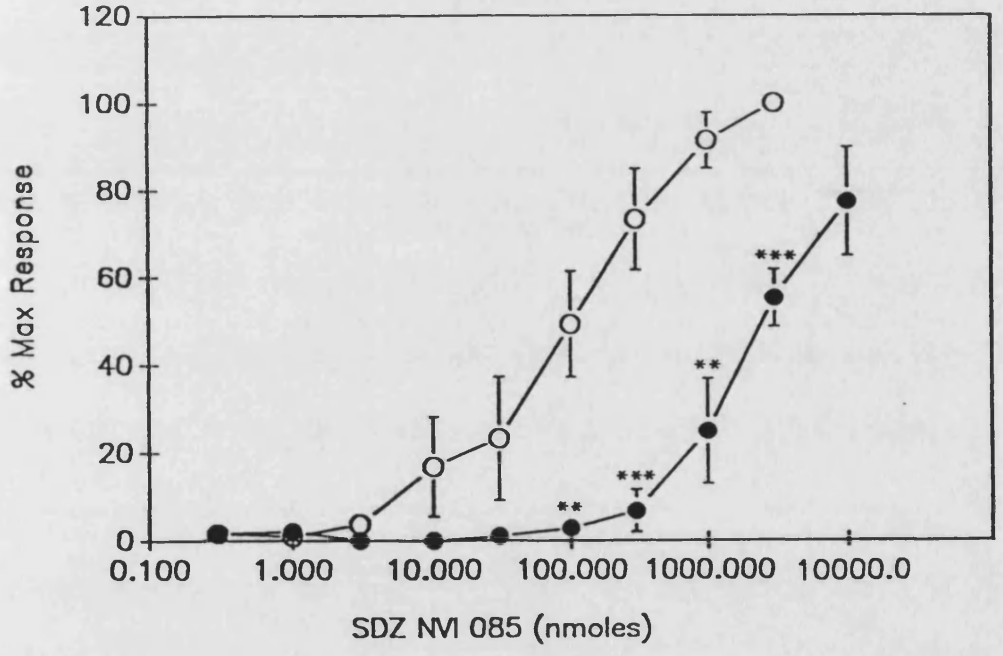


Fig. 45 Effect of SZL-49, $5x10^{-6}$M (●) on methoxamine- and SDZ NVI 085-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=5).
(* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)

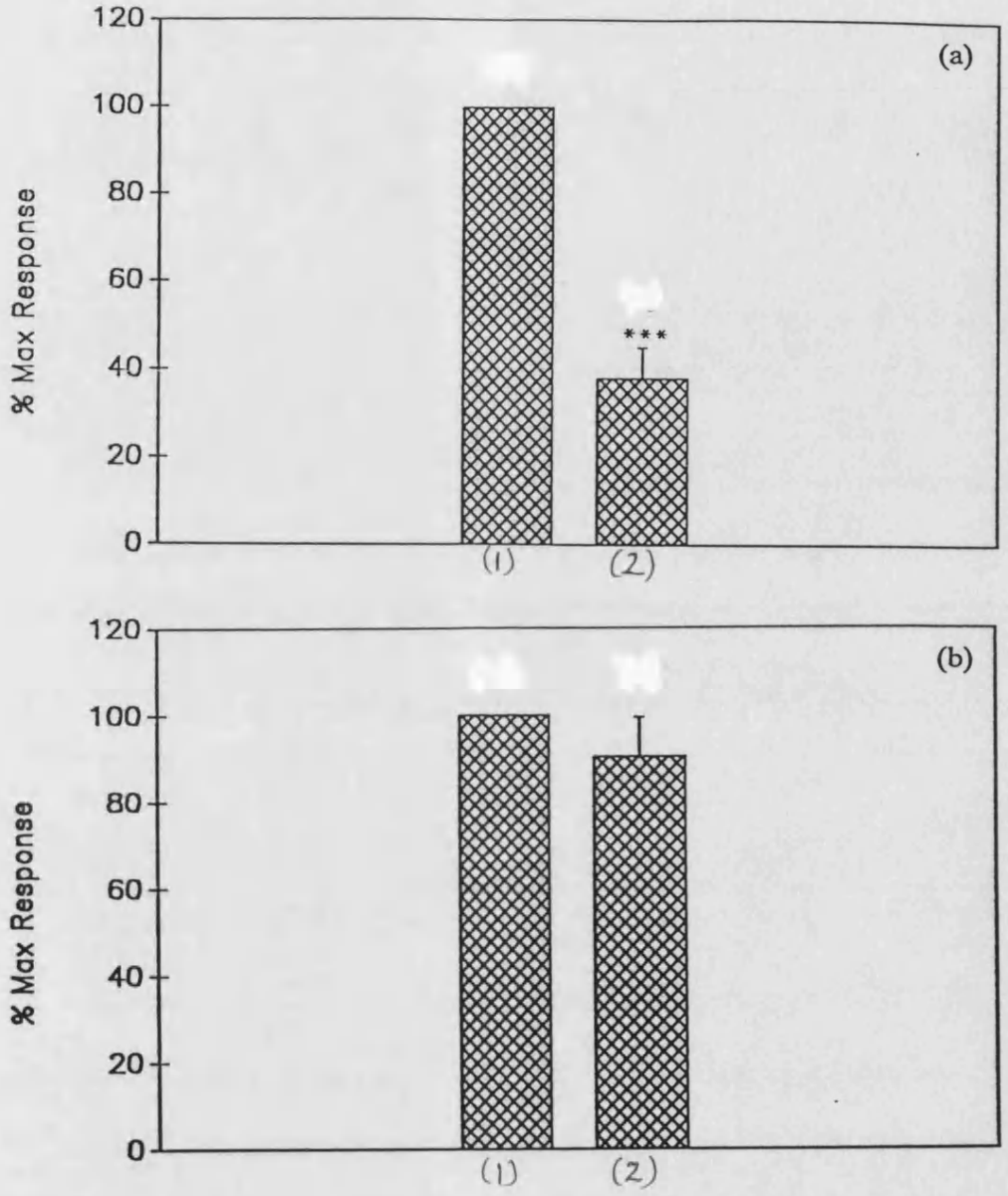


Fig. 46 Vasoconstrictor response of the perfused ovarian vascular bed to electrical stimulation (1) before and (2) after irreversible antagonists; (a) SZL-49 $5x10^{-6}$M and (b) CEC $3x10^{-5}$M (mean±se, n=4). (*** $p < 0.001$)

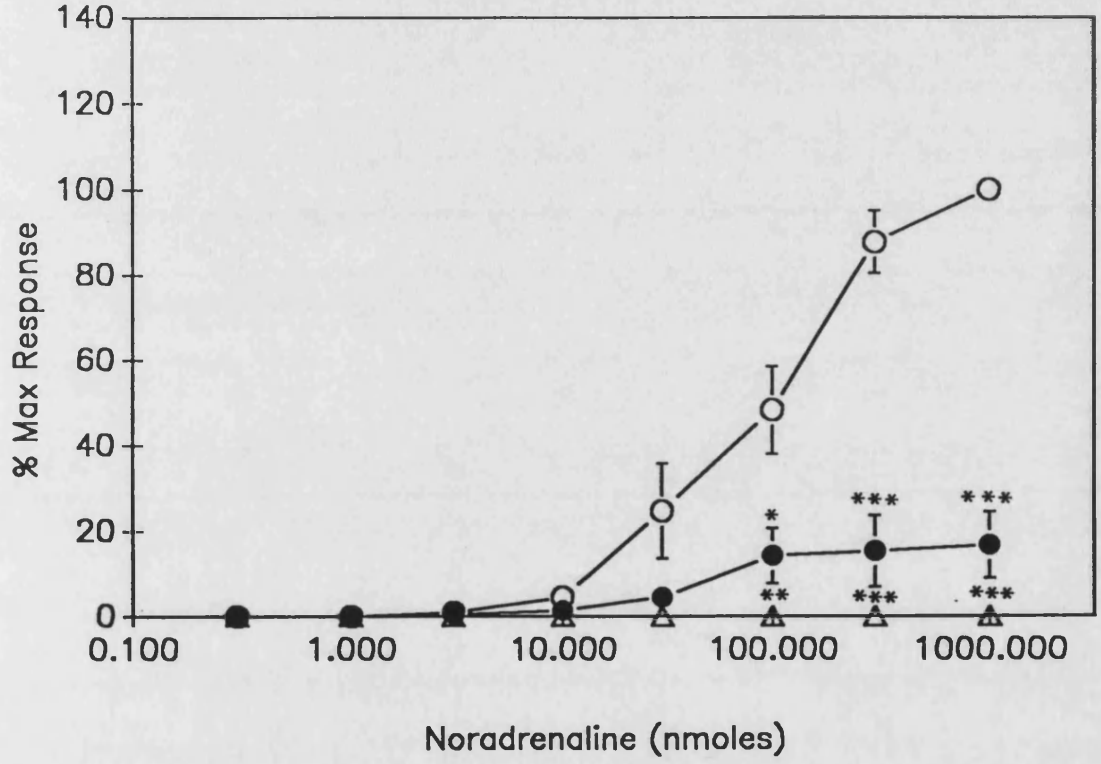


Fig. 47 Effect of Ca^{2+}-free KH-solution with (Δ) or without (●) EGTA on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).
(* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)

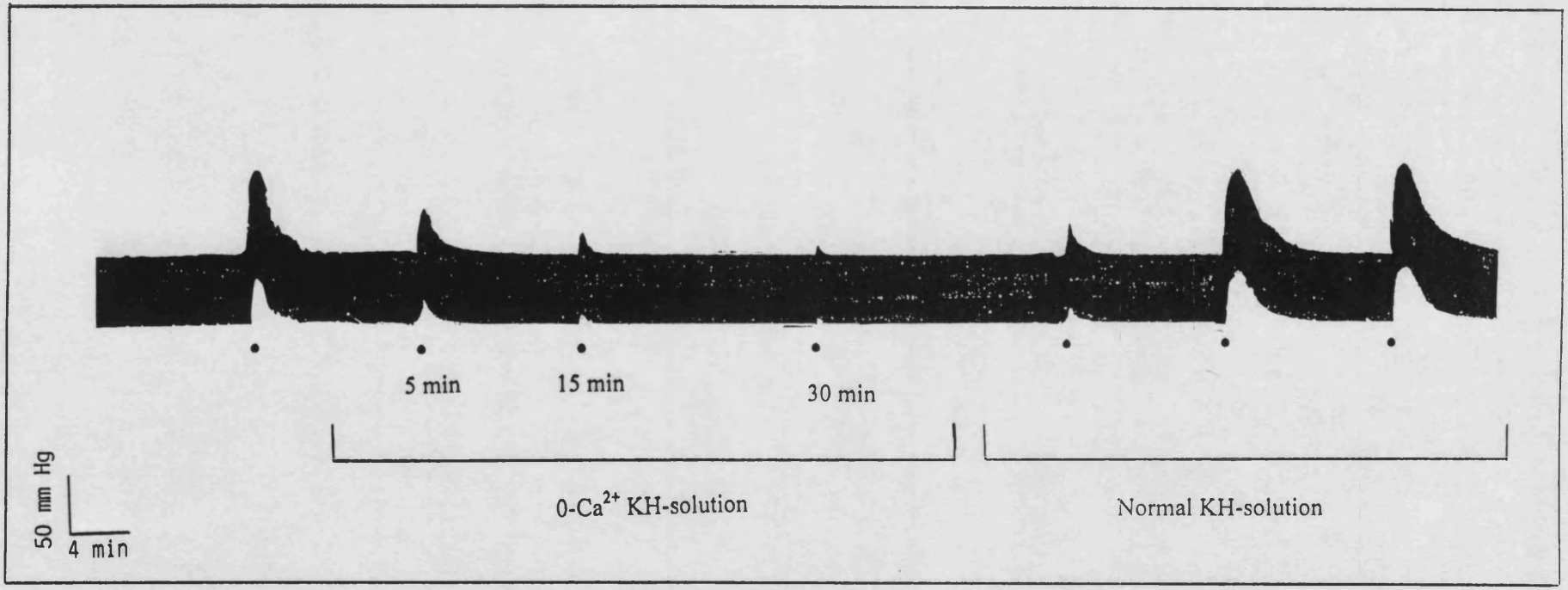


Fig. 48 A typical trace of time-dependent loss of NA-induced vasoconstriction (• = 300 nmoles NA) in Ca^{2+}-free KH-solution.

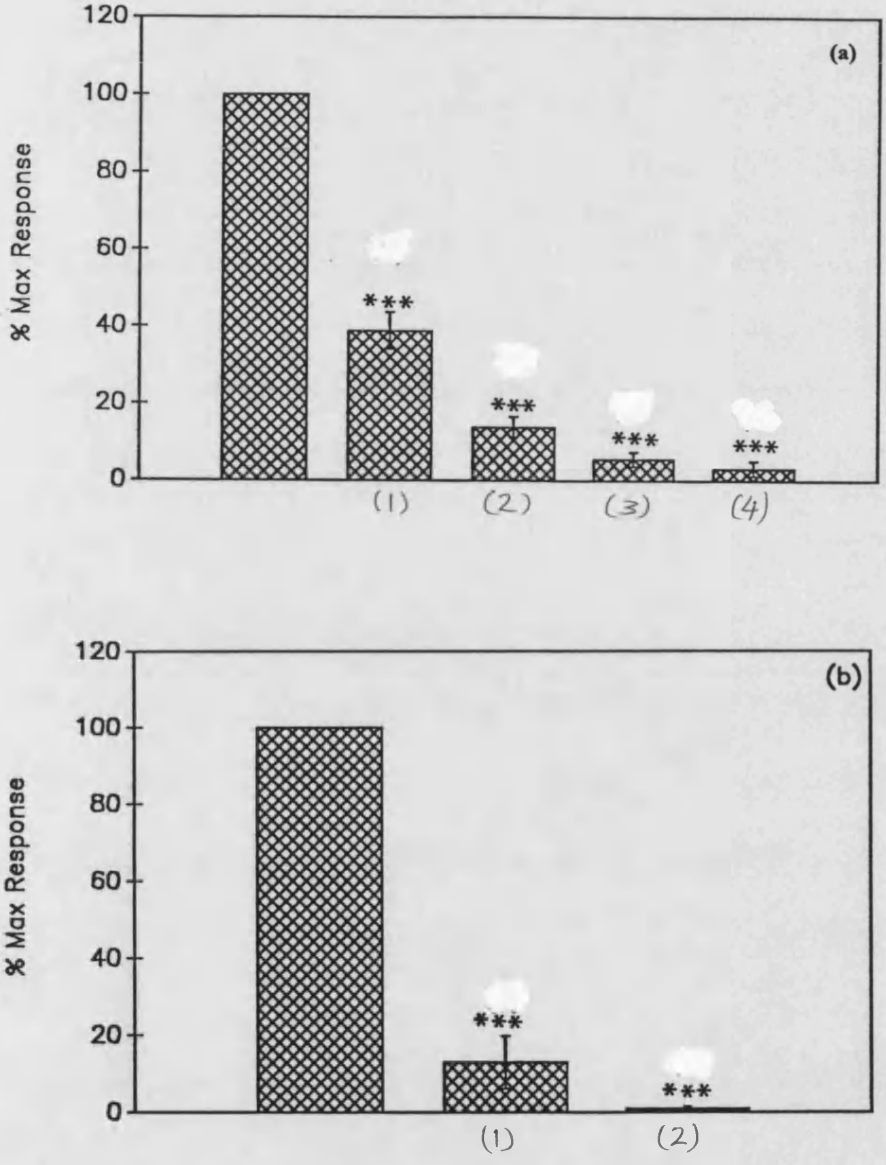


Fig. 49 Time-dependent loss of NA-induced vasoconstriction in Ca^{2+}-free KH-solution, with (b) or without (a) EGTA (2mM) (mean±se, n=6).
[after 5 min (1), 15 min (2), 30 min (3) & 45 min (4)] (*** $p < 0.001$)

responsiveness to NA was 2.5 min.

3.4.2 Calcium channel blocking agents

Neither verapamil nor nifedipine, modified the basal perfusion pressure of the ovarian vascular bed. Verapamil (10^{-7}M) significantly reduced the response to higher doses of NA (>30 nmoles). Hence, as shown in Figure 50, there was no rightward displacement of the NA-dose response curve. Increasing the concentrations of verapamil to 10^{-6}M did not result in increased blockade of NA response. However, at 10^{-5}M, verapamil produced a reduction in the response to NA. At this concentration, verapamil reduced the vasoconstrictor response to low and high doses of NA.

Similarly, nifedipine at concentrations of (10^{-8}M-10^{-5}M) produced a shift of the dose-response curve to NA accompanied by a reduction in the maximal response. The reduction was only statistically significant at the high doses of NA ($p<0.05$) (Fig. 50).

SDZ NVI 085-induced vasoconstriction was affected in a concentration dependent manner by verapamil and nifedipine. The reduction in the contractile response was observed at the higher concentrations of the inhibitors (Fig. 51) However, vasoconstrictor responses to low doses of SDZ NVI 085 were significantly reduced by verapamil and nifedipine.

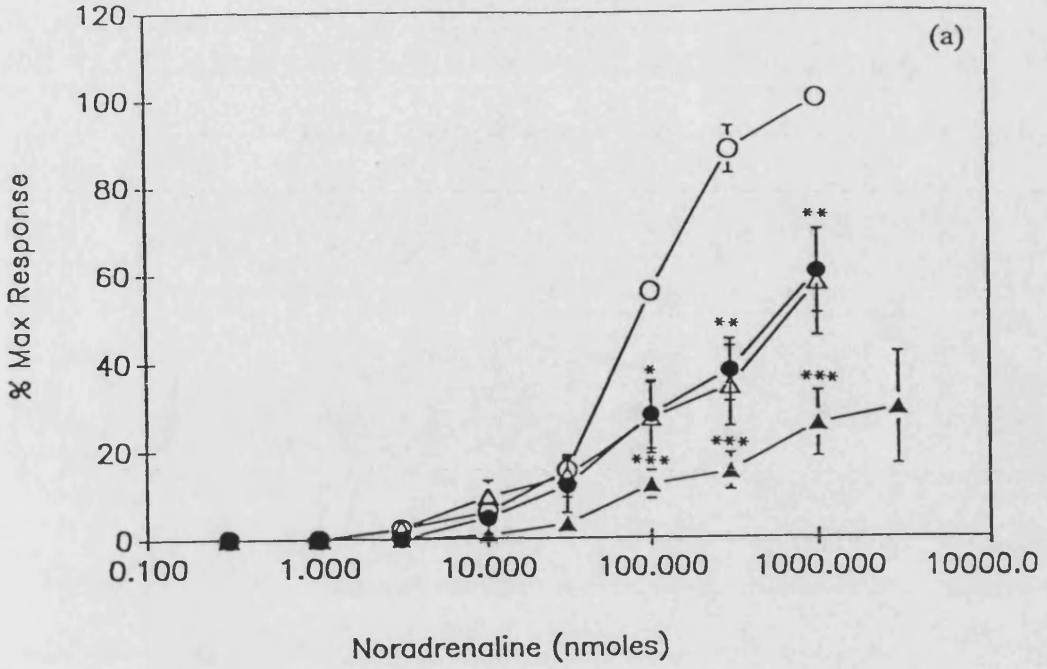
In another set of experiments, the effects of verapamil and nifedipine on KCl-induced vasoconstriction were examined. The KCl-induced vasoconstriction was significantly ($p<0.001$) reduced by verapamil and nifedipine (Fig. 52).

3.4.3 Intracellular calcium antagonist

Perfusion of the ovarian vascular bed with KH-solution containing ryanodine 10^{-5}M did not affect the basal perfusion pressure of the preparations. Neither did it produce any significant change in response to NA ($p > 0.05$) (Fig. 53).

3.4.4 Protein kinase C inhibitors

HA-1077 ($3x10^{-7}$M) produced a significant suppression ($p < 0.01$) of maximal response to NA. A further significant reduction in response to NA was observed at $3x10^{-6}$M ($p<0.05$) (Fig. 54). However, polymyxin B (10^{-6}M-10^{-4}M) did not cause any significant reduction in the pressor response to NA ($p > 0.05$). There was a slight but non-significant enhancement of NA dose-response curve at concentrations



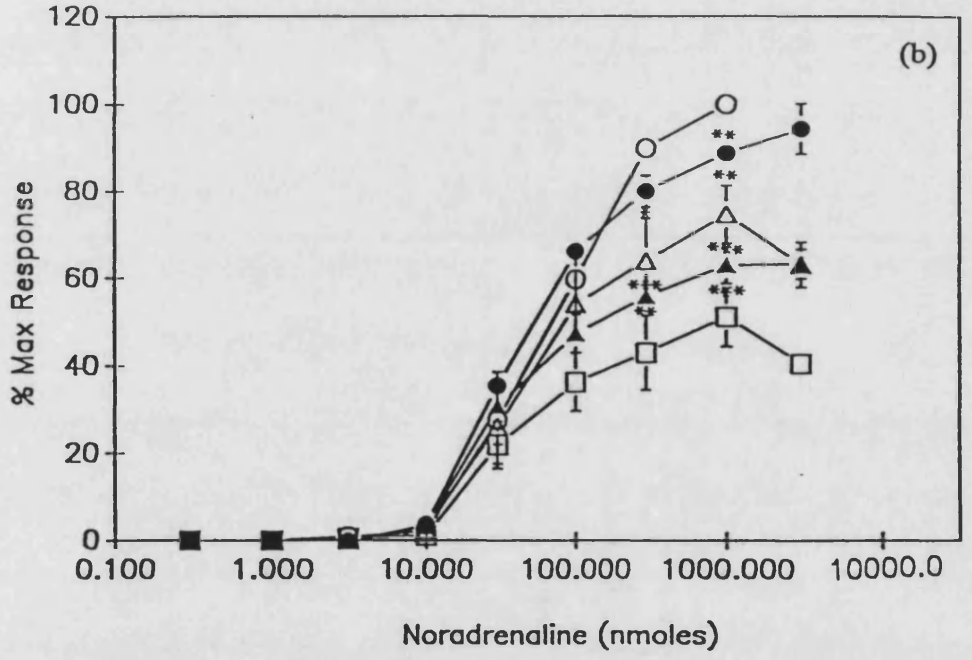
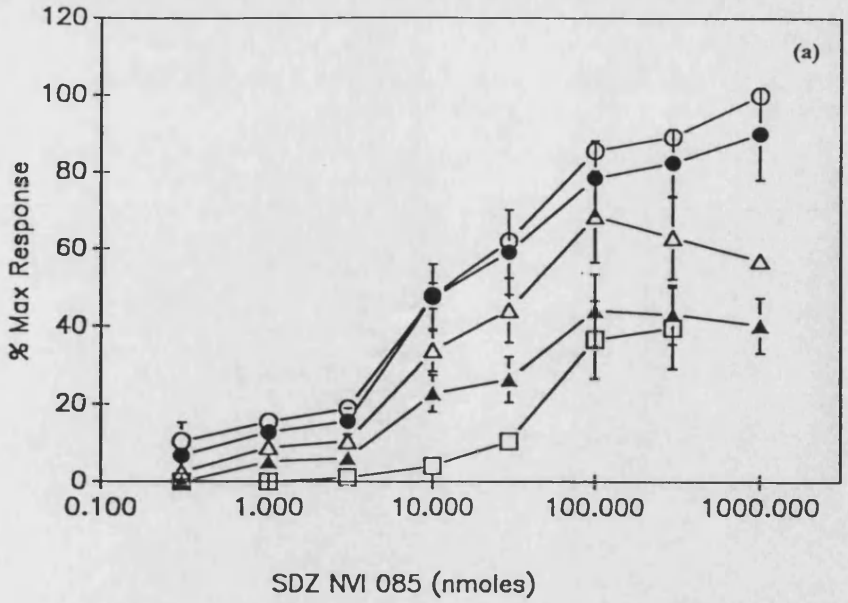


Fig. 50a Effect of verapamil 10^{-7}M (●) , 10^{-6}M (Δ) and 10^{-5}M (▲) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).

Fig. 50b Effect of nifedipine 10^{-8}M (●), 10^{-7}M(Δ) , 10^{-6}M (▲) and 10^{-5}M (□) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4). (* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)



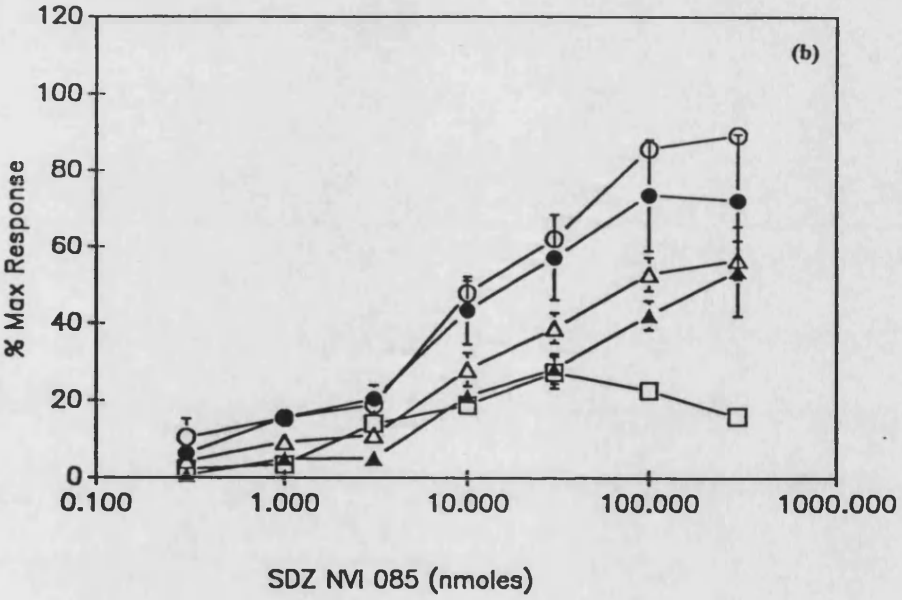


Fig. 51 Effect of (a) verapamil and (b) nifedipine; 10^{-8}M (●), 10^{-7}M (Δ), 10^{-6}M (▲) and 10-5M (□) on SDZ NVI 085-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4). [vasoconstrictor effects to SDZ NVI 085 ≥ 10 nmoles are significant at concentrations of 10^{-7} M, 10^{-6} M & 10^{-5} M of verapamil & nifedipine, $p < 0.01$]

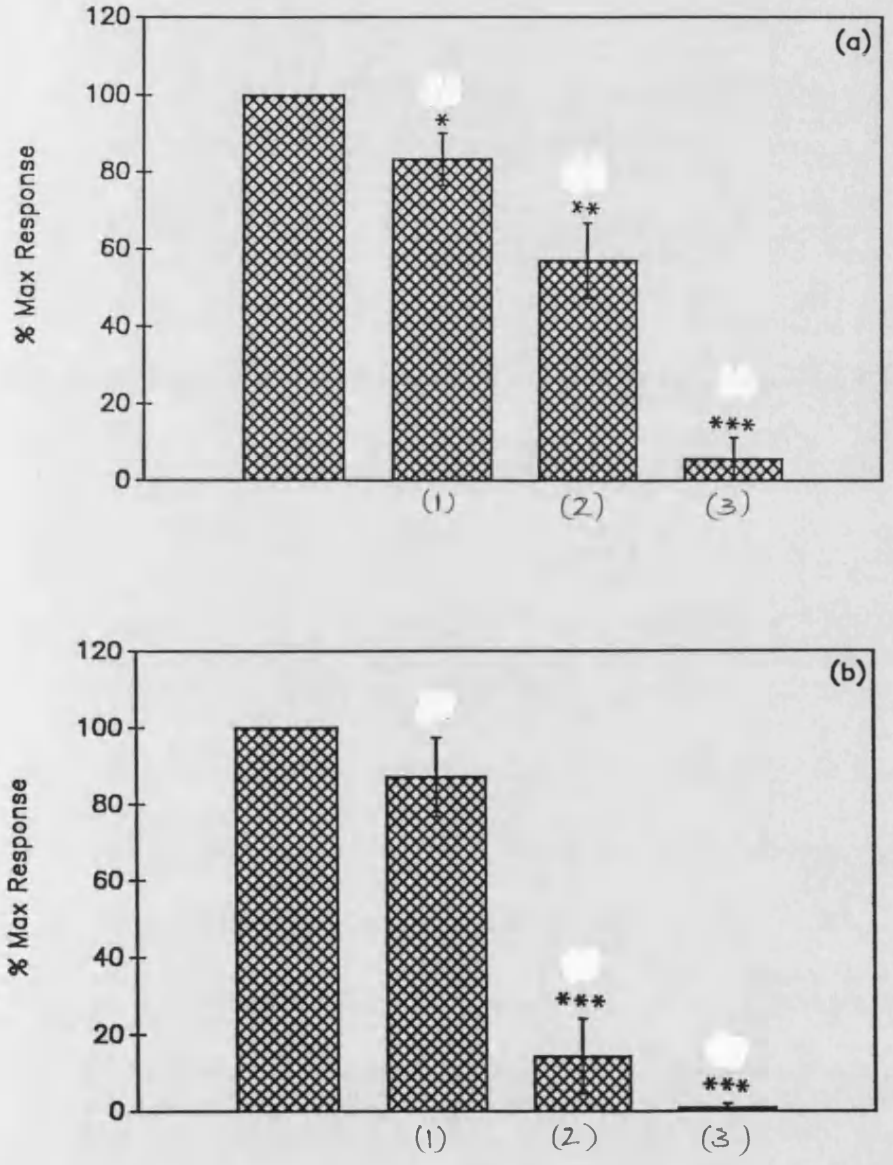


Fig. 52 Effect of verapamil (a) and nifedipine (b); 10^{-8}M (1), 10^{-7}M (2) and 10^{-6}M (3) on KCl-induced vasoconstriction in the perfused rabbit ovarian vascular bed (mean±se, n=4). (* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)

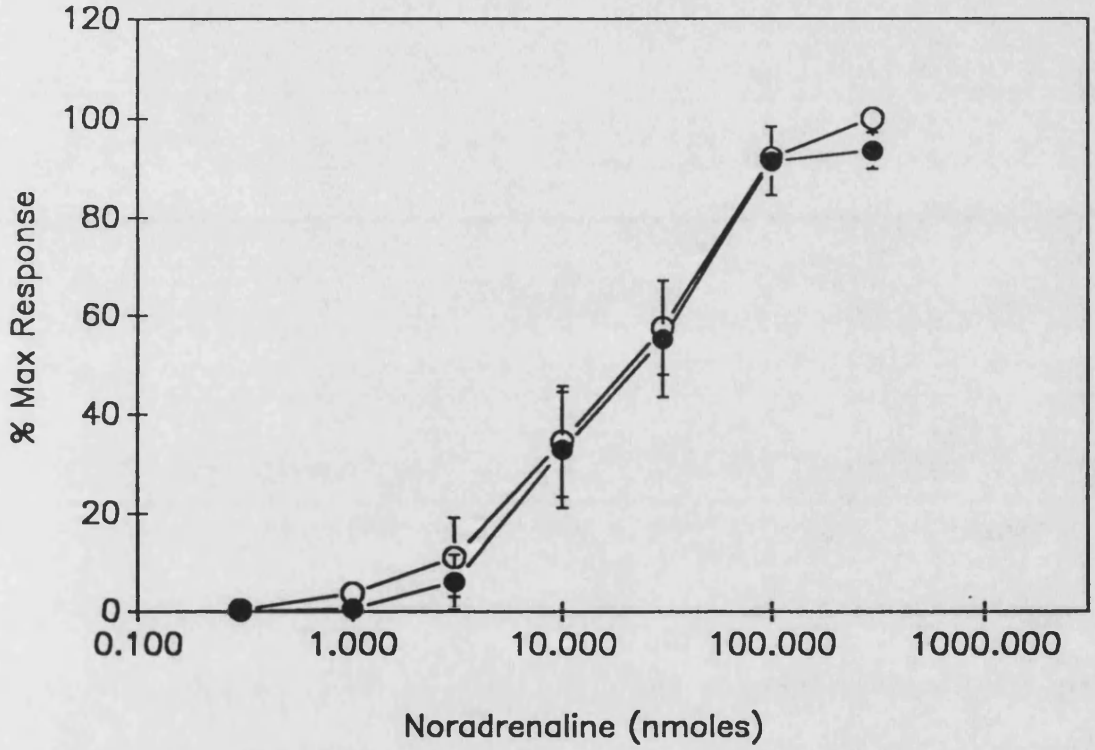


Fig. 53 Effect of ryanodine, 10^{-5}M (●)on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).

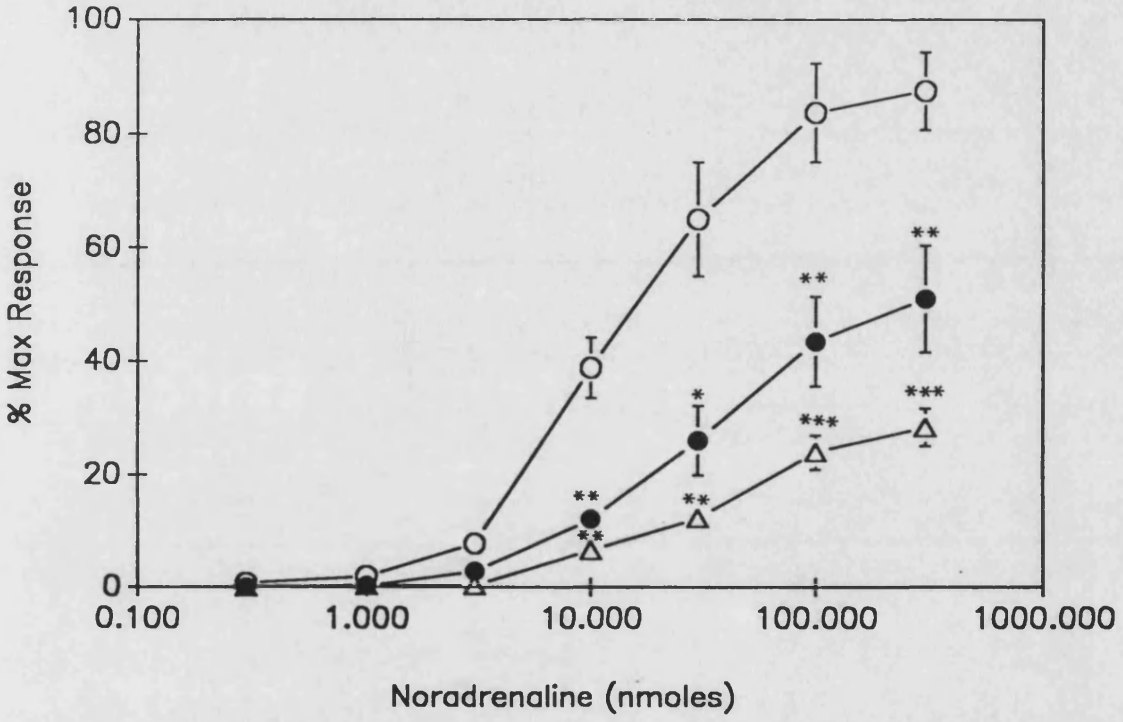


Fig. 54 Effect of HA-1077 $3x10^{-7}$M (●) and $3x10^{-6}$M (Δ) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).
(* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)

of 10^{-6}M-10^{-4}M. (Fig. 55). As shown in Figure 56, vasoconstrictor response to $CaCl_2$ (100 μM) was significantly attenuated after treatment with HA-1077 ($p < 0.05$), while responses were not affected by polymyxin B, even at the concentration of 10^{-4} M.

3.4.5 Tyrosine kinase inhibitor

Incubation of the ovarian vascular bed with genistein (10^{-6}M-10^{-5}M) produced a significant ($p < 0.01$) suppression of the NA dose-response curve accompanied by a reduction of the maximal response (Fig. 57).

3.5 Effects of hormones and pregnancy

3.5.1 Ovulation induction with HCG

In this group of rabbits, the isolated ovaries were reddish and swollen, indicating increased permeability and blood supply. Ovulation points (follicles) observed were 6.7±0.8 in a group of 6 rabbits. The basal perfusion pressure for HCG-treated rabbits was 27.5 ± 5.3 mmHg. This was significantly ($p < 0.05$) different from corresponding values in control rabbits (Table 8). NA and oxymetazoline produced dose-dependent vasoconstriction of the ovarian vascular bed (Fig. 58). The ED_{50} values were 4.5±1.4 and 3.8±1.7 nmoles respectively. These values were significantly lower than corresponding values obtained for NA ($p < 0.05$) and oxymetazoline ($p < 0.05$) in control rabbits (Table 9) indicating enhanced sensitivity. In order to determine whether the increased sensitivity of the ovarian vascular bed to NA and oxymetazoline was due to a specific alteration in α_1-adrenoceptor sensitivity or a non-specific increase in tissue sensitivity, the pressor effect of histmine was examined. As shown in Figure 59, the sensitivity of the preparations to histamine was not altered by HCG treatment. The ED_{50} values were 51.9±5.4 and 53.5±9.0 in control and HCG treated rabbits respectively ($p > 0.05$). The maximum increases in perfusion pressure recorded for NA, oxymetazoline and histamine (Table 10) were not significantly different between control and HCG treated rabbits ($p > 0.05$). Oxymetazoline remained as a partial agonist.

The vasoconstrictor responses to NA were slightly but not significantly ($p > 0.05$) potentiated by L-NOARG (10^{-5}M) (Fig. 60).

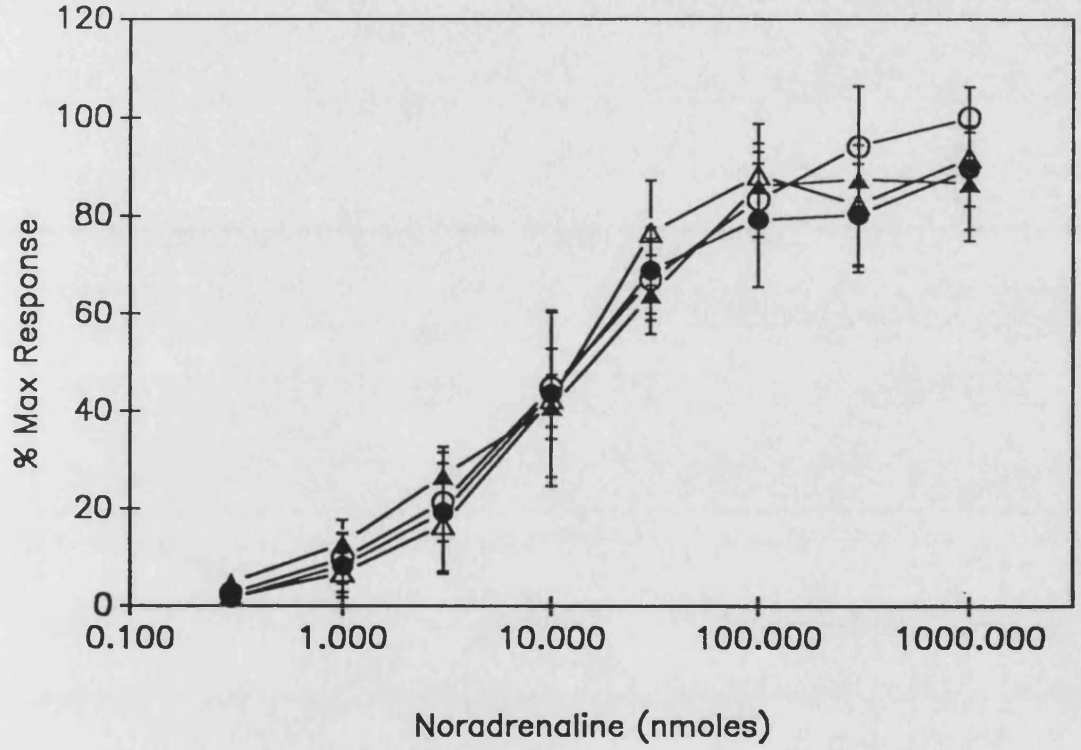


Fig. 55 Effect of polymyxin B 10^{-6}M (●), 10^{-5}M (Δ) and 10^{-4}M (▲) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=5).

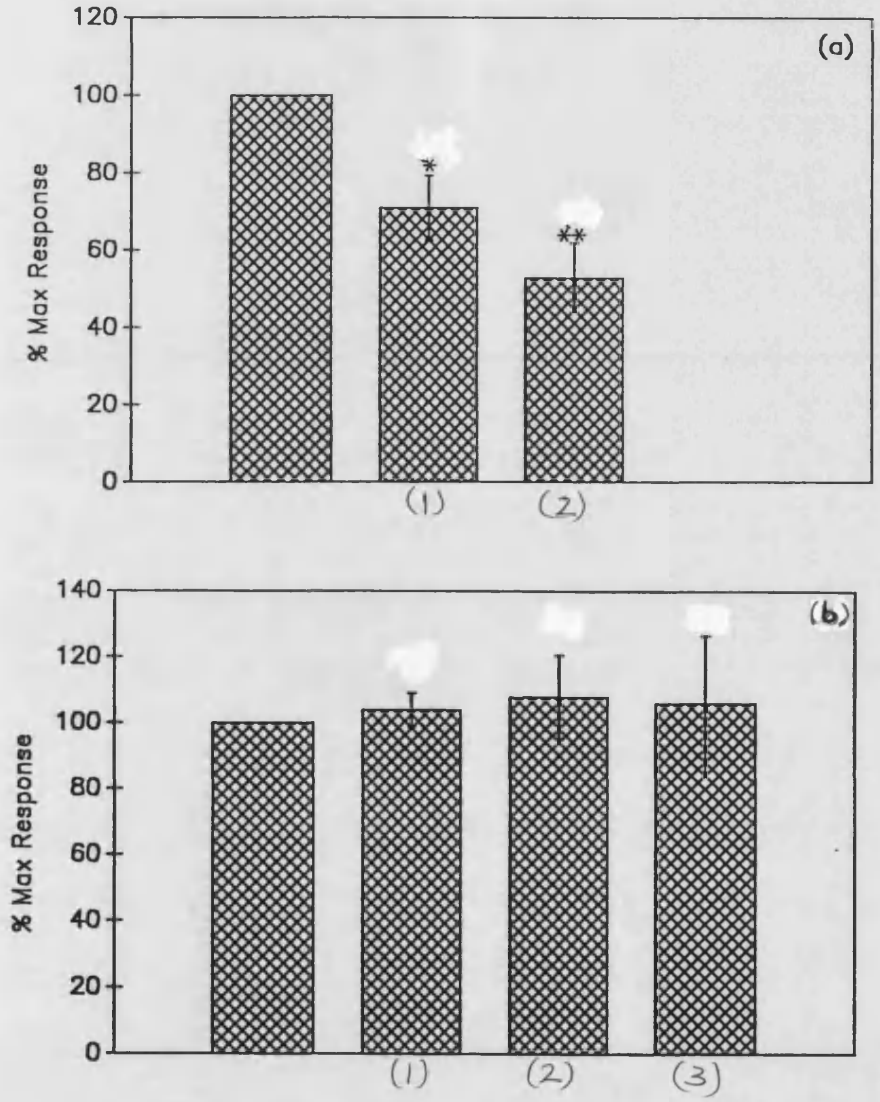


Fig. 56 Vasoconstrictor responses to $CaCl_2$ (100 μmoles) in 80 mM KCl depolarized KH-solution before and after treatment with PKC inhibitors (mean±se, n=3).

(a) HA-1077, (1) $3x10^{-7}$ M and (2) $3x10^{-6}$ M.

(b) Polymyxin B , (1) 10^{-6} M, (2) 10^{-5} M and (3) 10^{-4} M.

(* $p < 0.05$ & ** $p < 0.01$)

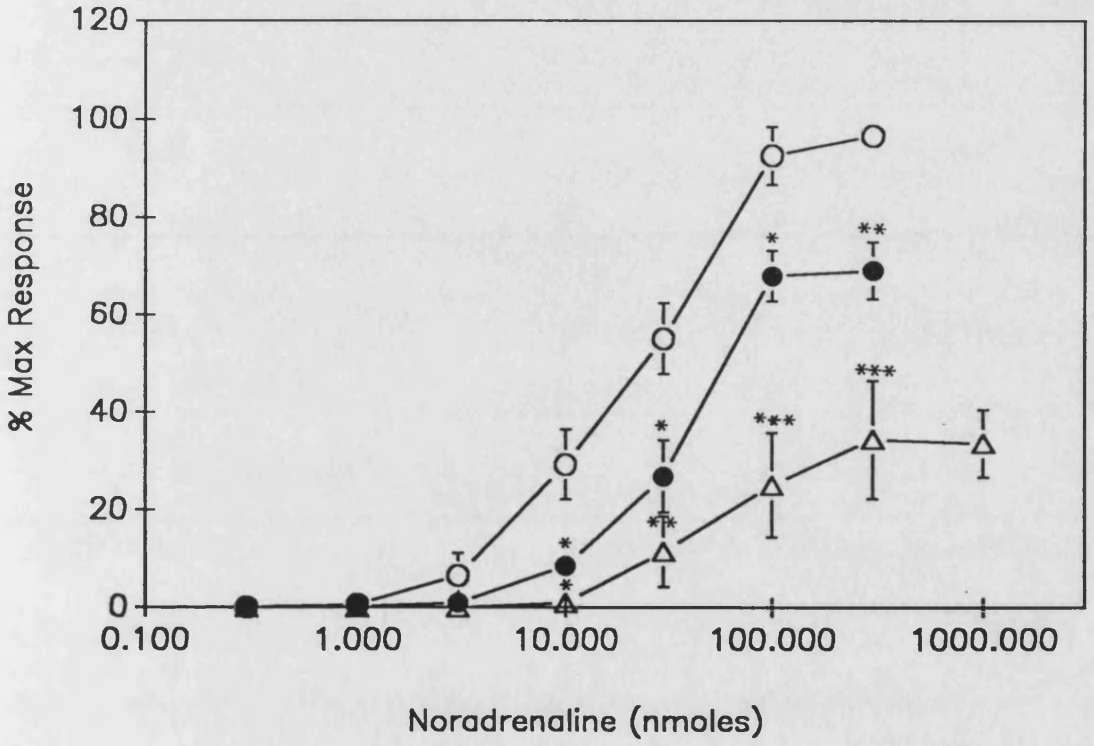


Fig. 57 Effect of genistein, 10^{-6}M (●) and 10^{-5}M (Δ) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=4).

(* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)

Table 8 Basal perfusion pressures (B.P.P. mmHg), of the perfused ovarian vascular bed in control and treated rabbits, (mean ± se, n=4-8). (* = $p<0.05$)

Animal group	B.P.P.
Control	41.7±3.3
HCG-treated	27.5±5.3*
Oestrogen-treated	33.0±3.4
Pregnant	27.8±4.1*

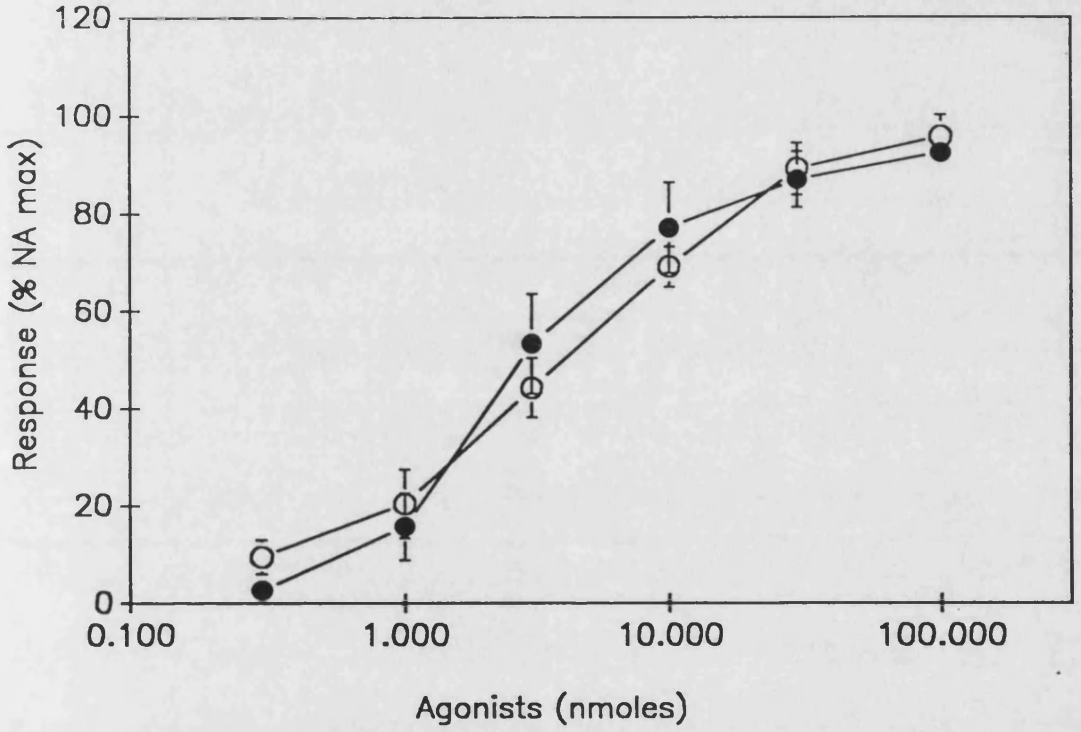


Fig. 58 NA- (O) and oxymetazoline-induced vasoconstriction (●) in the perfused ovarian vascular bed of HCG-treated (100 i.u.) rabbits (mean±se, n=5).

Table 9 ED_{50} values (nmoles) for different agonists in the perfused ovarian vascular bed of control and treated rabbits, (mean ± se, n=4-8).

(* = $p<0.05$ & ** = $p<0.01$)

	NA	Oxymetazoline	Histamine
Control	25.1±6.0	53.4±15.6	51.9±5.4
HCG-treated	4.5±1.4**	3.8±1.7*	53.5±9.0
Oestrogen-treated	14.7±5.3	3.3±1.8*	45.4±7.8
Pregnant	10.0±3.0*	6.2±2.0*	147.4±24.4**

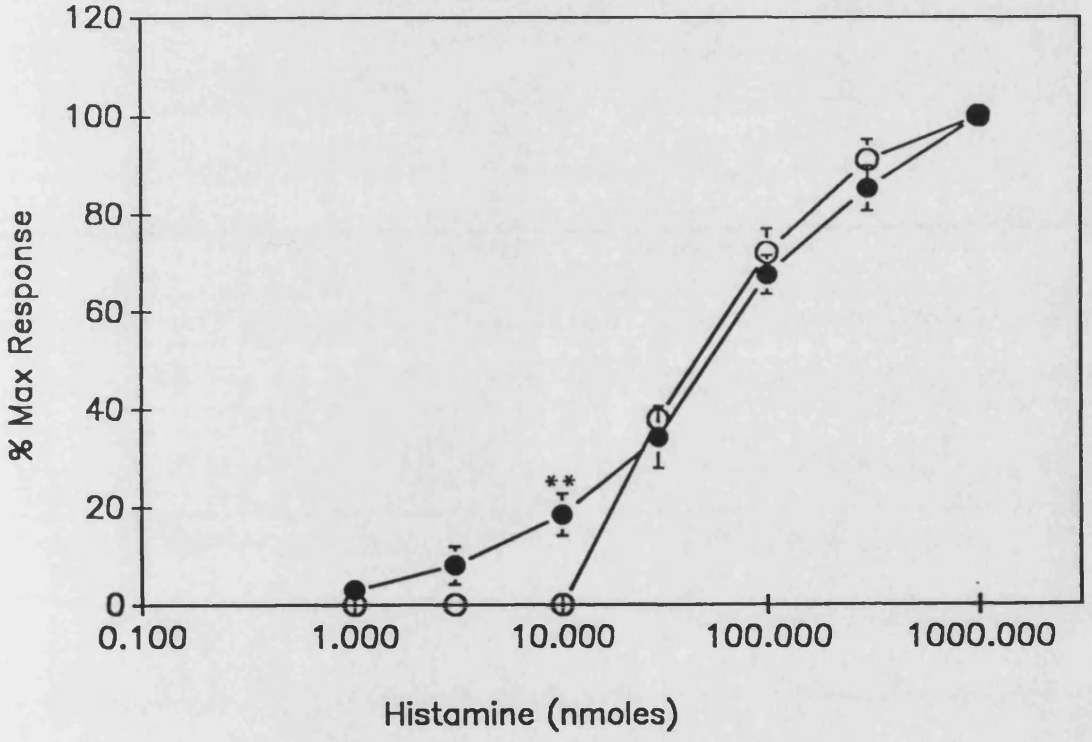


Fig. 59 Histamine-induced vasoconstriction in the perfused ovarian vascular bed of control (O) and HCG-treated (100 i.u.) rabbits (●) (mean±se, n=4). (** $p < 0.01$)

Table 10 Maximal increases in perfusion pressure (mmHg) produced by different agonists in the perfused ovarian vascular bed of control and treated rabbits, (mean ± se, n=4-8).

(* = $p<0.05$)

	Noradrenaline	Oxymetazoline	Histamine
Control	198.8±11.2	135.4±12.2	205±18.7
HCG-treated	206.7±14.0	176.3±13.3	171.9±29.7
Oestrogen-treated	214.6±10.9	187.0±16.1*	181.3±10.1
Pregnant	192.7±12.4	75.0±8.4*	182.5±12.0

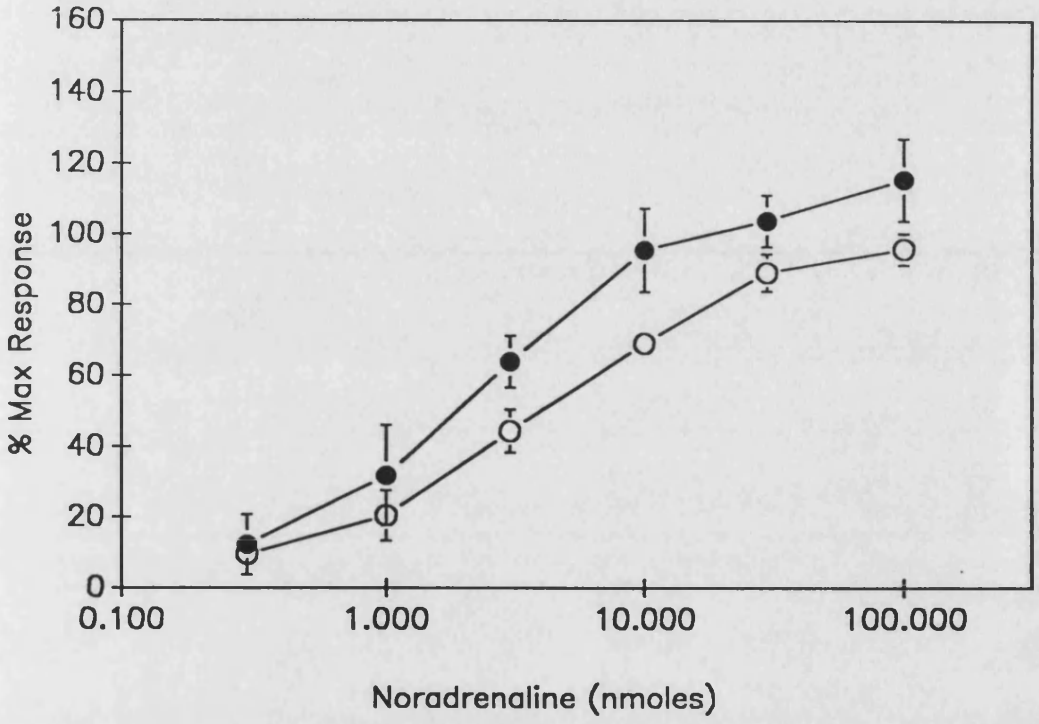


Fig. 60 Effect of L-NOARG 10^{-5}M (●) on NA-induced vasoconstriction (O) in the perfused ovarian vascular bed of HCG-treated (100 i.u.) rabbits (mean±se, n=4).

Relaxant responses for carbachol and SNP were established before and after incubation with L-NOARG. Carbachol (0.1-1000 nmoles) dose-dependently reduced the perfusion pressure. The ED_{50} value of carbachol was 8.4±3.9 nmoles and the maximum relaxation was 42.6±11.3 % of basal tone. Carbachol-induced relaxation was suppressed significantly after incubation with L-NOARG 10^{-5}M ($p<0.05$). SNP (0.1-1000 nmoles) also produced a dose-dependent reduction in perfusion pressure. However, L-NOARG (10^{-5}M) had no effect on SNP-induced responses (Fig. 61) (Table 11). There was no significant difference in ED_{50} values or maximal relaxation produced by carbachol or SNP in the control and HCG-treated rabbits ($p > 0.05$).

3.5.2 Effects of 17-ß-oestradiol

The basal perfusion pressure in the ovarian vascular bed isolated from oestrogen-treated rabbits was 33.0 ± 3.4 mmHg, which was not significantly different from corresponding values in control rabbits ($p > 0.05$). NA and oxymetazoline produced dose-dependent vasoconstriction of the ovarian vascular bed (Fig. 62). The ED_{50} values were 14.7±5.3 and 3.3±1.8 nmoles respectively. These values were lower than those in control rabbits. The ED_{50} value for oxymetazoline was significantly reduced ($p < 0.05$), indicating enhanced sensitivity. The sensitivity of the preparations to histamine was not affected (ED_{50} values were 51.9±5.4 and 45.4±7.8 nmoles in control and oestrogen-treated rabbits, respectively). There was no significant difference in the maximum perfusion pressures recorded for NA or histamine in control and oestrogen treated rabbits, while the corresponding value for oxymetazoline was significantly enhanced ($p < 0.05$) (Table 10). Vasoconstrictor responses to NA were not significantly potentiated by L-NOARG (10^{-5}M) (Fig. 63). Carbachol (0.1-1000 nmoles) produced a dose-dependent reduction in perfusion pressure. At the highest concentration of cabachol used, the reduction in pressure was 41.7±8.4 % of basal tone. The ED_{50} value was 3.3±0.2 nmoles. The relaxant response to carbachol was significantly reduced after incubation with L-NOARG 10^{-5}M ($p < 0.01$). SNP also produced a dose-dependent reduction in perfusion pressure. However, the response to SNP was not affected by L-NOARG 10^{-5}M. There was no significant difference in ED_{50} values or maximal relaxation produced by carbachol or SNP in the control and oestrogen-treated rabbits ($p > 0.05$).

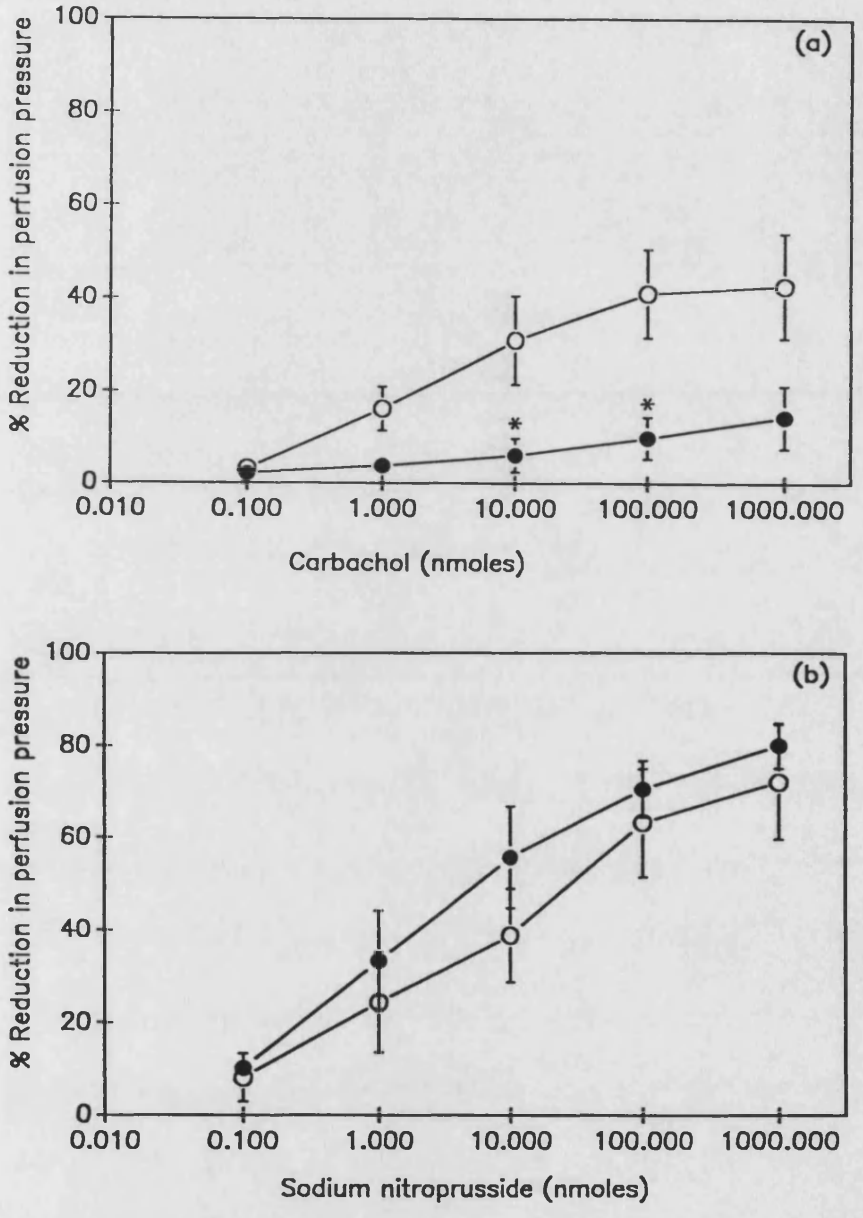


Fig. 61 Effect of L-NOARG 10^{-5}M (●) on vasodilator responses to (a) carbachol and (b) sodium nitroprusside in the perfused ovarian vascular bed of HCG-treated (100 i.u.) rabbits (mean±se, n=4). (* $p < 0.05$)

Table 11 ED_{50} values and maximal reduction in perfusion pressures for carbachol and SNP in the perfused ovarian vascular bed of control and treated rabbits, (mean±se, n=4-5).

a = nmoles

P.P. = perfusion pressure

	Carbachol		SNP	
	ED_{50}[a]	% Reduction P.P.	ED_{50}[a]	% Reduction P.P.
Control	3.2±0.9	69±9.5	2.9±1.1	74.4±14.1
HCG-treated	8.4±3.9	42.6±11.3	9.9±5.9	72.0±12.5
Oestrogen-treated	3.3±0.2	41.7±8.4	6.3±1.2	81.0±4.2
Pregnant	7.2±3.9	61.0±11.7	5.2±2.3	70.6±7.6

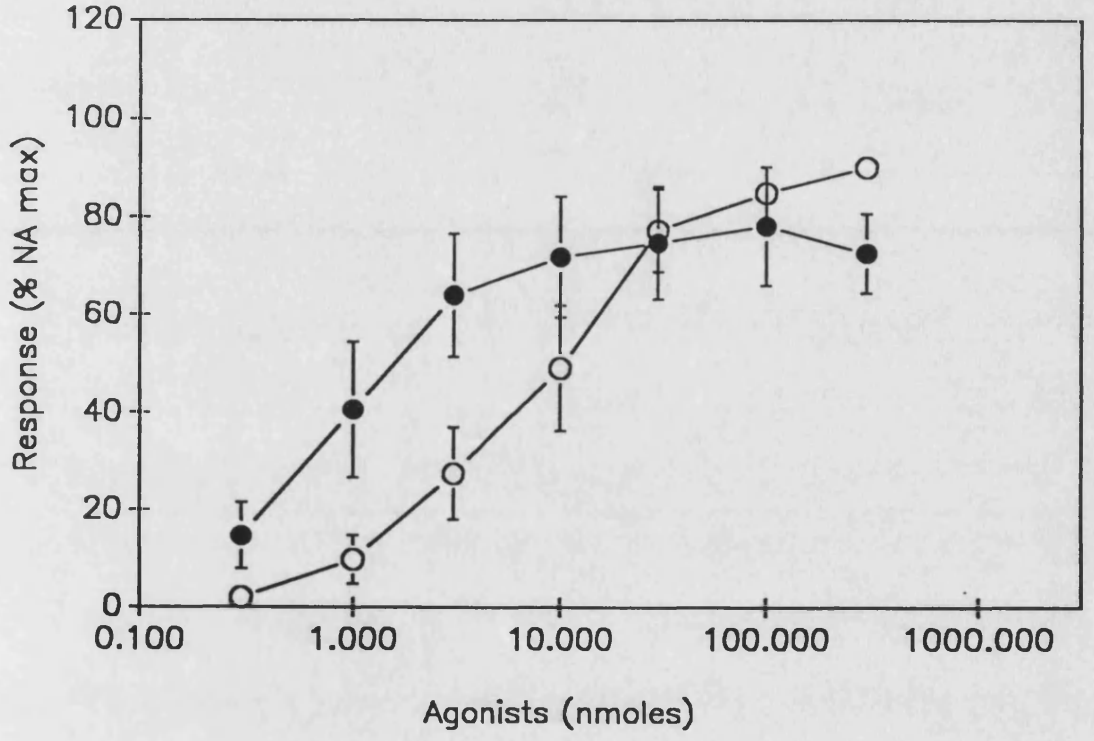


Fig. 62 NA- (O) and oxymetazoline-induced vasoconstriction (●) in perfused ovarian vascular bed of ß-oestradiol 17-propionate-treated (5 mg) rabbits (mean±se, n=5).

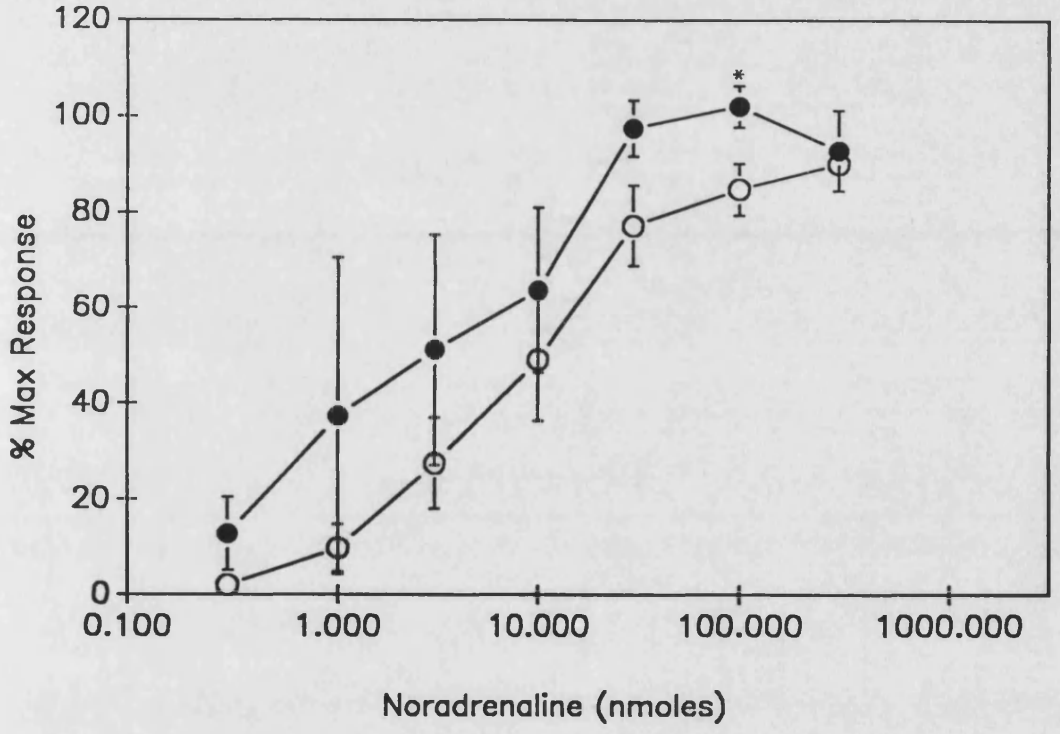


Fig. 63 Effect of L-NOARG 10^{-5}M (●) on NA-induced vasoconstriction (O) in the perfused ovarian vascular bed of ß-oestradiol 17-propionate-treated (5 mg) rabbits (mean±se, n=4).

NA-induced vasoconstriction was not affected at lower concentrations of 17-ß-oestradiol, when applied to the perfusing medium. However, at higher concentrations of 17-ß-oestradiol, NA-induced vasoconstriction was attenuated (Fig. 64). In addition, there was a significant suppression in the maximal perfusion pressure ($p < 0.001$).

3.5.3 Effects of pregnancy

The mean basal perfusion pressure for the ovarian vascular bed in pregnant rabbits at day 22 - 24 of gestation was 27.8 ± 4.1 mmHg and this was significantly different ($p < 0.05$) from corresponding values in control rabbits. NA and oxymetazoline produced dose-dependent vasoconstriction of the ovarian vascular bed (Fig. 65). The ED_{50} values were 10.0±3.0 and 6.2±2.0 nmoles respectively. These values were significantly lower ($p < 0.05$) than corresponding values obtained for NA and oxymetazoline in control rabbits (Table 9), indicating that sensitivity to these agents was enhanced. The sensitivity of the preparations to histamine was significantly reduced during pregnancy ($p < 0.01$) (Fig. 66). The ED_{50} values were 51.9±5.4 and 147.4±24.4 nmoles in control and pregnant rabbits respectively. The maximum perfusion pressures recorded for NA and histamine were not significantly different compared to control animals ($p > 0.05$), while the corresponding value for oxymetazoline was significantly reduced ($p < 0.05$) (Table 10). Vasoconstrictor responses to NA were not significantly potentiated by L-NOARG 10^{-5}M. The ED_{50} value for NA after L-NOARG (10^{-5}M) in the pregnant rabbit was 5.5±2.9 nmoles and the maximum response was 87.3%. However, the vasoconstrictor response to oxymetazoline was significantly potentiated by L-NOARG (10^{-5}M) ($p < 0.05$) (Fig. 67). Yohimbine (10^{-5}M) did not affect the NA dose-response curve (Fig. 68). In addition, B-HT 920 did not produce any vasoconstrictor response either at basal perfusion pressure or when it was raised with U46619, indicating that α_2-adrenoceptors were not functional.

Prazosin (10^{-7}M) shifted the NA dose-response curve without suppressing the maximum response (Fig. 69). The pK_B value was calculated to be 9.6 ± 0.3. Vasoconstrictor responses to NA were not affected after incubation with CEC ($3x10^{-5}$M), however, there was a significant reduction ($p < 0.001$) after incubation with SZL-49 ($5x10^{-6}$M) (Fig. 70).

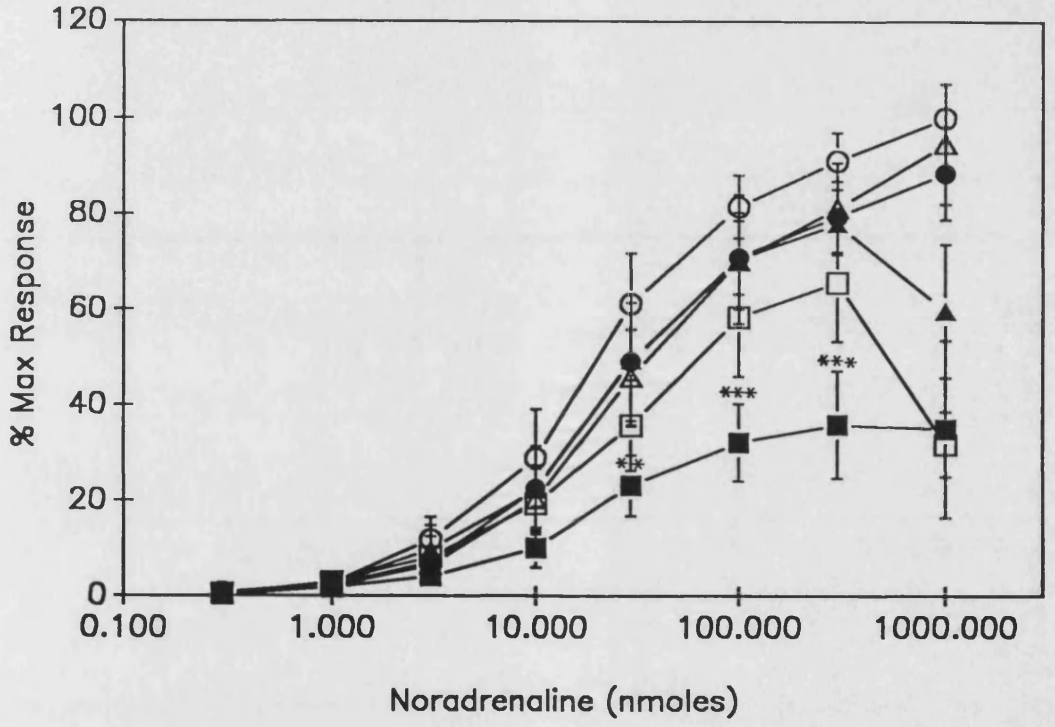


Fig. 64 Effect of 17-ß-oestradiol (in vitro) $3x10^{-9}$M (●), $3x10^{-8}$M (Δ), $3x10^{-7}$M (▲), $3x10^{-6}$M (□) and $3x10^{-5}$M (■) on NA-induced vasoconstriction (O) in the perfused rabbit ovarian vascular bed (mean±se, n=7). (** $p < 0.01$ & *** $p < 0.001$)

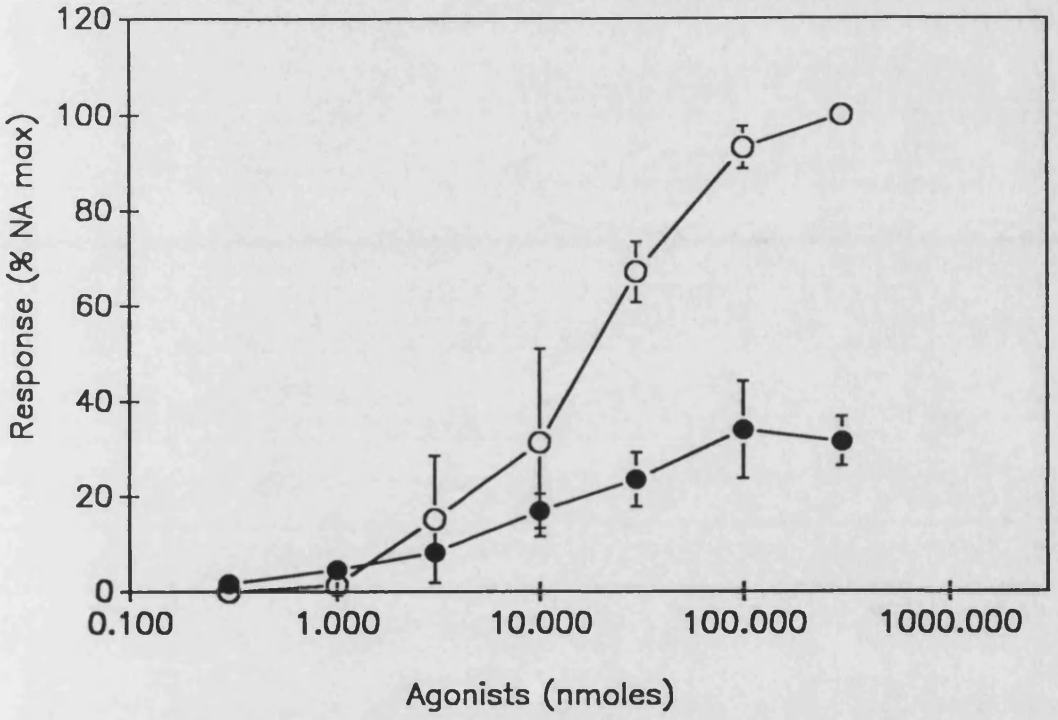


Fig. 65 NA- (O) and oxymetazoline-induced vasoconstriction (●) in the perfused ovarian vascular bed of pregnant rabbits (mean±se, n=4-5).

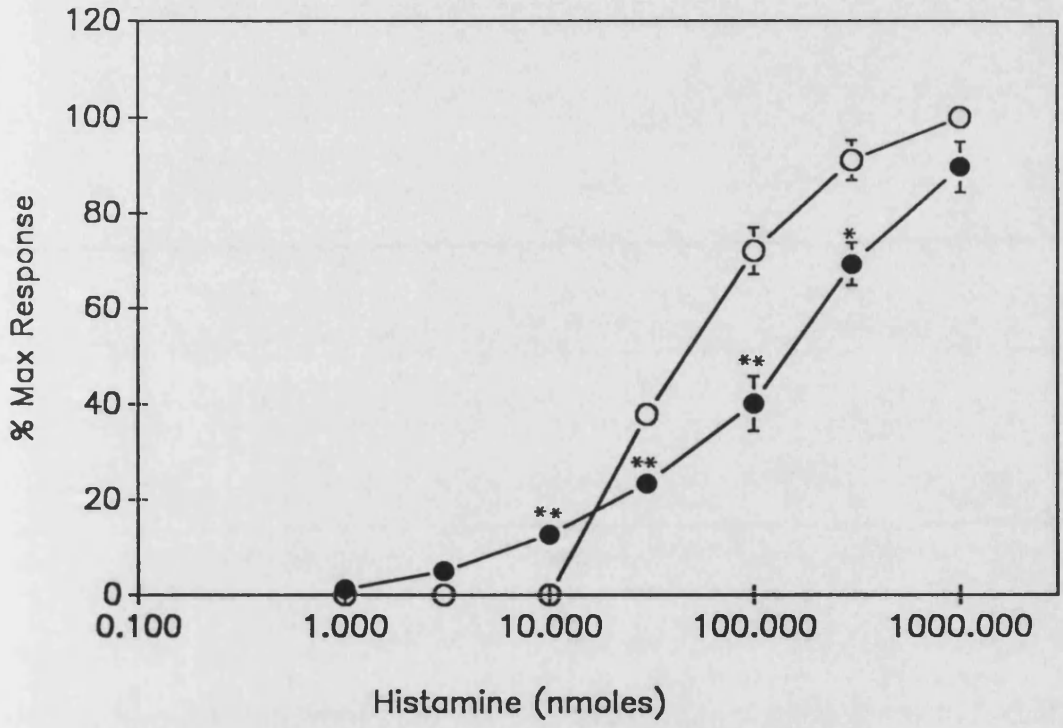


Fig. 66 Histamine-induced vasoconstriction in perfused ovarian vascular bed of control (O) and pregnant rabbits (●) (mean±se, n=4). (* $p < 0.05$ & ** $p < 0.01$)

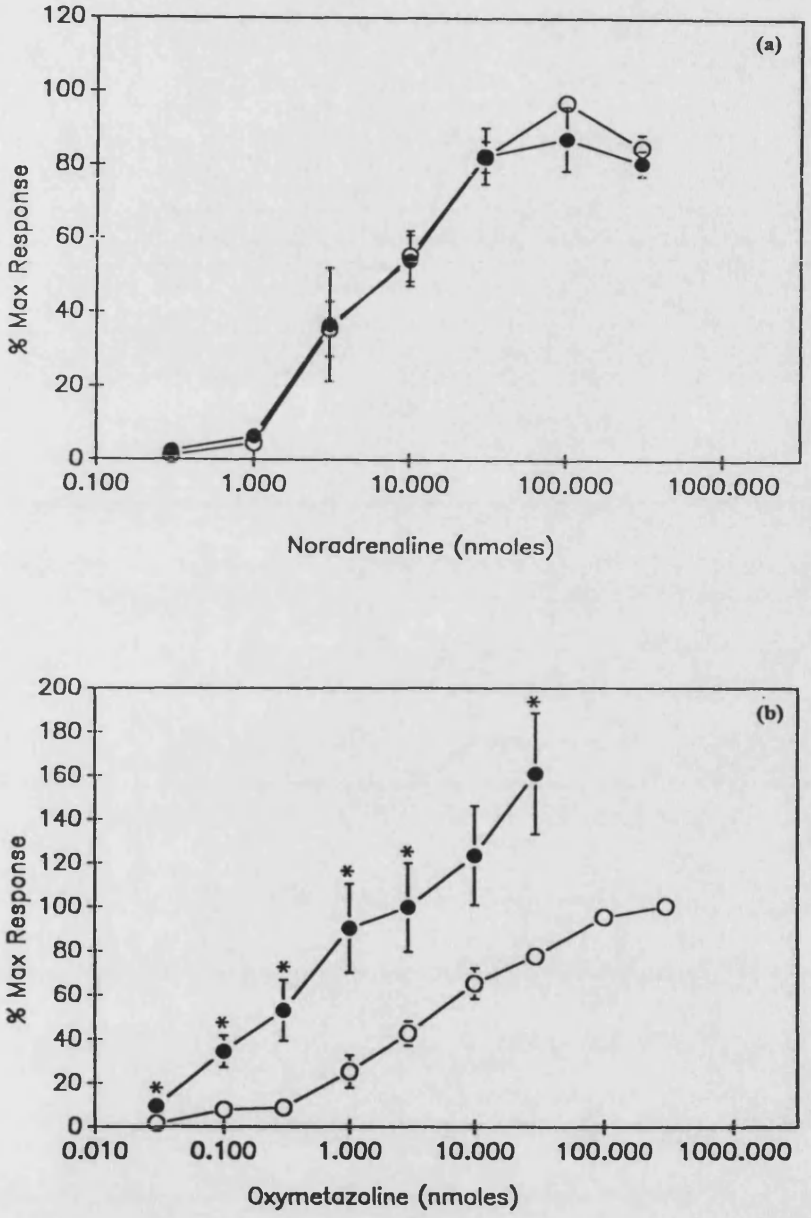


Fig. 67 Effect of L-NOARG 10^{-5}M (●) on (a) NA- and (b) oxymetazoline-induced vasoconstriction (b) in the perfused ovarian vascular bed of pregnant rabbits (mean±se, n=4). (* $p < 0.05$)

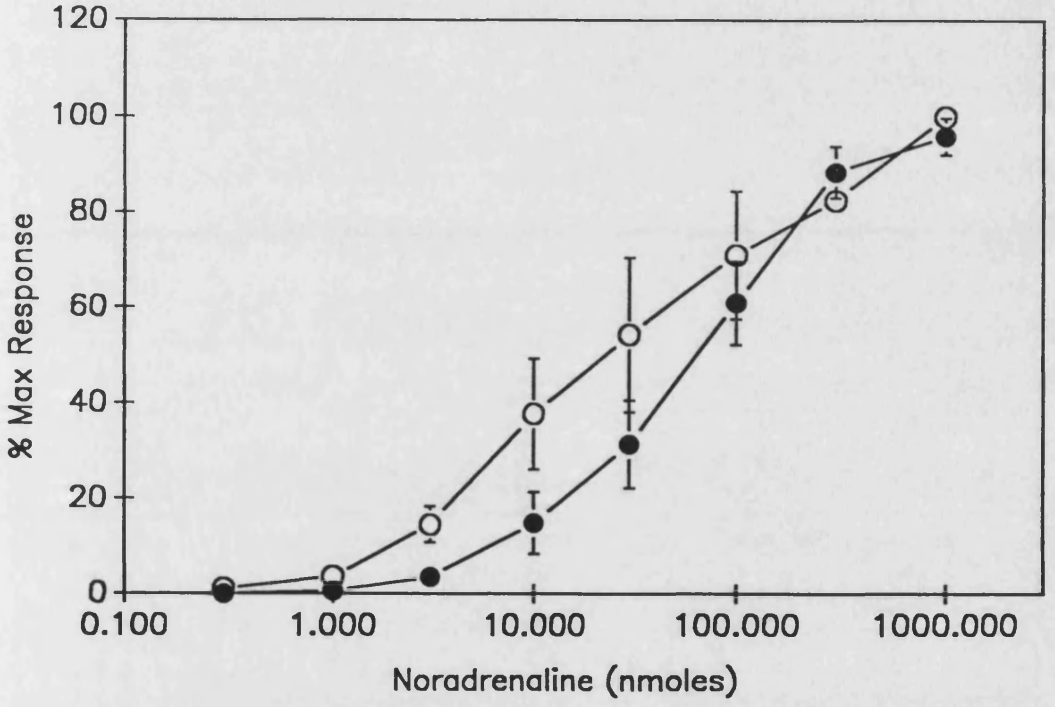


Fig. 68 Effect of yohimbine, 10^{-5}M (●) on NA-induced vasoconstriction (O) in the perfused ovarian vascular bed of pregnant rabbits (mean±se, n=4).

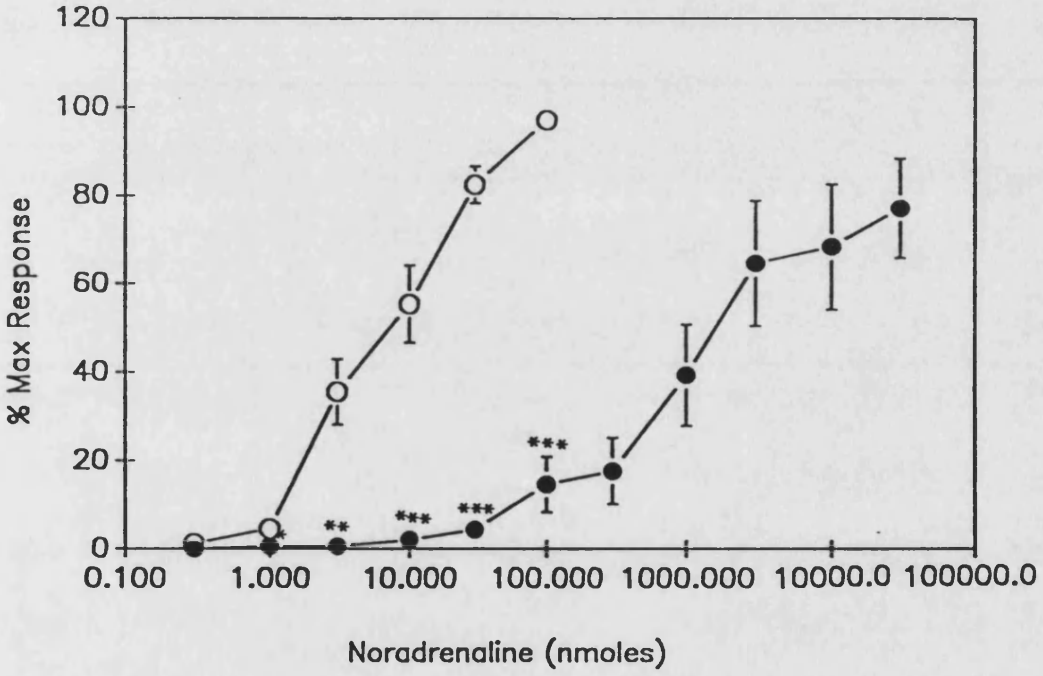
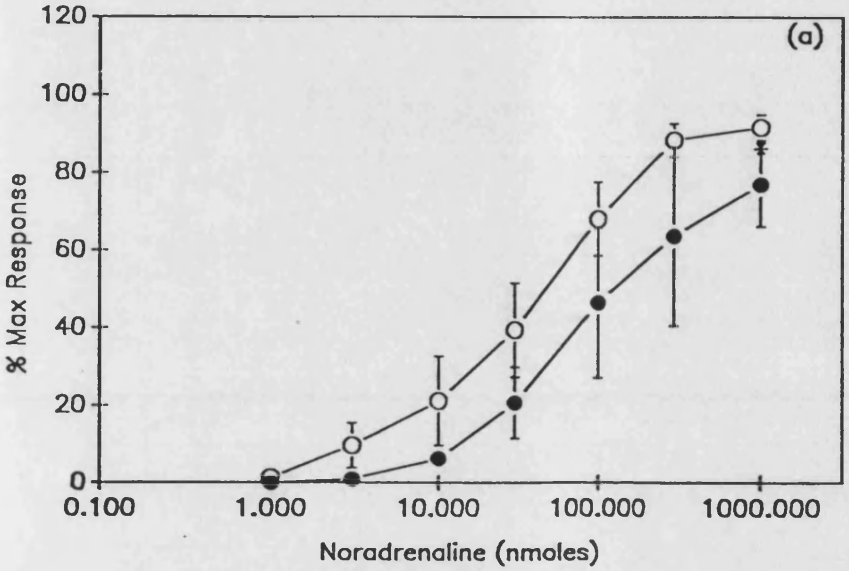


Fig. 69 Effect of prazosin, 10^{-7}M (●) on NA-induced vasoconstriction (O) in the perfused ovarian vascular bed of pregnant rabbits (mean±se, n=5).

(* $p < 0.05$, ** $p < 0.01$ & *** $p < 0.001$)



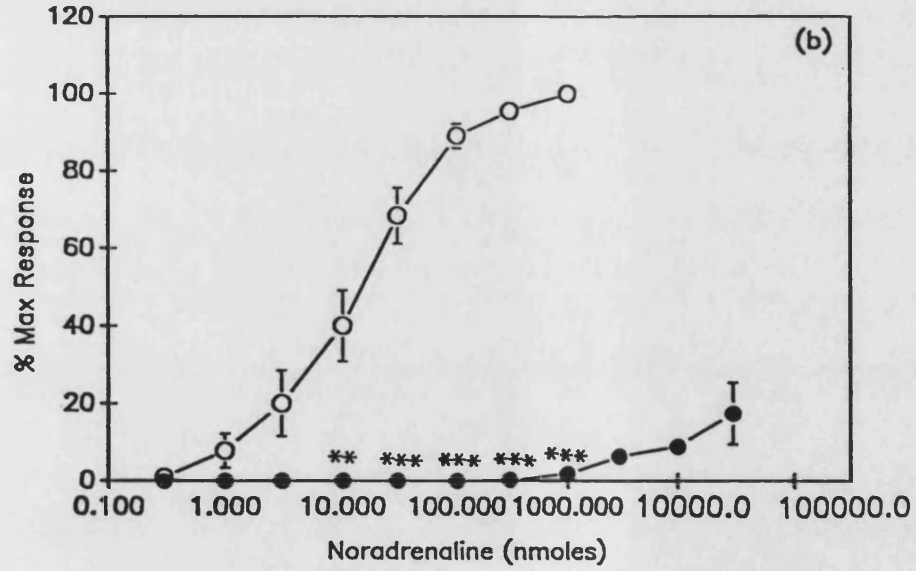


Fig. 70 Effect of irreversible antagonists (●) on NA-induced vasoconstriction (O) in the perfused ovarian vascular bed of pregnant rabbits, (a) CEC $3x10^{-5}$M & (b) SZL-49 $5x10^{-6}$M (mean±se, n=4). (** $p < 0.01$ & *** $p < 0.001$)

Relaxant responses for carbachol and SNP were established before and after incubation with L-NOARG. Carbachol (0.1-1000 nmoles) dose-dependently reduced the perfusion pressure. The ED_{50} value of was 7.2±3.9 nmoles and the maximum relaxation was 61.0±11.7% of basal tone. Carbachol-induced relaxation was suppressed significantly after incubation with L-NOARG 10^{-5}M ($p<0.05$), while SNP-induced responses were not affected (Figs. 71 and 72). There was no significant difference in ED_{50} values or maximal relaxation produced by carbachol or SNP in the control and pregnant rabbits ($p > 0.05$).

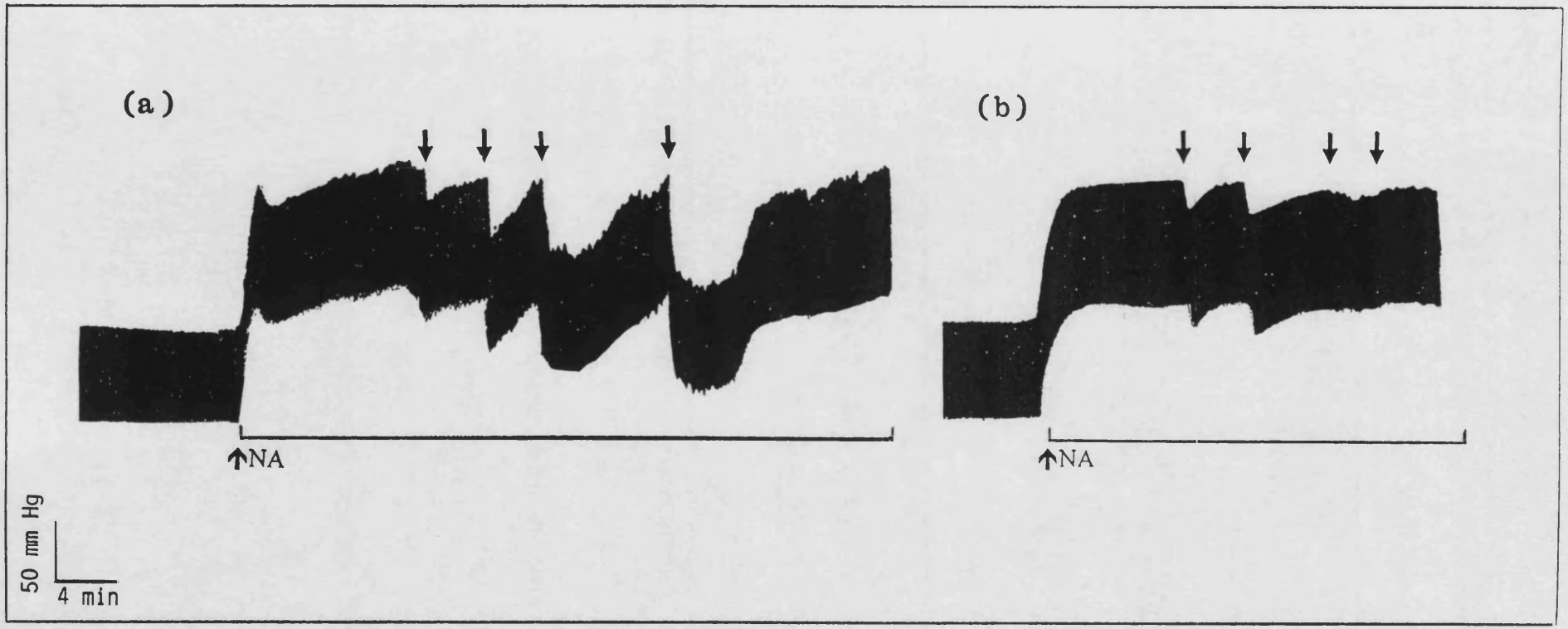
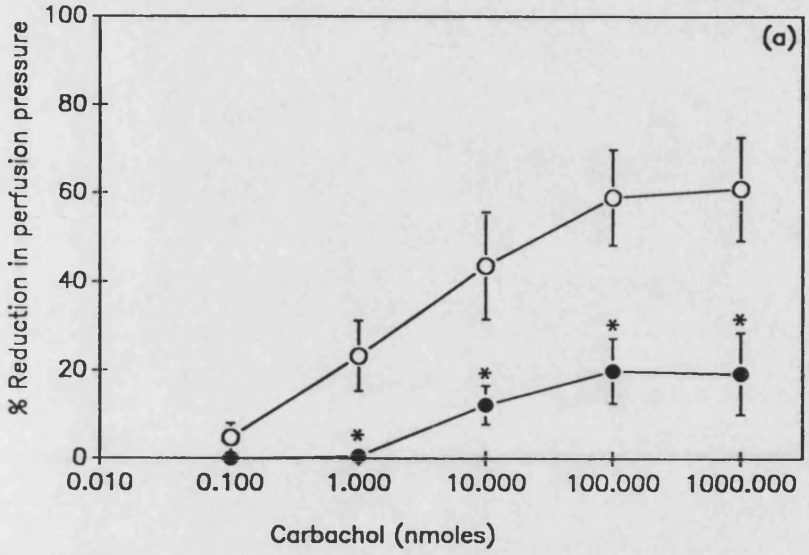


Fig. 71 A typical trace of carbachol-induced vasodilatation in pregnant rabbits, (a) before and (b) after L-NOARG (10^{-5} M). Perfusion pressure was raised with NA (10^{-5} M). [↓ represents 1, 10, 100 & 1000 nmoles carbachol respectively]



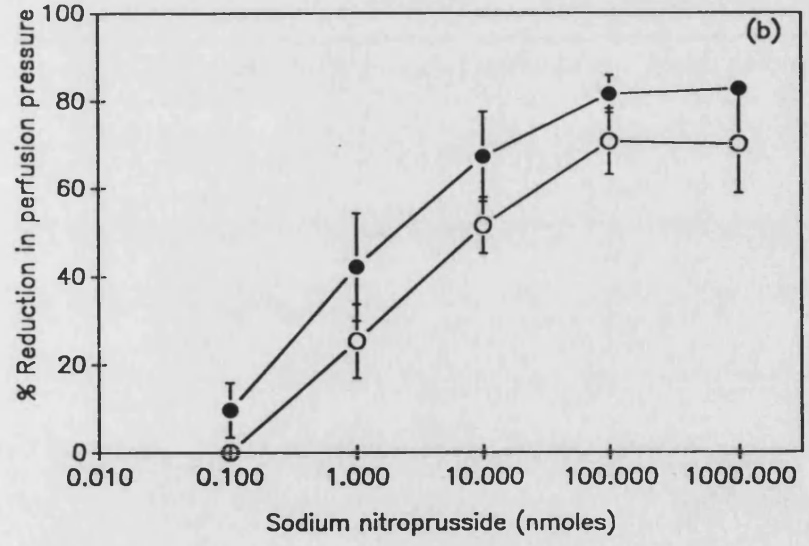


Fig. 72 Effect of L-NOARG 10^{-5}M (●) on vasodilator responses to (a) carbachol and (b) sodium nitroprusside in the perfused ovarian vascular bed of pregnant rabbits (mean±se, n=5). (* $p < 0.05$)

CHAPTER FOUR

DISCUSSION

4.1 Introduction

The mammalian ovary is well supplied with autonomic nerves of both sympathetic and parasympathetic origin (Owman et al., 1979). The nerve terminals innervate blood vessels and also form networks around follicles in various stages of development. In the follicle walls, they establish close contacts with smooth muscle cells in the theca externa layer (Burden, 1978; Owman et al., 1979). Thus the adrenergic nerves may play a role in regulating blood flow to the ovary, ovulation and also serve a neuroendocrine function. Previous studies have shown that noradrenaline evoked dose-dependent vasoconstrictor responses in the rabbit (Graham & Sani, 1971), human (Varga et al., 1979) and ewe (Kuhl et al., 1974) ovarian artery, but the receptors mediating such responses were not analysed. Oriowo and Bevan (1986), showed that the α_1-adrenoceptors mediated noradrenaline-induced contractions of ring segments of the rabbit ovarian artery. However, there is now evidence in the literature, indicating that, events taking place in muscular arteries may not always reflect what happens in the resistance vessels. For example, Cauvin et al., (1984) showed that the effectiveness of Ca^{2+} channel antagonists, in inhibiting NA-induced contractions was inversely related to the vessel diameter. Thus mesenteric resistance vessels are more sensitive to nifedipine than the muscular superior mesenteric artery. The difference (i.e. between resistance arteries and muscular arteries) is not limited to signal transduction mechanism. It has been shown that both α_1- and α_2-adrenoceptors are involved in NA-induced contractions of small diameter rabbit intrarenal arteries (Owen & Patel, 1994; 1996). In contrast, only α_1-adrenoceptors mediated NA-induced contraction of the main renal artery (Oriowo et al., 1989). Similarly, while rat renal artery responses to electrical stimulation and exogenous α-adrenergic agonists are exclusively mediated via α_1-adrenoceptors (Schmitz et al., 1981; Wolff et al., 1987); Bohmann et al, (1995) have uncovered α_2-adrenoceptors, mediating vasoconstriction in spontaneously hypertensive rat kidneys in the presence of low concentrations of angiotensin II. Thus under certain conditions, α_2-adrenoceptors activity could be unmasked. Recent studies have also shown that noradrenaline-induced vasoconstriction in the perfused mesenteric vascular bed was resistant to inhibition by CEC indicating mediation via α_{1A}-adrenoceptor subtype. This is in

contrast to NA-induced contractions of ring segments of the superior mesenteric artery which were CEC sensitive indicating α_{1B} (α_{1D}?) receptors were involved.

Therefore, the main objective of this study was to characterize α-adrenergic receptors mediating vasoconstriction in the ovarian vascular bed and how these may be influenced by the endothelium and changes in hormonal status following ovulation and pregnancy. In addition, experiments were also carried out to identify the signal transduction mechanisms involved in the response to adrenergic receptor activation.

The perfused whole vascular bed preparation is a set-up designed to mimic the in situ situation. The perfusion pressure can be controlled and set to the level that can be obtained in vivo. Also, the preparation can be perfused at a constant flow rate or pressure. Both of these techniques are relevant to in vivo situations. In the present study, perfusion at a constant flow rate was the method of choice mainly because techniques for studying ovulation, in vitro, using the perfused rabbit ovary utilized a constant flow rate approach (Uchida et al., 1967; Lambertsen et al., 1976; Wallach et al., 1978; Kobayashi et al., 1981). One disadvantage of this system (i.e. perfused whole bed) is that, with time, the perfused vascular bed becomes oedematous which could affect the results. For example, in the rat isolated perfused lung preparation, there was a time dependent gain in lung weight (oedema) increasing from 6% after 2hr to 96% after 4hr of perfusion (Lal et al., 1994). Thus the first set of experiments were designed to study the time-dependence of the response of the ovarian vascular bed to NA. This was essential since in antagonist studies, each preparation would serve as its own control. The results showed that at least 4 consecutive dose-response curves (at 45-60 min interval) could be obtained in each preparation, without a difference in potency or maximum response. There was a significant increase in weight of the preparation at the end of the experiment which could be attributed to the fluid trapped in the fatty tissue surrounding the ovarian artery close to its point of entry into the ovary. It was observed that the more the fat, the more was the gain in weight. This oedema, however, had no effect on the basal perfusion pressure which remained constant throughout the experiment and therefore, it was a reliable preparation.

Noradrenaline is the main neurotransmitter in sympathetic nerves and therefore it was chosen to be the reference agonist with which other agonists were compared. The dominant mechanism terminating the action of NA in sympathatically innervated

tissues is the uptake process (Iversen & Salt, 1970). This process is inhibited by cocaine and tricyclic antidepressants (Hughes, 1972). Later studies have shown that influence of the uptake system on NA-induced vasoconstriction is directly proportional to the density of innervation (Bevan et al., 1974). Therefore, in the present study, failure of uptake blockers (cocaine and DOCA), singly or in combination to significantly enhance NA-induced vasoconstriction could be due to the low level of adrenergic innervation of the ovarian vasculature (Stefenson et al., 1981). The uptake process is also affected by the width of the synaptic cleft. There is evidence, showing that the concentration of neurotransmitter in the cleft of medially innervated tissues (small width of synaptic cleft) is several fold higher than was found in the cleft of junctionally innervated tissues (Bevan, 1977) and this was attributed to the fact that diffusion away from the cleft is much higher in junctionally innervated tissues. Even though there is no indication of the width of the synaptic cleft in the ovarian vasculature, the possibility that diffusion away from the cleft rather than uptake into the nerves could be responsible for terminating the action of NA cannot be excluded.

The reactivity of an isolated vascular smooth muscle preparation to NA is also influenced by a simultaneous activation of ß-adrenoceptors and a release of prostanoids/EDRF from the endothelium. NA activates ß-adrenoceptors, being more selective for $ß_1$-adrenoceptor subtype (Lands et al., 1967). Although it was earlier assumed that $ß_2$-adrenoceptors mediate vasodilatation, it is now known that $ß_1$-adrenoceptor activation can also result in vasodilatation. This has been shown in several arterial preparations (Edvinsson & Owman, 1974; Edvinsson et al., 1976; Nakane et al., 1988; Yamada et al., 1988; Purdy et al., 1988). Thus a simultaneous activation of ß-adrenoceptors could limit the response of the arterial smooth muscle to NA by a process of physiological antagonism. However, results obtained in this study showed that NA-induced vasoconstriction in the ovarian vascular bed was not enhanced by blocking ß-adrenoceptors with propranolol, indicating that NA-induced vasoconstriction in this preparation was not limited by a simultaneous activation of ß-adrenoceptors.

In many vascular smooth muscle preparations, the vasoconstrictor response to NA is accompanied by a release of prostanoids including $PGF_{2\alpha}$, PGE_2, TXB_2 and PGI_2 from the endothelium and smooth muscle (McGiff et al., 1976; Boeynaems et al., 1987). $PGF_{2\alpha}$ and TXB_2 are vasoconstrictor agents while PGE_2 and PGI_2 are vasodilators. Thus the overall modulatory effect of the prostanoids would depend on the relative proportions of dilator and constrictor prostanoids. In the perfused rabbit ovarian vascular bed (this study), indomethacin ($5x10^{-6}M$) did not affect NA-induced vasoconstrictor responses indicating that NA-induced vasoconstriction was not modulated by the prostanoids. This could be due to one or both of two possibilities, either that the prostanoids were not generated in enough concentrations to produce a response or that the effects of vasodilator and vasoconstrictor prostanoids on NA-induced vasoconstriction neutralize each other. There is no evidence to support either of these alternatives. In subsequent experiments therefore, none of these inhibitors (propranolol, indomethacin, DOCA and cocaine) were routinely used.

Transmural electrical stimulation at supramaximal voltage induced a frequency-dependent vasoconstriction of the ovarian vascular bed. The response was abolished by 6-OHDA indicating that it was adrenergic in nature. However, the vasoconstrictor response to electrical stimulation was weak when compared to the maximum response to exogenous NA. This is consistent with a sparse adrenergic innervation of the rabbit ovarian arteries and low content of NA in the ovarian nerves (Stefenson et al., 1981).

4.2 Influence of the vascular endothelium

In several vascular beds, perfusion with CHAPS is an effective way of removing the endothelium. Successful de-endothelialization was evidenced by an increased sensitivity to contractile agonists and loss of endothelium-dependent vasodilation (Tesfamariam & Halpren, 1987; Moore et al., 1990). This procedure was therefore employed to remove the endothelium in the ovarian vascular bed in an attempt to assess the influence of the endothelium on vasoconstrictor effects of NA. The concentration of CHAPS (4.7 mg/ml) used in this study has been shown to effectively remove the endothelium in several vascular beds, including mesenteric vascular bed (Tesfamariam & Halpern, 1987) based on the failure of acetylcholine to dilate the vessel after CHAPS, and electron microscopic examination. In the present study,

there was a small increase in perfusion pressure during the infusion of CHAPS which would probably suggest denudation of the endothelium. Loss of endothelial function was confirmed by the failure of carbachol to evoke a dilatation following CHAPS. However, vasoconstrictor responses to low doses of NA were not enhanced by CHAPS treatment whereas responses to higher doses of NA were reduced significantly. The reduction in vasoconstrictor responses could not have been due to a non-specific destruction of the vascular smooth muscle since the vasoconstrictor effects of KCl were not affected by CHAPS treatment. It is therefore possible that the reduction in NA-induced vasoconstriction following endothelium removal by CHAPS could suggest an involvement of an endothelium-derived contracting factor in the vasoconstricting effect of NA. On the other hand, NA-induced vasoconstriction was potentiated by L- NOARG, an NO-synthase inhibitor (Moore et al., 1990) indicating that NA-induced vasoconstriction was accompanied by a release of endothelium-derived relaxing factor. L-NOARG, has been shown to produce a reversible inhibition of the vasoconstrictor effect of Ach in rabbit aortic rings and the rat perfused mesentery (Moore et al., 1989; 1990). An explanation was that NA had two sites of activity on the normal vessel wall; to contract the smooth muscle and to release EDRF. EDRF would then behave as a functional antagonist of the contractile signal following α-adrenoceptor activation. L-NOARG also has some affinity for the inducible NOS (iNOS) (McCall et al., 1991). Therefore, there is the possibility that L-NOARG could have potentiated NA-induced vasoconstriction by inhibiting iNOS activity. If this were to be true, aminoguanidine, a more selective inhibitor of iNOS (Misko et al., 1993), (with no activity on cNOS), should also enhance NA-induced vasoconstriction. However, no enhancement of NA-induced responses was observed following perfusion of the vascular bed with aminoguanidine. Thus inhibition of iNOS is not involved in L-NOARG enhancement of NA-induced vasoconstriction in this vascular bed. This is supported by the following observations: (1) four consecutive NA dose-response curves (at 1hr intervals) could be obtained without any reduction in potency or maximum response (the absolute response tended to increase), and (2) L-NOARG had no effect in endothelium-denuded preparations. So it is quite possible that both endothelium derived relaxing and contracting factors (EDRF & EDCF) modulate the response to NA (in particular at the high doses) in the perfused

ovarian vascular bed with a predominance of the EDCF contribution. Thus removal of the endothelium would remove the modulating effect of both EDCF and EDRF, and hence the response to NA would be reduced. On the other hand, treatment with NO-synthase inhibitor would remove only the inhibitory effect of EDRF leading to an enhanced response to NA. In the perfused rabbit ovarian vascular bed (this study), carbachol induced a dose-dependent vasodilator effect of the ovarian vascular bed pre-constricted by NA. This contradicts earlier reports by Graham & Sani (1971) who only observed vasoconstriction to acetylcholine even when perfusion pressure was raised with NA. The reason for this discrepancy is not known. However, in the present study, NA (10^{-5}M) was used to raise the perfusion pressure as opposed to a concentration of $2x10^{-6}$M used by Graham & Sani (1971). It is possible that carbachol-induced vasodilation is dependent on the level of tone existing in the preparation. Carbachol-induced vasodilatation was abolished by inhibitors of cyclicGMP production, LY 83583 (Mulsch et al., 1988; Fleming et al., 1991) and methylene blue (Mayer et al., 1993), confirming that it is mediated via generation of cyclicGMP. The results so far would confirm that carbachol-induced an endothelium-dependent vasodilation which involves cyclicGMP generation. The same concentration of L-NOARG which enhanced NA-induced pressor responses reduced but did not abolish the vasodilator responses to carbachol indicating that carbachol-induced vasodilation is only partly dependent on NO generation. Frew et al. (1993) also reported an inhibition of ACh-induced relaxations by L-NOARG in rat aorta. Considerable evidence shows that, in addition to endothelium-derived nitric oxide, Ach and a variety of other agents release a factor that causes vascular smooth muscle relaxation through the release of an hyperpolarizing factor, EDHF (Chen et al., 1988; Komori & Vanhoutte, 1990). It has been demonstrated that ACh and other muscarinic agonists stimulate endothelium-dependent smooth muscle hyperpolarization (Bolton et al., 1984; Garland & McPherson, 1992; Chen et al., 1988). ACh-induced hyperpolarization was not blocked by EDRF inhibitors such as methylene blue and oxyhaemoglobin (Chen et al., 1988; Taylor & Weston, 1988). Even though both carbachol and SNP act via NO accumulation, only carbachol stimulates NO production through cNOS activity. Thus it is to be expected that L-NOARG would not affect vasodilatation-induced by SNP. This was observed in the

ovarian vascular bed as vasodilatation induced by SNP was not affected by L-NOARG. Interestingly, LY 83583 and methylene blue, also had no effect on SNP-induced vasodilatation, suggesting that cyclicGMP generation may not be involved in this action of SNP. Earlier studies have shown that SNP is a receptor-operated Ca^{2+} channel antagonist (Karaki et al., 1985; 1988b). This could account for SNP-induced vasodilatation observed in this study. Similarly, isoprenaline induces vasodilatation mainly by activating adenyl cyclase leading to accumulation of cyclicAMP (Furchgott & Vanhoutte, 1989). Recent reports, however; tend to indicate that isoprenaline can also stimulate guanylate cyclase leading to accumulation of cyclicGMP. It has been shown, at least in ring segments of the rat aorta that isoprenaline-induced relaxation is accompanied by elevated levels of cyclicGMP (Gray & Marshall, 1992). The same authors also showed that L-NOARG significantly reduced isoprenaline-induced relaxation confirming that NO release is involved. In the ovarian vascular bed, however; isoprenaline-induced vasodilatation was not affected by L-NOARG and not significantly affected by LY 83583. This would indicate that isoprenaline induced vasodilatation of the rabbit ovarian vascular bed independently of NO and cyclicGMP. It is therefore concluded that there is little or no basal release of NO in the perfused ovarian vascular bed, since L-NOARG alone did not raise the basal perfusion pressure. However, NA-induced contractions are accompanied by NO release which limited the contractions since inhibition of cNOS led to increased response to NA. Carbachol induced vasorelaxant responses, though dependent on cyclicGMP accumulation, were significantly reduced but not abolished by L-NOARG, indicating that it was not totally mediated via NO. It is therefore, concluded that the relaxant effects may be mediated via both endothelium-derived NO and EDHF.

4.3 Adrenergic receptors in the ovarian circulation

4.3.1 α- or ß-Adrenoceptors

NA responded with a vasoconstriction in the rabbit ovarian vascular bed. The profound inhibitory effect of phentolamine on NA-induced vasoconstriction, clearly demonstrated the involvement of α-adrenoceptors. The failure of propranolol to modulate NA-induced vasoconstriction could be due to either of two possibilities; (1) that there are no ß-adrenoceptors in the rabbit ovarian circulation or (2) that ß-

adrenoceptors, though present are not functional. Experiments were therefore performed to test for the presence or otherwise of ß-adrenoceptors in the ovarian vascular bed. When the perfusion pressure was raised with NA (or methoxamine), isoprenaline (at high doses) induced dose-dependent reductions in perfusion pressure probably suggesting that there are ß-adrenoceptors in the ovarian vascular bed. Varga et al, (1979) have also observed ß-adrenoceptor mediated vasodilation in the human ovarian bed. However, isoprenaline-induced vasodilation in the ovarian vascular bed was not significantly affected by propranolol (10^{-6}M and 10^{-5}M), indicating that atypical rather than conventional $ß_1$- and $ß_2$-adrenoceptors probably mediated this response. It is also possible that isoprenaline-mediated vasorelaxation could be due to α-adrenoceptor blockade. In order to test this possibility, perfusion pressure was raised with U46619 (a thromboxane mimetic agent) or histamine instead of NA. Under this condition, isoprenaline produced pressor responses at all doses. The pressor responses were blocked by phentolamine, indicating involvement of α- rather than ß-adrenoceptors, thus confirming the absence of ß-adrenoceptors in the rabbit ovarian vascular bed.

4.3.2. α_1- or α_2-Adrenoceptors

It is now widely accepted that the post-junctional effects of α-adrenergic agonists are mediated by α_1- and α_2-adrenoceptors, both of which are equipotently antagonized by phentolamine (Nichols & Ruffolo, 1991). As described above, NA-induced vasoconstriction was potently antagonized by phentolamine. Therefore, in order to identify the α-adrenergic receptors present in the ovarian vascular bed, the interactions between selective antagonists prazosin (α_1) and yohimbine (α_2) with adrenergic agonists were investigated. NA, methoxamine, phenylephrine, SDZ NVI 085 and oxymetazoline produced dose-dependent vasoconstriction of the perfused ovarian vascular bed, while clonidine, tizanidine, B-HT 920 and UK 14,304 were not effective. This would probably suggest that α_1-adrenoceptors mediated vasoconstriction. However, the possibility of a minor contribution of α_2-adrenoceptors can not be ruled out. Thus a simultaneous activation of α_2-adrenoceptors could have contributed to the effect of NA. Although α_2-adrenoceptor-mediated vasoconstriction is readily demonstrable in vivo and in vitro using venous smooth muscle (Matthews et al., 1984;), it is generally difficult to demonstrate α_2-

adrenoceptor mediated responses in arterial smooth muscles in vitro (Skarby et al., 1983; Sulpizio & Hieble, 1987). However, a definite and concentration dependent response is usually unmasked in the presence of an elevated tone using constrictor agents like vasopressin, angiotensin and Bay K 8644 (Templeton et al., 1989; Bohmann et al., 1995). This protocol was also used in this study in an attempt to unmask any α_2-adrenoceptor mediated vasoconstriction. Clonidine, tizanidine, B-HT 920 and UK 14,304 did not produce any significant vasoconstrictor effect in preparations in which perfusion pressure was raised with methoxamine, NA or U46619. It is therefore unlikely that there are postjunctional α_2-adrenoceptors mediating vasoconstriction in the ovarian vascular bed. Blue and Clarke (1990) also could not demonstrate α_2-adrenoceptors mediated responses in the perfused rat kidney even when perfusion pressure was elevated with angiotensin II. Further support for α_1-adrenoceptor mediated responses in the ovarian vascular bed was obtained from antagonist studies. Prazosin, concentration-dependently antagonized NA-induced vasoconstrictions. There was a parallel rightward shift of the NA dose-response curve without a reduction in the maximum response. The pA_2 value was 7.35 and the slope 1.0 ± 0.1, the latter was not significantly different from 1, indicating competitive antagonism. This observed pA_2 value was very low compared to values reported in the literature (Agrawal et al., 1984). The reason for this low potency is at present not known. However, a similar low pA_2 value (<8.0) was obtained in ring segments of the ovarian artery (Oriowo & Bevan, 1986), probably indicating that α_1-adrenoceptors in the ovarian circulation have a low affinity for prazosin. Oxymetazoline, phenylephrine, methoxamine and SDZ NVI 085 were all antagonized by prazosin. Except for oxymetazoline, the pA_2 / pK_B values recorded for these agonists were found to be similar to the value for NA. The pA_2 value for prazosin against oxymetazoline was lower than its potency against the other agonists. The reason for this is not known. However, a similar observation has been made in the rabbit prostate gland (Sulpizio A., personal communication). Yohimbine, a selective α_2-adrenoceptor antagonist, antagonized NA-induced vasoconstriction, but only at a high concentration. The k_B value (obtained using 10^{-5}M yohimbine) was 7.1×10^{-6}M. The prazosin/yohimbine ratio was approximately 100, which is still consistent with activation of α_1-adrenoceptors in spite of the low potency of prazosin in this

preparation. Phenylephrine was also not antagonized by yohimbine, therefore, α_1- but not α_2-adrenoceptors mediated vasoconstriction in the perfused rabbit ovarian vascular bed.

The results also showed that α_1-adrenoceptors-mediated vasoconstrictor responses to electrical stimulation. Transmural electrical stimulation at supramaximal voltage induced a frequency-dependent vasoconstriction of the ovarian vascular bed. The vasoconstrictor responses were abolished by 6-OHDA indicating that they were adrenergic in origin. Prazosin produced a significant concentration-dependent reduction of the electrically evoked response suggesting that α_1-adrenoceptors were involved. Yohimbine at 10^{-5}M, reduced the responses to electrical stimulation by 15%. However, this concentration of yohimbine is similar to the concentration at which yohimbine blocks α_1-adrenoceptors.

4.3.3 Subtype(s) of α_1-adrenoceptors

Within the past ten years, there have been a number of reports showing heterogeneity of α_1-adrenoceptors in different tissues, vascular and non-vascular (McGrath, 1982; Morrow & Creese, 1986; Flavahan & Vanhoutte, 1986). Based on the differential affinities of vascular smooth muscle preparation to prazosin and yohimbine, Flavahan and Vanhoutte (1986) subdivided α_1-adrenoceptors into α_{1H}- and α_{1L}-subtypes. This sub-classification was later expanded by Muramatsu and coworkers (Muramatsu et al., 1991) to include a third subtype, α_{1N} which also has a low affinity for prazosin. This existence of this subtype was based on the varying affinity of the tissues for HV 723; which is selective for α_{1N}-subtype. α_1-Adrenoceptors were also classified into α_{1A}-and α_{1B}-subtypes based on their differential sensitivities to a number of antagonists (Han et al., 1987; Minneman 1988). α_{1A}-adrenoceptors are activated by phenylephrine, methoxamine and SDZ NVI 085 (Eltze & Boer, 1992) and are potently antagonized by WB-4101 (K_B= 9.0), 5-methylurapidil and (+)-niguldipine (Han et al., 1987). They are not inactivated by the clonidine analogue CEC, but are inactivated by SZL-49 (Piascik et al., 1990). α_{1B}-adrenoceptors, on the other hand, are less sensitive to WB-4101 (K_B < 8) but are inactivated by CEC (Han et al., 1987). Later studies have identified two more subtypes of the α_1-adrenoceptors. These are α_{1C}-(Schwinn et al., 1991) and α_{1D}-subtypes (Perez et al.,1991). So far, the functional significance of these α_1-adrenoceptor subtypes are not known. Both α_{1C}- and α_{1D}-subtypes are potently

antagonized by WB-4101 (=α_{1A}) (Lomasney et al., 1991) and are also inactivated by CEC (α_{1C}- not significantly). In the perfused rabbit ovarian vascular bed, NA-induced vasoconstriction was competitively antagonized by WB-4101. But the pA_2 value (7.93) was much lower than what would be expected at α_{1A}-, α_{1C}- or α_{1D}-adrenoceptor subtypes indicating that α_{1B} receptors might mediate NA-induced vasoconstriction in the ovarian vascular bed. However, NA-induced vasoconstriction was not affected by CEC but was remarkably inhibited by SZL-49. This would suggest that α_{1B}- and α_{1D}-adrenoceptor subtypes are possibly not involved in NA-induced responses in the ovarian vascular bed. The reason for the low pA_2 value for WB-4101 is unknown. Perhaps the same reason might explain the low prazosin pA_2 value too. The α_1-adrenoceptors were found to be activated by phenylephrine, oxymetazoline, methoxamine and SDZ NVI 085 and the agonists-induced vasoconstriction were competitively antagonized by WB-4101. The α_{1B}-selective antagonist CEC, did not have any effect on NA- and oxymetazoline-dose response curves. The agonists-induced vasoconstriction was inhibited by the α_{1A}-selective antagonist SZL-49. There was a highly significant rightward shift of the agonists-dose response curve, indicating involvement of α_{1A}-adrenoceptor subtype. Since both α_{1C}- and α_{1D}-subtypes are potently antagonized by WB 4101 (Lomasney et al., 1991) and are also inactivated by CEC, this would suggest that α_{1B}-, α_{1C}- and α_{1D}-adrenoceptor subtypes are not involved in agonist-induced responses in the ovarian vascular bed. The fact that SZL-49 significantly reduced agonist-induced vasoconstrictor responses would strengthen the suggestion that α_{1A}-adrenoceptor subtype mediated agonist-induced vasoconstrictor response in the ovarian vascular bed. (+)-Niguldipine is highly selective antagonist of the α_{1A}-adrenoceptor subtype (Gross et al., 1988). Therefore, the observation in this study that (+)-niguldipine at a low concentration (10^{-9}M) abolished the pressor response to NA would further confirm that α_{1A}-subtype mediated agonist responses in the ovarian vascular bed.

WB-4101, concentration-dependently reduced the electrically-induced response. This together with the fact that SZL-49 but not CEC reduced the response, would confirm that α_{1A}-adrenoceptors mediated the vasoconstrictor response to electrical stimulation.

4.4 Signal transduction mechanism

The relative role of extracellular calcium influx and intracellular calcium release in the coupling of α_1-adrenoceptor activation to response have been the subject of much controversy for many years. Pressor responses in vivo mediated by postjunctional vascular α_1-adrenoceptors were found to be resistant to inhibition by calcium channel antagonists (Cavero et al., 1983 & van Meel et al., 1981), suggesting that, α_1-adrenoceptors may not rely heavily upon extracellular calcium to produce vasoconstriction. However, partial α_1-adrenoceptor agonists evoked responses that are affected by Ca^{2+} channel antagonists indicating that α_1-adrenoceptor activation could also be linked to transmembrane influx of Ca^{2+}. This has been supported by studies measuring Ca^{2+}fluxes in vascular smooth muscles which showed that α_1-adrenergic agonists promote Ca^{2+} influx and release of intracellular Ca^{2+}(Deth & Lynch, 1981; Cauvin et al., 1982; Chiu et al., 1986). Experiments were therefore performed to determine the source(s) of activator Ca^{2+}utilized by adrenergic agonists in ovarian vascular bed.

The results showed that there was a significant reduction in response to NA when perfused with a nominally Ca^{2+}-free KH-solution and also that the residual responses were abolished in Ca^{2+}-free KH-solution containing EGTA, suggesting the involvement of both influx of extracellular Ca^{2+} and release of (probably membrane bound) intracellular Ca^{2+} in the vasoconstrictor response to NA. The results also showed that vasoconstrictor response to a fixed dose (300 nmoles) of NA was lost within 60 min in a Ca^{2+} -free medium. The half life was 4 min, which was reduced to 2.5 min in the presence of EGTA (2mM). This could probably suggest that Ca^{2+} stored in a labile pool (and probably of limited capacity) contributed to the response to NA. On returning to a normal KH-solution, more than 40% of the response to NA was restored within the first 5 min while 100% responsiveness was attained within 15 min indicating that the store is easily filled with Ca^{2+}. The calcium channel blockers verapamil and nifedipine produced a concentration dependent reduction in the vasoconstrictor response to NA. However, even at a concentration of 10^{-5}M, none of these agents abolished NA-induced vasoconstriction. This would be consistent with the assumption that both extra- and intracellular sources of Ca^{2+} are involved in NA-induced responses. In support of this, is the observation that KCl-induced

vasoconstrictor responses were abolished by verapamil and nifedipine at a concentration of 10^{-6}M, confirming that KCl-induced vasoconstriction was solely dependent on an influx of extracellular Ca^{2+}. Bolton (1979) originally identified two types of Ca^{2+} channels, voltage operated (VOC) and receptor-operated (ROC) channels differing in their sensitivities to Ca^{2+} channel antagonists. VOCs are more susceptible to Ca^{2+} channel antagonists (eg. verapamil) than are ROCs. Ca^{2+} mobilization by NA in the ovarian vascular bed is consequent upon its combining with α_1-adrenoceptors on the membrane and thus involves receptor operated channels. The fact that verapamil and nifedipine are more potent against KCl (which involves depolarization)-induced vasoconstriction would tend to support this hypothesis. In addition, SNP, a ROC inhibitor (Karaki et al., 1985) at 1000 nmoles, abolished NA-induced vasoconstrictor response, would confirm that NA evoked vasoconstrictor responses by activating ROCs. There are reports in the literature that partial agonists are more sensitive to inhibition by Ca^{2+} channel inhibitors than full agonists (Nichols & Ruffolo, 1988; Ruffolo et al., 1984; Oriowo et al., 1990, 1992), suggesting that agonist efficacy determines the sensitivity of a response to Ca^{2+} channel inhibitors. This is further supported by the observation that pretreatment with phenoxybenzamine to reduce the efficacy of full agonists, resulted in enhanced sensitivity of the full agonsit to the antagonist effect of Ca^{2+} channel inhibitors (Ruffolo, 1984; Nichols & Ruffolo, 1988). This was tested in this study by examining the effect of verapamil and nifedipine on SDZ NVI 085-induced vasoconstriction. This agent was a partial agonist in the ovarian vascular bed (this study) producing 74% of NA-maximum. The results showed that verapamil and nifedipine, inhibited SDZ NVI 085-induced vasoconstrictions. However, neither verapamil nor nifedipine was more effective against SDZ NVI 085 relative to NA, indicating that agonist efficacy does not affect sensitivity to Ca^{2+} channel inhibitors in this preparation.

Similar observations have been made in the rabbit thoracic aorta segments where clonidine-induced contractions (Emax = 54% of NA maximum) were not more sensitive to nifedipine than were NA-induced contractions (Oriowo, personal communications).

Functional assessment of the role of SR in contraction is difficult. It has often involved measurements of contraction in Ca^{2+}-free media, which eliminates Ca^{2+} entry

(Devine et al., 1972) but may also deplete Ca^{2+} stores and , in the presence of caffeine, unloads the SR Ca^{2+} store (Leijten & van Breemen, 1984). Ryanodine, a plant alkaloid, depletes the SR Ca^{2+} store by opening SR Ca^{2+} release channels (Hwang & van Breemen, 1987). Ryanodine does not affect plasma membrane Ca^{2+} channels (Lattanzio et al., 1987), nor does it affect the contractile apparatus or the sarcolemmal Ca^{2+} transport mechanisms (Sutko et al., 1985). Therefore, this alkaloid appears to be useful for assessing the relative role of the SR in vascular smooth muscle contraction (Shima & Blaustein, 1992) and was chosen in this study to assess the role of intracellular calcium in mediating the vasoconstrictor responses to NA. The results showed that ryanodine (up to 10^{-5}M) had little or no effect on the vasoconstrictor response to NA. This would therefore suggest that NA-induced vasoconstrictions in the ovarian vascular bed is not dependent on Ca^{2+} release from the SR.

α_1-Adrenoceptor activation is associated with the hydrolysis of membrane-bound inositol phosphate resulting in the generation of $Ins(1,4,5)P_3$ and DAG. $Ins(1,4,5)P_3$ releases Ca^{2+} from intracellular stores (Berridge, 1984; 1987) while DAG activates PKC (Nishizuka, 1986). Activation of PKC produces contractions via an influx of extracellular Ca^{2+}. Since it has been shown in the previous sections that α_1-adrenoceptor activation in the rabbit ovarian vascular bed promotes an influx of extracellular Ca^{2+} , an attempt was made to determine whether activation of PKC was involved in this phenomenon. This was done by examinig the effects of two inhibitors of PKC, HA-1077 (Asano et al., 1989) and polymyxin B (Miller et al., 1986; Khalil & van Breemen, 1988) on NA-induced vasoconstriction in the ovarian vascular bed. The results showed that HA-1077 produced a concentration-dependent inhibition of NA-induced responses associated with a suppression of the maximum response. Perhaps this would suggest an involvement of PKC activation in NA-induced vasoconstrictor response. However, this is not supported by the failure of polymyxin B even at a concentration of 10^{-4} M, to significantly reduce NA-induced vasoconstriction. It is quite possible that other factors could have been responsible for the inhibitory effect of HA-1077. For example, HA-1077 has been shown to inhibit Ca^{2+} influx as well as reduce sensitivity of the contractile elements to Ca^{2+} (Asano et al., 1987). This has been confirmed in the present study on the ovarian vascular bed, where Ca^{2+}-induced

contractions of high K^+-depolarized preparations were significantly attenuated by HA-1077 but not by polymyxin B.

Evidence have accumulated over the past ten years that tyrosine kinases can also participate in signal transduction mechanisms. They have been shown to be involved in cell proliferation and transformation (Bishop, 1987; Draetta et al., 1988), regulation of neurotransmitter receptors (O'Dell et al., 1991) and initiation of mitogenic responses by certain growth factors (Dvir et al., 1991; Ullrich & Schlessinger, 1990). Di Salvo et al (1988; 1989) have shown that smooth muscles contain unusually high levels of TK activity, raising the possibility that TK activity could be important for the growth response and possibly contractile response of smooth muscles. Later studies have shown that TK inhibitors, e.g. genistein (Akiyama et al., 1987; Casnellie, 1991) and tyrphostin (Levitzki & Gilon, 1991; Casnellie, 1991) inhibited agonist-induced contractions in several smooth muscles including carotid artery, guinea pig taenia coli and mesenteric microvessels, rat mesenteric resistance arteries and thoracic aorta (Toma et al., 1995; Di Salvo et al., 1993; Adeagbo & Triggle, 1993). The possible involvement of TK activation, in signal transduction pathway in the ovarian vascular bed, was examined by studying the effect of genistein on NA-induced vasoconstrictor responses. The results showed that genistein concentration-dependently inhibited NA-induced vasoconstriction. Genistein and other TK inhibitors have been shown to be specific in their action. They do not affect MLCK or cyclicAMP-dependent kinases, and also had no effect on direct activation of the contractile apparatus (Di Salvo et al., 1993). Therefore, inhibition of NA-induced vasoconstriction by genistein in the ovarian vascular bed would suggest an involvement of TK activation in the NA-induced vasoconstriction.

4.5 Hormonal influences on the ovarian vasculature

A greater predominance of cardiovascular diseases in young women would suggeset a role for sex hormones in the pathogenesis of these disorders. Women taking oral contraceptives are more prone to hypertension (Laragh et al., 1972; Fregly, 1972; Kaplan, 1978). This is believed to be due to the oestrogenic component of the pill (Wallace, 1971). In support of this, is the observation that chronic treatment of rats with oestrogens induces and maintains hypertension (Bhatt & Gulati, 1986). In vitro,

vascular smooth muscle reactivity has been shown to be enhanced by oestrogen pretreatment. Altura (1972) showed that terminal mesenteric arterioles from female rats were more potently contracted by catecholamines than were similar preparations from male rats. Farhat and Ramwell (1992) have observed that perfused lung preparations from female rats were significantly more responsive to U46619 than similar preparations from age-matched male rats. This have been confirmed by other investigators including Bento and DeMoreas, 1992; Austin and Chess-Williams, 1995; Vargas et al., 1995 and Ma etal., 1996. This would provide further evidence for the role of female sex hormones in modulating vascular smooth muscle reactivity.

The effect of oestrogen pretreatment, in vitro and in vivo, on reactivity of the ovarian vascular bed to α_1-adrenergic agonists was therefore examined. The results obtained in these studies showed that oestradiol-17ß, administered in vitro produced a significant concentration dependent reduction in NA-induced vasoconstrictor responses. This was characterized more by a depression of the maximum response, while there was only a slight rightward shift of the dose-response curves. This could probably be due to an inhibitory effect of oestradiol-17ß on Ca^{2+} influx. Oestradiol-17ß has been shown to inhibit Ca^{2+} influx in several preparations including rat aorta (Vargas et al., 1989), rabbit heart (Raddino et al., 1986) and uterine arterial smooth muscle cells (Stice et al., 1987). However, ovarian vascular bed, isolated from rabbits treated with oestradiol-17ß propionate, exhibited significantly enhanced pressor response to NA and oxymetazoline indicating that oestradiol might be acting at the genomic level. Unlike in the mesenteric bed (Vargas et al., 1995) or lung preparation (Farhat & Ramwell, 1992) where oestradiol produced a leftward shift of NA dose-response curve and an increase in maximum response, oestrogen-induced enhancement of NA response in the ovarian vascular bed was characterized only by a leftward shift of the dose-response curve without a significant change in the maximum response. In addition, the increased sensitivity appeared to be specific for α-adrenergic agonists. Thus while the potencies of NA and oxymetazoline were increased, the potency of histamine in inducing vasoconstriction was unchanged.

Two other situations associated with increased plasma concentrations of oestradiol, ovulation induction and pregnancy also enhanced vasoconstrictor responses to NA and oxymetazoline but not histamine.

The increased sensitivity of the ovarian vascular bed to α_1-adrenergic agonists under conditions of oestrogen dominance could be due to a number of factors including, (1) oestrogen inhibition of uptake mechanisms, (2) increase in agonist affinity and/or receptor density or (3) increased availability of Ca^{2+}.

It is well recognized that steroids inhibit uptake$_2$ process (Iversen & Salt, 1970) in adrenergically innervated tissues. It is however unlikely that inhibition of uptake$_2$ would explain oestradiol-induced potentiation of NA response, since in preliminary experiments, it was shown that an inhibition of uptake$_2$ (DOCA) did not modulate NA-induced vasoconstrictor responses.

The potency of α_1-adrenergic agonists in inducing vasoconstriction is determined by two factors, affinity for the receptors and receptor density. Bevan et al (1986) showed that there is a direct correlation between potency of NA and its affinity for α_1-adrenergic receptors in a number of rabbit arteries. In the cat however, receptor density rather than affinity seems to be the main determinant of agonist potency (Oriowo et al., 1989). Radioligand binding studies have shown that oestrogen-induced potentiation of NA vasosonstriction in rat mesenteric arteries is associated with an increase in agonist affinity but not receptor density (Colucci et al., 1982). Functional studies on the same tissue showed that NA dose-response curve was shifted to the left, however, the maximum response remained unchanged. In the perfused rabbit ovarian vascular bed, no radioligand binding studies have been done and so no definitive statement can be made about changes (or not) in agonist-affinity or receptor density. However, the characteristics of the change, leftward shift in NA dose-response curve without an increase in the maximum response, is characteristic of an enhanced sensitivity due to an increase in agonist-affinity. Laher and Bevan (1985) have shown that receptor number (density) determines the maximum response to an agonist. Thus it is most likely that the increased sensitivity of the ovarian vascular bed to α_1-adrenergic agonists following treatment with oestradiol-17ß-propionate, or ovulation induction and pregnancy could be due to oestradiol-induced increase in agonist affinity for the receptors.

Austin and Chess-Williams (1995) observed a significant increase in vascular α_2-but not α_1-adrenoceptor responsiveness following treatment of rats with 17ß-oestradiol. In order to test whether functional α_2-adrenoceptors were unmasked following

hormone manipulation, the effect of yohimbine and B-HT 920 were tested against NA-induced vasoconstriction in preparations obtained from pregnant rabbits only (since there was no qualitative or quantitative difference in the responses to NA in different groups of the rabbits). It was observed that B-HT 920, an α_2-adrenoceptor agonist, did not produce vasoconstrictor responses. This coupled with the weak antagonist potency of yohimbine against vasoconstriction induced by NA, would confirm that α_2-adrenoceptors do not contribute to NA-induced responses. Further studies also showed that there was no change in α_1-adrenoceptor subtype. NA-induced vasoconstriction was resistant to CEC but attenuated by SZL-49 indicating that α_{1A}-adrenoceptors (as in the control rabbits) mediated NA responses in conditions of oestrogen dominance.

Endothelial dysfunction could also be a contributing factor to enhanced vasoconstrictor activity. This has been shown in experimental hypertensive rats, where impaired endothelial function was associated with increased vascular reactivity (Sunano et al., 1989). In the perfused ovarian vascular bed, treatment with oestrogens, ovulation induction and pregnancy are associated with increased sensitivity of the preparations to NA (this study). L-NOARG did not significantly enhance NA-induced vasoconstrictions in any of these situations. This is contrary to the significant potentiation of NA responses by L-NOARG in control rabbits. Perhaps this observation would suggest endothelial dysfunction in these hormonal states. However, this is unlikely to be the case. There are several reports in the literature, that oestrogen treatment and pregnancy improves endothelium-dependent vasodilatation. For example, Bell (1973) and Graham and Sani (1971) have shown that endothelium-dependent vasodilator responses to acetylcholine in the guinea pig uterine artery and rabbit uterine and ovarian arteries, were unmasked during pregnancy. In addition, it has been shown that oestrogen-treatment increases basal release of nitric oxide in ring segmants of the rat aorta (Rahimian et al., 1996) and increases arterial relaxation in mesenteric arteries (Ferrer & Osol, 1996).

In this study, the vasoconstrictor response to oxymetazoline, a partial α_1-adrenoceptors agonist, was enhanced by L-NOARG in the ovarian vascular bed preparations from pregnant rabbits (in the same preparation where L-NOARG failed to enhance NA responses). In addition, there was no significant difference between the

vasodilator potencies of carbachol in control rabbits and hormone-treated (or pregnant) rabbits. The maximum relaxation was also not different. Perhaps more significantly, carbachol-induced vasodilation was abolished by L-NOARG, indicating that NO is totally responsible for the vasodilator effect of carbachol.

Therefore, failure of L-NOARG to enhance NA-induced vasoconstriction in the hormone treated rabbits (including pregnancy) could possibly be due to the fact that under these conditions, the response to NA was already maximally potentiated, so that no further potentiation could be obtained in the presence of L-NOARG. In support of this, is the fact that ED_{50} (for NA) obtained in preparations from hormone-treated rabbits was not significantly different from the value obtained in control rabbits, in the presence of L-NOARG.

4.6 Conclusion

Based on the results obtained in this study, it was concluded that:

(1) The ovarian vascular bed responded to adrenergic agonists with a vasoconstriction.

(2) There is little or no basal release of NO. However, the vasoconstrictor response to NA was accompanied by a release of NO resulting in reduced response to NA.

(3) α_1- But not α_2-adrenoceptors mediated NA-induced vasoconstriction in this vascular bed.

(4) The α_1-adrenoceptor is of the α_{1A}-subtype.

(5) α_{1A}-adrenoceptor activation in the rabbit ovarian vascular bed is linked to an influx of extracellular Ca^{2+} through activation of tyrosine kinase.

(6) Oestrogen treatment, ovulation induction and pregnancy are all associated with an α_1-adrenoceptor specific increase in sensitivity of the ovarian vascular bed to NA and oxymetazoline. However, there was no change in receptor characteristics, i.e. α_2-adrenoceptor was not unmasked and the vasoconstrictor responses were still mediated via α_{1A}-adrenoceptor subtype.

(7) There was no enhanced vasodilator response to carbachol indicating that there was no increased release of EDRF accompanying the changes in

hormone status. However, L-NOARG abolished carbachol-induced vasodilatation indicating that NO mediated the response.

In summary, the enhanced sensitivity of the ovarian vascular bed to noradrenaline following induction of ovulation and pregnancy could be of physiological relevance. The ovarian artery gives off a branch which supplies the oviduct and continues to join the uterine artery. Development of the ovum begins in the proximal part of the oviduct and it is therefore essential that there must be an adequate blood supply to this region to provide nutrients to the developing ovum. Therefore, increased vasoconstriction in the ovarian vascular bed would divert blood flow towards the proximal part of the oviduct. This would result in increased supply of nutrients to the developing embryo. Similarly, during pregnancy, there is no need for ovulation to take place and so diversion of blood flow away from the ovary would lead to increased perfusion of the uterus and hence supply of nutrients to the growing fetus. This would perhaps be more significant in humans where a good part of the blood supply to the uterus is through the ovarian artery.

REFERENCES

Abel, P., Zeng, W., Porter, J., Scofield, M., Liu, F., Gonzalez-Cabrera, I., Dowd, F. and Jeffries, W. (1995) The atypical α_1-adrenoceptor. Pharmac. Communic. 6, 29-38.

Adam, L. P., Haeberle, J.and Hathaway, D. (1989) Phosphorylation of caldesmon in arterial smooth muscle. J. Biol. Chem. 264, 7698-7793.

Adam, L. P., Kalinowski, E. and Hathaway, D. (1994) Mitogen-activated protein kinase and contractility in carotid arteries: effects of the tyrosine kinase inhibitor, genistein. Circulation 90, 356.

Adelstein, R. and Sellers, J. (1987) Effects of calcium on vascular smooth muscle contraction. Am. J. Cardiol. 59, 4B-10B.

Agrawal. D. K., Triggle, G. R. and Daniel, E. E. (1984) Pharmacological characterization of the postsynaptic α-adrenoceptors in vascular smooth muscle from canine and rat mesenteric arterial beds. J. Pharmacol. Exp. Ther. 229, 831-838.

Akiyama, T., Ishida, J., Nakagawa, S., Ogawara, H., Watanabe, S., Itoh, N., Shibuya, M. and Fukami, Y. (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. J. Biol. Chem. 262, 5592-5595.

Altura, B. M. (1972) Sex as a factor influencing the responsiveness of arterioles to catecholamines. Eur. J. Pharmacol. 20, 261-265.

Arunlakshana, O. & Schild, H. O. (1959) Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14, 48.

Asano, T., Ikegaki, I., Satoh, S., Suzuki, Y., Shibuya, M., Takayasu, T. and Hidaka, H. (1987) Mechanism of action of a novel antivasospasm drug, HA1077. J. Pharmacol. Exp. Ther. 241, 1033-1040.

Asano, T., Suzuki, T., Tsuchiya, M., Satoh, S., Ikegaki, I., Shibuya, M., Suzuki, Y. and Hidaka, H. (1989) Vasodilator actions of HA 1077 in vitro and in vivo putatively mediated by the inhibition of protein kinase. Br. J. Pharmacol. 98, 1091-1100.

Austin, C. and Chess-Williams, R. (1995) The influence of 17-ß-oestradiol and the natural oestrous cycle on α-adrenoceptor-mediated responses of the cardiovascular system in the rat. J. Pharm. Pharmacol. 47, 656-660.

Babbedge, R., Bland-Ward, P., Hart, S. and Moore, P (1993) Inhibition of rat cerebellar nitric oxide synthase by 7-nitro indazole and related substituted indazoles. Br. J. Pharmacol. 110, 225-228.

Bahr, J., Kao, L. and Nalbandov, A. (1974) The role of catecholamines and nerves in ovulation. Biol. Reprod. 10, 273-290.

Bell, C. (1973) Oestrogen-induced sensitization of the uterine artery of the guinea-pig to acetylcholine. Br. J. Pharmac. 49, 595-601.

Bento, A. C. and DeMoraes, S. D. (1992) Effects of estrogen pretreatment of the spare α_1-adrenoceptors and the slow and fast components of the contractile response of the isolated female rat aorta. Gen. Pharmac. 23, 565-570.

Berridge, M. J. (1983) Rapid accumulation of inositol triphosphate reveals that agonists hydrolyse polyphosphoinositides instead of phosphatidylinositol. Biochem. J. 212: 849-858.

Berridge, M. J. (1984) Inositol triphosphate and diacylglycerol as second messengers. Biochem. J. 220: 345-360.

Berridge, M. J. (1987) Inositol triphosphate and diacylglycerol: two interacting second messengers. Annu. Rev. Biochem. 56, 159-193.

Berridge, T. R., Doxey, J. C., Roach, A. G., Strachan, D. A. and Virdee, N. K. (1984) Antagonist effects of idazoxan and yohimbine against several α_2-adrenoceptor agonists in pithed rats. Br. J. Pharmacol. 81, 86P.

Berthelsen, S. and Pettinger, W. A. (1977) A functional basis for classification of α-adrenergic receptors. Life Sci. 21, 595-606.

Bevan, J. A. (1977) Some functional consequences of variation in adrenergic synaptic cleft width and in nerve density and distribution. Fed. Proceed. 36 (10), 2439-2443.

Bevan, J., Hosmer, D., Ljung, B., Pegram, B. and Su, C. (1974) The innervation pattern and neurogenic response of rabbit veins. Blood Vessels, 11, 172-182.

Bevan, J. A., Oriowo, M. A. and Bevan, R. D. (1986) Relation between physiological variation in α-adrenoceptomediated arterial sensitivity and agonist affinity. Science, 234, 187-196.

Bhatt, J. D. and Gulati, O. D. (1986) The role of the sympathetic nervous system in oestrogen-induced hypertension in rats. Br. J. Pharmacol. 89, 685-691.

Bishop, J. M. (1983) Cellular oncogens and retroviruses. Annu. Rev. Biochem. 52, 301-354.

Bishop, J. M. (1987) The molecular genetics of cancer. Science, 235, 305-311.

Bjersing, L. and Cajander, S. (1974) Ovulation and the mechanism of follicle rupture. VI. Ultrastructure of theca interna and the inner vascular network surrounding rabbit graafian follicles prior to induced ovulation. Cell. Tiss. Res. 153, 31-44.

Blasco, L., Wu, C. H., Flickinger, G. L., Pearlmutter, D. and Mikhail, G. (1975) Cardiac output and genital blood flow distribution during the preovulatory period in rabbits. Biol. Reprod. 13, 581-586.

Blue, D. R. and Clarke, D. E. (1990) Characterization and modulation of vascular α-adrenoceptor responses in rat kidney. Br. J. Pharmacol. 99, 204P.

Boer, R., Grassegger, A., Schudt, C. and Glossmann, H. (1989) (+)-Niguldipine binds with very high affinity to Ca^{2+} channel and to a subtype of α_1-adrenergic receptors. Eur. J. Pharmacol. 172, 131.

Boeynaems, J. and Pirotton, S (1994) Phospholipase C and phosphoinositide metabolism. In. Regulation of the vascular endothelium: signals and transduction mechanisms. Ch 2, pp. 25-39. R. G. Landes Company, Austin.

Boeynaems, J., Demolle, D. and Galand, N. (1987) Adrenergic stimulation of vascular prostacyclin: role of alpha-1 receptors in smooth muscle cells. Eur. J. Pharmacol. 144 (2), 193-200.

Bohmann, C., Rist, W., Schollmeyer, P. and Rump, L. (1995) Low concentrations of angiotensin II unmask vasoconstrictory α_2-adrenoceptors in isolated perfused kidneys of spontaneously hypertensive rats. Cardiov. Res. 30, 857-865.

Bolton, T. B. (1979) Mechanisms of action of transmitters and other substances on smooth muscle. Physiol. Rev. 59, 606-718.

Bolton, T. B., Lang, R. J.& Takewaki, T. (1984) Mechanisms of action of noradrenaline and carbachol on smooth muscle of guinea-pig anterior mesenteric artery. J. Physiol. 351, 549-572.

Brown, G. L. and Gillespie, J. S. (1957) The output of sympathetic transmitter from the spleen of the cat. J. Physiol. 138, 81-102.

Burden, H. W. (1972) Ultrastructural observations on ovarian perifollicular smooth muscle in the cat, guinea pig, and rabbit. Am. J. Anat. 133, 125-142.

Burden, H. W. (1973) The distribution of smooth muscle in the cat ovary with a note on its adrenergic innervation. J. Morph. 140, 467-476.

Burgess, G. M., Gofrey, P. P., McKinney, J. S., Berridge, M. J., Irvine, R. F. and Putney, J. (1984) The second messenger linking receptor activation to internal Ca release in liver. Nature, 309: 63-66.

Burnstock, G. (1975) Innervation of vascular smooth muscle: histochemistry and electron microscopy. Clin. Exp. Pharm. Physiol. Suppl. 2, 7-20.

Burr, J. H. and Davies, J. I. (1951) The vascular system of the rabbit ovary and its relationship to ovulation. Anat. Rec. III. 273-297.

Bylund, D. and Ray-Pregner, C. (1989) Alpha-2A and alpha-2B adrenergic receptor subtypes: Attenuation of cyclic AMP production in cell lines containing only one receptor subtype. J. Phrm. Exp. Ther. 251, 640-644.

Bylund, D. B. (1985) Heterogeneity of alpha-2 adrenergic receptors. Pharmac. Biochem. Behav. 22, 835-843.

Bylund, D. B., Eikenberg, D. C., Hieble, J. P., Langer, S. Z., Lefkowitz, R. J., Minneman, K. P., Molinoff, P. B., Ruffolo, R. R. and Trendelenburg, U. (1994) IV. International union of pharmacology nomenclature of adrenoceptors. Pharmac. Rev. 46: (2), 121-136.

Bylund, D., Blaxall, H., Iversen, L., Caron, M., Lefkowitz, R. and Lomasney, J. (1992) Pharmacological characterization of α_2-adrenergic receptors: comparison of pharmacologically defined subtypes with subtypes identified by molecular cloning. Molec. Pharmac. 42, 1-5.

Bylund, D., Ray-Pregner, C. and Murphy, T. (1988) Alpha-2A and alpha-2B adrenergic receptor subtypes: Antagonist binding in tissues and cell lines containing only one subtype. J. Pharmac. Exp. Ther. 245, 600-607.

Casnellie, J. E. (1991) Protein kinase inhibitors: probes for the functions of protein phosphorylation. Adv. Pharmacol. 22, 167-205.

Cauvin, C. & Malik, S. (1984) Induction of Ca^{++}influx and intracellular Ca^{++} release in isolated rat aorta and mesenteric resistance vessels by norepinephrine activation of α_1-adrenoceptors. J. Pharmacol. Exp. Ther. 230, 413-418.

Cauvin, C., Loutzenhiser, R., Hwang, O. and van Breemen, C. (1982) α_1-Adrenoceptors induce Ca influx and intracellular Ca release in isolated rabbit aorta. Eur. J. Pharmac. 84: 233-235.

Cauvin, c., Saida, K. & van Breemen, C. (1984) Extracellular Ca^{2+} dependence and diltiazem inhibition of contraction in rabbit conduit arteries and mesenteric resistance vessels. Blood vessels, 21, 23-31.

Cavero, I., Shepperson, N., Lefevre-Borg, F. and Langer, S. Z. (1983) Differential inhibition of vascular smooth muscle responses to α_1-and α_2-adrenoceptor agonists by diltiazem and verapamil. Circ. Res. 52 (suppl. 1): 69-76.

Chen, G., Suzuki, H. & Weston, A. (1988) Acetylcholine releases endothelium-derived hyperpolarizing factor and EDRF from rat blood vessels. Br. J. Pharmacol. 95, 1165-1174.

Chen, G., Yamamoto, Y., Miwa, K. & Suzuki, H. (1991) Hyperpolarization of arterial smooth muscle induced by endothelial humoral substances. Am. J. Physiol. 260, H1888-H1892.

Cherney, D., Didio, L. and Motta, P. (1975) The development of rabbit ovarian follicles following copulation. Fertil. Steril. 26, 257-271.

Chiu, A. T., Mc Call, D. E., Thoolen, M. J. M. and Timmermans, P. B. (1986) Ca^{++} utilization in the constriction of rat aorta to full and partial $alpha_1$-adrenoceptor agonists. J. Pharmac. exp. Ther. 238: 224-231.

Cho, H. J., Xie, Q. W., Calaycay, J, Mumford, R. A., Swiderek, K. M., Lee, T. D. and Nathan, C. (1992) Calmodulin is a subunit of nitric oxide synthase from macrophages. J. Exp. Med. 176, 599-604.

Clarke, D. E., Vimont, R. L. and Blue, D. R. (1990) Vascular a_1-adrenoceptors in rat kidney: agonist and antagonist [prazosin, idazoxan, WB 4101, (+)-niguldipine] characterization. Eur. J. Pharmacol. 183 (suppl.), 773.

Cocks, T. M., Angus, J. A., Campbell, J. H. & Campbell, G. R. (1985) Release and properties of endothelium-derived relaxing factor (EDRF) from endothelial cells in culture. J. Cell. Physiol. 123, 310-320.

Colucci, W., Gimbrone, M., McLaughlin, M., Halpern, W. and Alexander, R. (1982) Increased vascular catecholamine sensitivity and α-adrenergic receptor affinity in female and estrogen-treated male rats. Circ. Res. 50, 805-811.

Cooke, J. P., Rimele, T. J., Flavahan, N. A. and Vanhoutte, P. M. (1985) Nimodipine and inhibition of alpha adrenergic activation of the isolated canine saphenous vein. J. Pharmac. Exp. Ther. 234, 598-602.

Corbett, J. A., Sweetland, M., Lancaster, J. and McDaniel, M. (1993) A 1-hour pulse with 1L-1ß induces formation of nitric oxide and inhibits insulin secretion by rat islets of Langerhans: evidence for a tyrosine kinase signalling mechanism. FASEB J. 7, 369-374.

Corbett, J. A., Tilton, R. G., Chang, K., Hasan, K. S., Ido, Y., Wang, J. L., Sweetland, M. A., Lancaster, J. R., Williamson, J. R. and McDaniel, M. L. (1992) Aminoguanidine, a novel inhibitor of nitric oxide formation, prevents diabetic vascular dysfunction. Diabetes, 41: 552556.

Cotecchia, S., Kobilka, B. K., Daniel, K. W., Nalon, R. D., Lapetina, E. Y., Caron, M. G., Lefkowitz, R. J. and Regan, J. W. (1990) Multiple second messenger pathways of α-adrenergic receptor subtypes expressed in eukaryotic cells. J. Biol. Chem. 265, 63-69.

Cotecchia, S., Schwinn, D., Randall, R., Lefkowitz, R., Caron, M. and Kobilka, B. (1988) Molecular cloning and expression of the cDNA for the hamster α_1-adrenergic receptor. Proc. Natl. Acad. Sci. USA 85, 7159-7163.

Danthuluri, N. R. and Deth, R. C. (1984) Phorbol ester-induced contraction of arterial smooth muscle and inhibition of α-adrenergic response. Biochem. Biophys. Res. Commun. 125: 1103-1109.

DeMey, J. G. & Vanhoutte, P. M. (1981) Role of the intima in cholinergic and purinergic relaxation of isolated canine femoral arteries. J. Physiol. 316,347-355.

DeMey, J. G. & Vanhoutte, P. M. (1982) Heterogenous behaviour of the canine arterial and venous wall. Importance of the endothelium. Circ. Res. 51, 439-447.

Deth, R. and Lynch, C. (1981) Inhibition of α-receptor-induced Ca^{2+} α_2-adrenoceptors influx by Mn^{2+} and La^{3+}. Eur. J. Pharmac. 71: 1-11.

Devine, C. E., Somlyo, A. V. and Somlyo, A. P. (1972) Sarcoplasmic reticulum and excitation-contraction coupling in mammalian smooth muscles. J. Cell Biol. 52, 690-718.

Di Salvo, J., Gifford, D. and Kokkinakis, A. (1988) $pp60^{c\text{-}src}$ Kinase activity in bovine coronary extracts is stimulated by ATP. Biochem. Biophys. Res. Commun. 153, 388-394.

Di Salvo, J., Gifford, D. and Kokkinakis, A. (1989) ATP- and polyphosphate-mediated stimulation of $pp60^{c\text{-}src}$ kinase activity in extracts from vascular smooth muscle. J. Biol. Chem. 264, 10773-10778.

Di Salvo, J., Steusloff, A., Semenchuk, L., Satoh, S., Kolquist, K. & Pfitzer, G. (1993) Tyrosine kinase inhibitors suppress agonist-induced contraction in smooth muscle. Biochem. Biophys. Res. Commun. 190, 968-974.

Docherty, J. R. and Hyland, L. (1984) Neuro-effector transmission through postsynaptic α_2-adrenoceptors in human saphenous vein. Br. J. Phrmac. 83, 362P.

Docherty, J. R. and Hyland, L. (1985) No evidence for differences between pre- and post-junctional α_2-adrenoceptors. Br. J. Phrmac. 86, 335-339.

Draetta, G., Piwnica-Worms, H., Morrison, D., Drucker, B., Roberts, T. and Beach, D. (1988) Human cdcz protein kinase is a major cell-cycle regulated tyrosine kinase substrate. Nature, 336, 738-744.

Drew, G. M. and Whiting, S. B. (1979) Evidence for two distinct types of postsynaptic α-adrenoceptor in vascular smooth muscle in vivo. Br. J. Pharmac. 67, 207-215.

Drummond, G. I. (1984) Cyclic GMP. In Cyclic neucleotides in the nervous system. pp. 40-125, Raven Press, New York.

Dvir, A., Milner, Y., Chomsky, O., Gilon, C., Gazit, A. and Levitzki, A. (1991) The inhibition of EGF-dependent proliferation of keratinocytes by tyrphostin tyrosine kinase blockers. J. cell Biol. 113, 857-865.

Edvinsson, L. and Owman, C. (1974) Pharmacological characterization of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries in vitro. Circ. Res. 35, 835-849.

Edvinsson, L., Owman, C. and Sjoberg, N. O. (1976) Autonomic nerves, mast cells, and receptors in human brain vessels: a histochemical and pharmacological study. Brain Res. 115, 377-393.

Ek, B., Westermark, B., Wasteson, A. and Heldin, C. -H. (1982) Stimulation of tyrosine-specific phosphorylation by platelet-derived growth factor. Nature, 295, 419-420.

Elhawary, A. M., Pettinger, W. A. and Wolff, D. W. (1992) Subtype-selective alpha-1 adrenoceptor alkylation in the rat kidney and its effect on the vascular pressor response. J. Pharmacol. Exp. Ther. 260, 709.

Eltze, M. & Boer, R. (1992) The adrenoceptor agonist, SDZ NVI 085, discriminates between α_{1A}- and α_{1B}-adrenoceptor subtypes in vas deferens, kidney and aorta of the rat. Eur. J. Pharmacol. 224, 125-136.

Eltze, M., Boer, R., Sanders, K. H. and Kolassa, N. (1991) Vasodilatation elicited by 5-HT_{1A} receptor agonists in constant-pressure-perfused rat kidney is mediated by blockade of α_{1A}-adrenoceptors. Eur. J. Pharmacol. 202, 33.

Espey, L. L. (1978) Ovarian contractility and its relationship to ovulation: A review. Biol. Reprod. 19, 540-551.

Espey, L. L. (1980) Ovulation as an inflammatory reaction. A hypothesis. Biol. Reprod. 22, 73-106.

Espey, L. L. (1974) Ovarian proteolytic enzymes and ovulation. Biol. Reprod. 10, 216-235.

Euler, U. and Lishajko, F. (1966) A specific kind of noradrenaline granules in the vesicular gland and the vas deferens of the bull. Life Sci. 5, 687-691.

Farhat, M. Y. and Ramwell, P. W. (1992) estradiol potentiates the vasopressor response of the isolated perfused rat lung to the thromboxane mimic U-46619. J. Pharmacol. Exp. Ther. 261, 686-691.

Feletou, M. & Vanhoutte, P. M. (1988) Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br. J. Pharmac. 93, 515-524.

Feletou, M., Canet, E. and Vanhoutte, P. (1994) Endothelial NO and vascular regulation. In. Endothelium-derived factors and vascular function. ed. T. Masaki. pp. 47-59. Excerpta Medica, Amsterdam.

Ferrer, M. and Osol, G. (1996) Chronic treatment with 17ß-estradiol enhances arterial relaxation induced by ß-adrenoceptor stimulation. FASEB J. 10, A707.

Flavahan, N. A. and Vanhoutte, P. M. (1986) α-Adrenoceptor subclassification in vascular smooth muscle. Trends Phamacol. Sci. 7, 347-349.

Fleming, I., Hecker, M. & Busse, R. (1994) Intracellular alkalinization induced by bradykinin sustains activation of the constitutive nitric oxide synthase in endothelial cells. Circ. Res. 74, 1220-1226.

Fleming, I., Julou-Schaeffer, G., Gray, G. A., Parrat, J. R. and Stoclet, J. C. (1991) Evidence that an L-arginine/nitric oxide dependent elevation of tissue cyclic GMP content is involved in depression of vascular reactivity by endotoxin. Br. J. Pharmacol. 103, 1047-1052.

Ford, A. P., Willliams, T. J., Blue, D. R. and Clarke, D. C. (1994) α_1-Adrenoceptor classification in: sharpening occam's razor. TiPS. 15: 167-169.

Forder, J., Scriabine, A. and Rasmussen, H. (1985) Plasma membrane calcium flux, protein kinase C activation and smooth muscle contraction. J. Pharmacol. Exp. Ther. 235, 267-273.

Forray, C., Bard, J., Wetzel, J., Chiu, G., Shapiro, E., Tang, R., Lepor, H., Hartig, P., Weinshank, R., Branchek, T. and Gluchowski, C. (1994) The α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1C} subtype. Mol. Pharmacol. 45, 703-708.

Forray, C., Borden, L., Bard, J., Chiu, G., Wetzel, J., Branchek, T., Weinshank, R., Gluchowski, C., Hartig, P., Tang, R., Shapiro, E. and Lepor, H. (1993) Characterization of α_1-adrenoceptor subtype that mediates human prostate contraction. Pharmacologist 35: 167.

Fregly, M. J. (1972) Effect of an oral contraceptive on spontaneously running activity, salt apetite and blood pressure of ovaryectomized rats. J. Pharmacol. Exp. Ther. 182, 335-342.

Frew, J., Paisley, K. and Martin, W. (1993) Selective inhibition of basal but not agonist-stimulated activity of nitric oxide in rat aorta by N^G-monomethyl-L-arginine. Br. J. Pharmacol. 110, 1003-1008.

Furchgott, R. F. (1984) The role of the endothelium in the responses of vascular smooth muscle to drugs. Annu. Rev. Pharmacol. Toxicol. 24, 175-197.

Furchgott, R. F. (1972) The classification of adrenoceptors (adrenergic receptors). an evaluation from the stand point of receptor theory. In. Handbook of experimental pharmacology. V. 33 Catecholamines. eds. M. Blaschko and E. Muscholl. p. 283, Springer-Verlag, New York.

Furchgott, R. F. and Zawadzki, J. V. (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature, 288: 373376.

Garcia-Sainz, J., Romero-Avila, M., Alcantara-Hernandez, R., Macias-Silva, M., Olivares-Reyes, J. and Gonzalez-Espinosa, C. (1992) Species heterogeneity of hepatic α_1-adrenoceptors: α1A, α1B and α1C subtypes. Biochem. Biophys. Res. Commun. 186, 760-767.

Garland, C. J. & McPherson, G. A. (1992) Evidence that nitric oxide does not mediate the hyperpolarization and relaxation to acetylcholine in the rat small mesenteric artery. Br. J. Pharmacol. 105, 429-435.

Gazit, A., Yaish, P., Gilon, C. and Levitzki, A. (1989) tyrphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. J. Med. Chem. 32, 2344-2352.

Graham, J. D. and Sani, D. A. (1971) Effect of stilboestrol on the response of the perfused uterine or ovarian vessels of the rabbit to catecholamines and acetylcholine. J. Physiol. (Lond.) 218, 64p-6p.

Gray, D. W. & Marshall, I. (1992) Novel signal transduction pathway mediating endothelium-dependent ß-adrenoceptor vasorelaxation in rat tharacic aorta. Br. J. Pharmacol. 107, 684-690.

Greenwald, G. (1963) In vivo recording of intraluminal pressure changes in the rabbit oviduct. Fertil. Steril. 14 (6), 666-674.

Griffith, T. M., Henderson, A. H., Hughes, E. D. & Lewis, M. J. (1984) Isolated perfused rabbit coronary artery and aorta strip preparations: the role of endothelium-derived relaxant factor. J. Physiol. 351, 13-24.

Gross, G., Hanft, G. and Rugevics, C. (1988) 5-Methyl-urapidil discriminates between subtypes of the α_1-adrenoceptor. Eur. J. Pharmacol. 151, 333.

Guyton, A. C. & Hall, J. E. (1996) Text book of medical physiology, 9th ed. pp. 203-204, W. B. Saunders company, London.

Hadley, M. E. (1984) Endocrinology, pp. 422-426, Prentice-Hall, Inc., New Jersey, U.S.A.

Hamada, Y., Wright, K. H. and Wallach, E. E. (1979) The effect of progesterone and human chorionic gonadotropin on ovulation in the in vitro perfused rabbit ovary. Ferti. Steril. 32, 335-9.

Han, C. H., Li, J. and Minneman, K. P. (1990) Subtypes of α_1-adrenoceptors in rat blood vessels. Eur. J. Pharmacol. 190, 97.

Han, C., Abel, P. W. and Minneman, K. P. (1987) α_1-Adrenoceptor subtypes linked to different mechanisms for increasing intracellular Ca^{2+} in smooth muscle. Nature 329, 333-335.

Harrison, J., D'Angelo, D., Zeng, D. and Lynch, K. (1991) Pharmacological characterization of rat α_2- adrenergic receptors. Molec. Pharmac. 40, 407-412.

Hieble, J. P., Sulpizio, A., Nichols, A., Willette, R. and Ruffolo, R. (1988) Pharmacologic characterizatin of SK&F 104078, a novel α_2-adrenoceptor antagonist which discriminates between pre- and postjunctional α_2-adrenoceptors. J. Pharm. Exp. Ther. 247, 645-652.

Hieble, J., DeMarinins, R., Fowler, P. and Matthews, W. (1986b) Selective alpha-2 adrenoceptor blockade by SK&F 86466: in vitro characterization of receptor selectivity. J. Pharm. Exp. Ther. 236, 90-96.

Hieble, J., Sulpizio, A., Nichols, A., DeMarinis, R., Pfeiffer, F., Lavanchy, P. and Ruffolo, R. (1986a) Pharmacological differentiation of pre- and postjunctional alpha$_2$ adrenoceptors. J. Hypertens. 4 (suppl 6), S189-S192.

Holck, M. I.; Jones, C. H. M. and Haeusler, G. (1983) Differential interactions of clonidine with postsynaptic α-adrenoceptors of rabbit main pulmonary artery. J. Cardiovasc. Pharmacol. 5, 240-248.

Hollenberg, M. D. (1994) Tyrosine kinase pathways and the regulation of smooth muscle contractility. TIPS, 15, 108-114.

Huckle, W., Prokop, C., Dy, R. C., Herman, B. and Earp, S. (1990) Angiotensin II stimulates protein-tyrosine phosphorylation in a calcium-dependent manner. Mol. Cell. Biol. 10, 6290-6298.

Hughes, J. (1972) Evaluation of mechanisms controlling the release and inactivation of the adrenergic transmitter in the rabbit portal vein and vas deferens. Br. J. Pharmacol. 44, 472-491.

Hunter, T. and Cooper, J. (1985) Protein tyrosine kinases. Annu. Rev. Biochem. 54, 897-930.

Hutchinson, P. J., Palmer, R. M. & Moncada, S. (1987) Comparative pharmacology of EDRF and nitric oxide on vascular strips. Eur. J. Pharmacol. 141, 445-451.

Hwang, K. S. and van Breemen, C. (1987) Ryanodine modulation of ^{45}Ca efflux and tension in rabbit aortic smooth muscle. Pflugers Arch. 408, 343-350.

Iversen, L. and Salt, P. J. (1970) Inhibition of catecholamine $uptake_2$ by steroids in the isolated rat heart. Br. J. Pharmacol. 40, 528-530.

Jacobs, D., Kull, F., Earp, H., Svoboda, M., Van Wyk, J. and Cautrecasas, P. (1983) J. Biol. Chem. 258, 9581-9584.

Jiang, M. and Morgan, K. (1987) Intracellular calcium levels in phorbol ester-induced contractions of vascular smooth muscle. Am. J. Physiol. 253, H1365-H1371.

Joseph, S. K., Thomas, A. P., Williams, R. J., Irvine, R. F. and Williamson, J. R. (1984) Myo-inositol 1,4,5,-triphosphte. A second messenger for the hormonal mobilization of intracellular Ca^{2+} in liver. J. Biol. Chem. 259: 3077-3081.

Kamm, K. and Stull, J. (1985) The function of myosin and myosin light chain kinase phosphorylation in smooth muscle. Annu. Rev. Pharmacol. Toxicol. 25, 593-620.

Kaplan, N. M. (1978) Cardiovascular complications of oral contraceptives. Ann. Rev. Med. 29, 31-40.

Karaki, H., Nagase, H., Ohizumi, Y., Satake, N. and Shibata, S. (1988a) Palytoxin-induced contraction and release of endogenous noradrenaline in rat tail artery. Br. J. Pharmacol. 95, 183-188.

Karaki, H., Nakagawa, H. and Urakawa, N. (1985) Age-related changes in the sensitivity to verapamil and sodium nitroprusside of vascular smooth muscle of rabbit aorta. Br. J. Pharmacol. 85, 223-228.

Karaki, H., Sato, K., Ozaki, H. & Murakami, K. (1988b) Effects of sodium nitroprusside on cytosolic calcium level in vascular smooth muscle. Eur. J. Pharmacol. 156, 259-266.

Kasuga, M., Zick, Y., Blithe, D., Crettaz, M. and Kahn, C. (1982) Insulin stimulates tyrosine phosphorylation of the insulin receptor in a cell-free system. Nature, 298, 667-669.

Khalil, R. A. and van Breemen, C. (1988) Sustained contraction of vascular smooth muscle: calcium influx or C-kinase activation? J. Pharmacol. Exp. Ther. 244, 537-542.

Kishimoto, A., Takai, A., Mori, T., Kikkawa, U. and Nishizuka, Y. (1980) Activation of calcium and phospholipid-dependent protein kinase by diacylglycerol, its possible relation to phosphatidylinositol turnover. J. biol. Chem. 255: 2273-2276.

Knowles, R. G., Palacios, M., Palmer, R. M. & Moncada, S. (1990) Kinetic characteristics of nitric oxide synthase from rat brain. Biochem. J. 269, 207-210.

Kobayashi, Y., Sjoberg, N.-O., Owman, Ch., Wright, K. H., Santulli, R. and Wallach, E. E. (1983a) The effect of adrenergic agents on the ovulatory process in the in vitro perfused rabbit ovary. Am. J. Obstet. Gynecol. 145, 857-64.

Kobayashi, Y., Wright, K. H., Santulli, R. and Wallach, E. E. (1981) Ovulation and ovum maturation in the rabbit ovary perfused in vitro. Biol. Reprod. 24, 283.

Kobayashi, Y., Wright, K. H., Santulli, R. and Wallach, E. E. (1983b) The effect of histamine and histamine blockers on the ovulatory process in the in vitro perfused rabbit ovary. Biol. Reprod. 28, 385.

Komori, K. & Vanhoutte, P. M. (1990) Endothelium-derived hyperpolarizing factor. Bld. Vessels. 27, 238-245.

kuhl, L. M., Stormshak, F. and Weber, L. J. (1974) Corpus luteum and oestrous cycle influence on physiological properties of ewe utero-ovarian arteries. Proc. Soc. Exp. Biol. Med. 147, 7-10.

Kuo, J. F., Andersson, R., Wise, B., Mackerlova, L., Salomonsson, I., Brackett, N., Katoh, N., Shoji, M. and Wrenn, R. (1980) Calcium-dependent protein kinase: widespread occurrence in various tissues and phyla of the animal kingdom and comparison of effects of phospholipid, calmodulin, and trifluoperazine. Proc. Natl. Acad. Sci. U. S. A., 72, 7039-7043.

Laher, I. and Bevan, J. A. (1985) Alpha adrenoceptor number limits response of some rabbit arteries to norepinephrine. J. Pharmacol. Exp. Ther. 233 (2), 290-297.

Lal, H., Woodward, B. and Williams, K. I. (1994) Differential effects of agents on bronchial and vascular tone and lung weight in rat isolated perfused lung. Can. J. Physiol. Pharmacol. 72 (suppl 1), 495.

Lambertsen, C., Greenbaum, D., Wright, K. and Wallach, E. (1976) In vitro studies of ovulation in the perfused rabbit ovary. Fertil. Steril. 27, 178-187.

Lands, A. M., Arnold, A., McAuliff, J., Luduena, F. and Brown, T. (1967) Differentiation of receptor systems activated by sympathomimetic amines. Nature, 214, 597-598.

Langer, S. Z.; Adler, E.; Enero, M. A. and Stefano, F. J. E. (1971) The role of the a-receptor in regulating noradrenaline overflow by nerve stimulation. Proc. Int. Union Physiol. Sci. 9, 335.

Langer, S., Pimoule, C., Faure, C. and Graham, D. (1994) Evidence that the classical α_{1A}- and cloned α_{1C}-adrenoceptors are the same subtype. Can. J. Physiol. Pharmacol. 72 (suppl 1), 554.

Laragh, J., Sealy, J. E., Ledingham, J. and Newton, M. A. (1972) Oral contraceptives, renin, aldosterone and high blood pressure. J. Am. Mec. Assoc. 201, 918-922.

Lattanzio, F. A., Schlatterer, R. G., Nicar, M., Campbell, K. P. and Sutko, J. L. (1987) The effect of ryanodine on passive calcium fluxes across sarcoplasmic reticulum membranes. J. Biol. Chem. 262, 2711-2718.

Lawrence, I, E. and Burden, H. W. (1976) The autonomic innervation of the interstitial gland of the rat ovary during pregnancy. Am. J. Anat. 147, 81-94.

Laz, T. M., Forray, C., Smith, K. E., Vacsse, P. J. J., Hartig, P. R., Gluchowski, C., Branchek, T. A. and Weinshank, R. L. (1993) Recombinant rat homolog of the bovine α_{1C}-adrenergic receptor exhibits an α_{1A}- like receptor pharmacology. Soc. Neurosci. Abstr. 19: 1788.

Laz, T., Forray, C., Smith, K., Bard, J., Vaysse, P., Branchek, T. and Weinshank, R. (1994) The rat homologue of the bovine α_{1C}-adrenergic receptor shows the pharmacological properties of the classical α_{1A}-subtype. Mol. Pharmacol. 46, 414-422.

LeClerc, G., Rouot, G., Schwartz, J., Velly, J. and Wemuth, C. G. (1980) Studies on some para-substituted clonidine derivatives that exhibit an α-adrenoceptor stimulant activity. Br. J. Pharmacol. 71, 5-9.

Lee, K., Toskas, K. and Villereal, M. (1993) Inhibition of bradykinin- and thapsigargin-induced Ca^{2+} entry by tyrosine kinase inhibitors. J. Biol. Chem. 268, 9945-9948.

Lee, M. and Severson, D. (1994) Signal transduction in vascular smooth muscle: diacylglycerol second messengers and PKC action. Am. J. Physiol. 267, C659-C678.

Lee, W. and Novy, M. J. (1978) Effects of luteinizing hormone and indomethacin on blood flow and steroidogenesis in the rabbit ovary. Biol. Reprod. 17, 799-807.

Leijten, P. A. and van Breemen, C. (1984) The effects of caffeine on the noradrenaline-sensitive calcium store in rabbit aorta. J. Physiol. (Lond) 357, 327-339.

Leijten, P., Cauvin, C., Lodge, N., Saida, K. and van Breemen, C. (1985) Ca^{2+} sources mobilized by α_1-receptor activation in vascular smooth muscle. Cli. Sci. 68 (Suppl. 10): 47S-50S.

Levitzki, A. and Gilon, C. (1991) Tyrphostins as molecular tools and potential antiproliferative drugs. T.I.P.S. 12, 171-173.

Liou, Y. -M. and Morgan, K. G. (1994) Redestribution of protein kinase C isoforms in association with vascular hypertrophy of rat aorta. Am. J. Physiol. 267, C980-C989.

Loirand, G., Faiderbe, S., Baron, A., Geffard, M. and Mironneau, J. (1992) Autoanti-phosphatidylinositide antibodies specifically inhibit noradrenaline effects on Ca^{2+} and Cl^- channels in rat portal vein myocytes. J. Biol. Chem. 267, 4312-4316.

Loirand, G., Pacaud, P., Mironneau, C and Mironneau, J. (1990) GTP-binding proteins mediate noradrenaline effects on calcium and chloride currents in rat portal vein myocytes. J. Physiol. 428, 517-529.

Lomasney, J. W., Cotecchia, S., Lorenz, W., Leung, W.-Y, Schwinn, D. A., Yang-Feng, T. L., Brownstein, M., Lefkowitz, R. J. and Caron, M. G. (1991) Molecular cloning and expression of the cDNA for the α_{1A}-adrenergic receptor, the gene for which is located on human chromosome 5. J. Biol. Chem. 266, 6365-6369.

Lomasney, J. W., Lorenz, W., Allen, L. F., King, K., Regan, J. W., Yang-Feng, T., Caron, M. G. and Lefkowitz, R. J. (1990) Expansion of the α_2-adrenergic receptor family: cloning and characterization of a human α_2-adrenergic receptor subtype, the gene for which is located on chromosome 2. Proc. Natl. Acad. Sci. USA 87, 5094-5098.

Luscher, T. F. & Vanhoutte, P. M. (1986) Endothelium-dependnet contractions to acetylcholine in the aorta of the spontaneously hypertensive rat. Hypertension, 8, 344-348.

Ma, L., Hsu, C. -H. Patterson, E., Robinson, C. P. and Thadani, U. (1996) 17-Beta-estradiol increases sensitivity to norepinephrine induced contractions and reduces pinacidil, a potassium channel opener, induced relaxations of isolated rabbit aortic rings. FASEB J. 10, A708.

Malhotra, R., Bhave, S., Wakade, T.D. and Wakade, A. R. (1988) Protein kinase C of sympathetic neuronal membrane is activated by phorbol ester: correlation between transmitter release, $^{45}Ca^{2+}$ uptake, and the enzyme activity. J. Neurochem., 51, 967-974.

Marczin, N., Papapetropoulos, A. and Catravas, J. (1993) Tyrosine kinase inhibitors suppress endotoxin- and 1L-1ß-induced NO synthesis in aortic smooth muscle cells. Am. J. Physiol. 265, H1014-H1018.

Markovits, J., Linassier, C., Fosse, P., Couprie, J., Pierre, J., Jacquemin-Sablon, A., Saucier, J., Le Pecq, J. and Larsen, A. (1989) Inhibitory effects of the tyrosine kinase inhibitor genistein on mammalian DNA topoisomerase II. Cancer Res. 49, 5111-5117.

Marshall, I., Burt, R. P., Anderson, P. O., Chapple, C. R., Greengrass, P. M., Johnson, G. I. and Wyllie, M. G. (1992) Human α_{1C}-adrenoceptor: functional characterization in prostate. Br. J. Pharmacol. 107: 327P.

Martin, W., Villani, G. M., Jothianandan, D., & Furchgott, R. F. (1985) Selective blockade of endothelium-dependent and glyceryl trinitrate-induced relaxation by hemoglobin and by methylene blue in the rabbit aorta. J. Pharm. Exp. Ther. 232, 708-716.

Matthews, W., Jim, K., Hieble, P. and DeMarinis, R. (1984) Postsynaptic α-adrenoceptors on vascular smooth muscle. Federation Proc. 43, 2923-2928.

Mayer, B., Brunner, F. and Schmidt, K. (1993) Inhibition of nitric oxide synthesis by methylene blue. Biochem. Pharmacol. 45, 367-374.

Mayer, B., Schmidt, K., Humbert, R. & Bohme, E. (1989) Biosynthesis of endothelium-derived relaxing factor: a cytosolic enzyme in procine aortic endothelial cells Ca^{2+} -dependently converts L-arginine into an activator of soluble guanylyl cyclase. Biochem. Biophys. Res. Commun. 164, 678-685.

McCall, T., Feelisch, M., Palmer, R. and Moncada, S. (1991) Identification of N-iminoethyl-L-ornithine as an irreversible inhibitor of nitric oxide synthase in phagocytic cells. Br. J. Pharmacol. 102, 234-238.

McGiff, J., Malik, K. and Terragno, N. (1976) Prostaglandins as determinants of vascular reactivity. Federation Proc. 35, 2382-2387.

McGrath, J. C. (1982) Evidence for more than one type of postjunctional α-adrenoceptor. Biochem. Pharmacol. 31, 467-484.

Medgett, I. C. and Langer, S. Z. (1984) Heterogeneity of smooth muscle α-adrenoceptors in rat tail artery in vitro. J. Pharmacol. Exp. Ther. 229, 823-830.

Michel, A. D., Loury, D. N. and Whiting, R. L. (1989a) Identification of a single α_1-adrenoceptor corresponding to the α_{1A}-subtype in rat submaxillary gland. Br. J. Pharmacol. 98, 883-889.

Michel, A. D., Loury, D. N. and Whiting, R. L. (1989b) Differences between the α_2-adrenoceptor in rat submaxillary gland and the α_{2A}- and α_{2B}-adrenoceptor subtypes. Br. J. Pharmacol. 98, 890-897.

Michel, M. C., Buscher, R., Phillip, T., and Brodde, O. E. (1993) α_{1A}-and α_{1B}-adrenoceptors enhance inositol phosphate generation in rat renal cortex. Naunyn Schmiedeberg's Arch. Pharmacol. 347, 180-185.

Michell, R. H. (1979) Inositol phospholipids in membrane function. Trends Biochem. Sci. 4: 128-131.

Miller, J., Hawkins, D. and Wells, J. (1986) Phorbol diesters alter the contractile response of porcine coronary artery. J. Pharmacol. Exp. Ther. 239, 38-42.

Miller, R. (1987) Multiple calcium channels and neuronal function. Science 235, 46-52.

Minneman, K. P. (1988) α_1-Adrenergic receptor subtypes, inositol phosphates and sources of cell Ca^{2+}. Pharmacol. Rev. 40, 87-119.

Misko, T. P., Moore, W. M., Kasten, T. P., Allen, N. G., Corbett, J. A., Tilton, R. G., McDaniel, M. L., Williamson, J. R. & Currie, M. G. (1993) Selective inhibition of the inducible nitric oxide synthase by aminoguanidine. Europ. J. Pharmacol. 233, 119-125.

Mohsin, S. and Pennefather, J. N. (1979) The sympathetic innervation of the mammalian ovary. A review of pharmacological and histological studies. Clin. Exp. Pharm. Physiol. 6, 335-354.

Moncada, S., Palmer, R. M. & Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. Pharm. Rev. 43, 109-142.

Moncada, S., Radomski, M. W. & Palmer, R. M. (1988) Endothelium-derived relaxing factor: identification as nitric oxide and role in the control of vascular tone and platelet function. Biochem. Pharmacol. 37, 2495-2501.

Moore, P. K., Al-Swayeh, O. A., Chong, N. W. S., Evans, R., Mirzazadeh, S. & Gibson, A. (1989) L-N^G-nitro arginine (NOARG) inhibits endothelium-dependent vasodilatation in the rabbit aorta and perfused rat mesentery. Br. J. Pharmacol. 98, 905P.

Moore, P., Wallace, P., Gaffen, Z., Hart, S. and Babbedge, R. (1993) Characterization of the novel nitric oxide synthase inhibitor 7-nitro indazole and related indazoles: antinociceptive and cardiovascular effects. Br. J. Pharmacol. 110, 219-224.

Morrow, A. L. and Creese, I. (1986) Characterization of α_1-adrenergic receptor subtypes in rat brain: A re-evaluation of [^{3}H] WB 4101 and [^{3}H] prazosin binding. Mol. Pharmacol. 29, 321-330.

Morrow, A. L., Battaglia, G., Norman, A. B. and Creese, I. (1985) Identification of subtypes of [^{3}H] prazosin labeled alpha-1 receptor binding sites in rat brain. Eur. J. Pharmacol. 109, 285-287.

Mulsch, A., Busse, R., Liebau, S. & Forstermann, U. (1988) LY 83583 interferes with the release of endothelium-derived relaxing factor and inhibits soluble guanylate cyclase. J. Pharmacol. Exp. Ther. 247, 283-288.

Munavvar, A. S. and Johns, E. J. (1991) Characteristics of α-adrenoceptors in the rat renal vasculature. Br. J. Pharmacol. 104 (suppl.) 318.

Muramatsu, I., Kigoshi, S. and Ohmura, T. (1991) Subtypes of α_1-adrenoceptors involved in noradrenaline-induced contractions of rat thoracic aorta and dog carotid artery. Jpn. J. Pharmacol. 57, 535-544.

Muramatsu, I., Ohmura, T., Kigoshi, S., Hashimoto, S., and Oshita, M. (1990) Pharmacological subclassification of α_1-adrenoceptors in vascular smooth muscle. Br. J. Pharmacol. 99, 197-201.

Murphy, T. and Bylund, D. (1988) Characterization of alpha-2 adrenergic receptors in the OK cell, an opossum kidney cell line. J. Pharm. Exp. Ther. 244, 571-578.

Nakane, T., Tsujimoto, G., Hashimoto, K. and Chiba, S. (1988) Beta adrenoceptors in the canine large coronary arteries: beta-1 adrenoceptors predominate in vasodilation. J. Pharmacol. Exp. Ther. 245, 936-943.

Nichols, A. J. and Ruffolo, R. (1991) Structure-activity relationships for α-adrenoceptor agonists and antagonists. In. α-Adrenoceptors: molecular biology, biochemistry and pharmacology. ed. Ruffolo, R. R. Prog. Basic Clin. Pharmacol. 8, 75-114. Basel, Karger.

Nichols, A. J. and Ruffolo, R. R. (1988) The relationship of α-adrenoceptor reserve and agonist intrinsic efficacy to calcium utilization in the vasculature. T.I.P.S., 9, 236-241.

Nilsson, H., Jensen, p. E., & Mulvany, M. J. (1994) Minor role for direct adrenoceptor-mediated calcium entry in rat mesenteric smaal arteries. J. Vas. Res. 31, 314-321.

Nishikawa, S. L., Sellers, J., Adelstein, R. and Hidaka, H. (1984) Protein kinase C modulates in vitro phosphorylation of the smooth muscle heavy meromyosin by myosin light chain kinase. J. biol. Chem. 259: 8808-8814.

Nishizuka, Y. (1986) Studies and perspectives of protein kinase C. Science, 233, 305-312.

O'Dell, T. J., Kandel, E. R. and Grant, S. G. (1991) Long-term potentiation in the hippocampus is blocked by tyrosine kinase inhibitors. Nature, 353, 558-560.

Oike, M., Kitamura, K. and Kuriyama, H. (1992) Histamine H_3-receptor activation augments voltage-dependent Ca^{2+} current via GTP hydrolysis in rabbit saphenous artery. J. Physiol. 448, 133-152.

Oriowo, M. A. & Bevan, J. A. (1986) α_1-Adrenoceptor subtype madiates norepinephrine-induced contraction of the rabbit isolated ovarian artery. J. Cardiov. Pharmacol. 8, 858-863.

Oriowo, M. A. & Bevan, J. A. (1990) Chloroethylclonidine unmasks a non α-adrenoceptor noradrenaline binding site in the rat aorta. Eur. J. Pharmacol. 178, 243-246.

Oriowo, M. A. and Ruffolo, R. R. (1992) Heterogeneity of postjunctional α_1-adrenoceptors in mammalian aortae; subclassification based on chloroethylclonidine, WB 4101 and nifedipine. J. Vas. Res. 29: 33-40.

Oriowo, M. A., Bevan, J. A. and Bevan, R. D. (1989) Variation in sensitivity of six cat and six rat arteries to norepinephrine can be related to differences in agonist affinity and receptor reserve. J. Pharmacol. Exp. Ther. 251, 16-20.

Oriowo, M. A., Hieble, J. P. and Ruffolo, R. R. (1991) Evidence for heterogeneity of prejunctional alpha-2 adrenoceptors. Pharmacology, 43, 1-13.

Oriowo, M. A., Nichols, A. J. and Ruffolo, R. R. (1990) A single α-adrenoceptor population coupled to two signal transduction processes may mediate contraction of the rat aorta. FASEB J. 4, A807.

Oriowo, M. A., Nichols, A. J. and Ruffolo, R. R. (1992) Receptor protection studies with phenoxybenzamine indicate that a single α_1-adrenoceptor may be coupled to two signal transduction processes in vascular smooth muscle. Pharmacology, 45, 17-26.

Owen, M. and Patel, C. (1996) α_2-Adrenoceptors mediate vasoconstriction in rabbit interlobular arteries. FASEB J. 10, A71.

Owen, M. and Patel, C. (1994) α_2-Adrenoceptors participate in mediation of norepinephrine-induced constriction in rabbit interlobular arteries. Can. J. Physio. Pharmacol. 72(suppl 1), 152.

Owman, C., Kannisto, P., Liedberg, F and Schmidt, G. (1991) Innervation of the ovary In. Local regulation of ovarian function. ed. N. -O. Sjoberg, L. Hamberger, P. Janson, Ch.

Owman and H. Coelingh Bennink. Ch. 14, pp, 149-169. The Parthenon Publishing group. New Jersey, USA.

Owman, C., Sjoberg, N. O., Wallach, E. E., Walles, B. and Wright, K. H. (1979) Neuromuscular mechanisms of ovulation. In: Human ovulation. Mechanisms, prediction and induction. ed. Hafez, E. S. pp. 57-100, Oxford, Pergamon press.

Palmer, R. M. & Moncada, S. (1989) A novel citrulline--forming enzyme implicated in the formation of nitric oxide by vascular endothelial cells. Biochem. Biophys. Res. Commun. 158, 348-352.

Palmer, R. M. J., Ferrige, A. G. & Moncada, S. (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature 327, 524-526.

Palmer, R. M., Ashton, D. S. & Moncada, S. (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature. 333,664-666.

Park, S. and Rasmussen, H. (1985) Activation of tracheal smooth muscle contraction: synergism between Ca^{2+} and C-kinase activators. Proc. Natl. Acad. Sci. USA, 82, 8835-8839.

Perez, D. M., Piascik, M. T. and Graham, R. M. (1991) Solution-phase library screening for the identification of rare clones; isolation of an α_{1D}-adrenergic receptor cDNA. Molecular pharmacology, 40, 876-883.

Petrash, A. and Bylund, D. (1986) Alpha-2 adrenergic receptor subtypes indicated by [^{3}H] yohimbine binding in human brain. Life Sciences 38, 2129-2137.

Price, D., Schwinn, D., Lomasney, J., Allen, L., Caron, M. and Lefkowitz, R. (1993) Identification, quantification and localization of mRNA for three distinct alpha$_1$ adrenergic receptor subtypes in human prostate. J. Urol. 150, 546-551.

Purdy, R. E., Stupecky, G. L. and Coulombe, R. (1988) Further evidence for a homogenous population of beta-1 adrenoceptors in bovine coronary artery. J. Pharmacol. Exp. Ther. 245, 67-71.

Raddino, R., Manca, C., Poli, E., Bolognesi, R. and Visioli, O. (1986) Effects of 17ß estradiol on the isolated rabbit heart. Arch. Int. Pharmacodyn. Ther. 281, 57-65.

Radomski, M. W., Palmer, R. M. & Moncada, S. (1990) Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. Proc. Natl. Acad. Sci. USA. 87, 10043-10047.

Radomski, M., Palmer, R. M. & Moncada, S. (1987a) Comparative pharmacology of endothelium-derived relaxing factor, nitric oxide and prostacyclin in platelets. Br. J. Pharmacol. 92, 181-187.

Radomski, M., Palmer, R. M. & Moncada, S. (1987b) The anti-aggregating properties of vascular endothelium: interactions between prostacyclin and nitric oxide. Br. J. Pharmacol. 92, 639-646.

Rahimian, R., van Breemen, C., Rusco, J., Li, L., Bolzen, B., Dube, G. and Laher, I. (1996) Greater stimulated and basal release of nitric oxide in the presence of estradiol. FASEB J. 10, A706.

Rapoport, R. & Murad, F. (1983) Endothelium-dependent and nitrovasodilator-induced relaxation of vascular smooth muscle: role of cyclic GMP. J. Cyclic Nucleo. Protein Phosphor. Res. 9, 281-296.

Rasmussen, H., Forder, J., Kajima, I. and Scriabine, A. (1984) TPA-induced contraction of isolated rabbit vascular smooth muscle. Biochem. Biophys. Res. Commun. 122: 776-784.

Rasmussen, H., Takuwa, Y. and Park, S. (1987) Protein kinase C in the regulation of smooth muscle contraction. FASEB J. 1, 177-185.

Rees, D. D., Palmer, R. M., Hodson, H. F. & Moncada, S. (1989) A specific inhibitor of nitric oxide formation from L-arginine attenuates endothelium-dependent relaxation. Br. J. Pharmacol. 96, 418-424.

Rees, D. D., Palmer, R. M., Schulz, R., Hodson, H. F. & Moncada, S. (1990a) Characterization of three inhibitors of endothelial nitric oxide synthase in vitro and in vivo. Br. J. Pharmacol. 101, 746-752.

Rees, D. D., Schulz, R., Hodson, H. F., Palmer, R. M. & Moncada, S. (1990b) Identification of some novel inhibitors of the vascular nitric oxide synthase in vivo and in vitro. In. Nitric oxide from L-arginine: a bioregulatory system, eds. Moncada, S. & Higgs, E. A. pp, 485-487, Elsevier, Amsterdam.

Reynolds, S. R. (1950) The vasculature of the ovary and ovarian function. Recent Progr. Horm. Res. 5, 65-100.

Rhoades, R. A. and Tanner, G. A. (1995) Medical physiology. fist ed. pp. 756-770, Little Brown Company, Boston.

Rosengren, E. and Sjoberg, N. O. (1967) The adrenergic nerve supply to the female reproductive tract of the cat. Am. J. Anat. 121, 271-284.

Rubanyi, G. M. & Vanhoutte, P. M. (1985) Hypoxia releases a vasoconstrictor substance from the canine vascular endothelium. J. Physiol. 364, 45-56.

Ruffolo, R. R. (1990) α_2-Adrenoceptor agonists and antagonists. Neurotrans., VI, 1-8.

Ruffolo, R. R. and Hieble, J. P. (1994) α-Adrenoceptors. Pharmac. Ther. 61: 1-64.

Ruffolo, R. R.; Nichols, A. J.; Stadel, J. M. and Hieble, J. P. (1991) Structure and function of α-adrenoceptors. Pharmacol. Rev. 43, 475-505.

Ruffolo, R., Morgan, E. and Messick, K. (1984) Possible relationship between receptor reserve and the differential antagonism of alpha-1 and alpha-2 adrenoceptor-mediated pressor responses by calcium channel antagonists in the pithed rat. J. Pharm. Exp. Ther. 230, 587-594.

Ruffolo, R., Patil, P. and Miller, D. (1983) Adrenoceptor-mediated effects of optically active catecholimidazolines in pithed rat. Naunyn-Schmiedeberg's Arch Pharmacol. 323, 221-227.

Ruffolo, R., Sulpizio, A., Nichols, A., DeMarinis, R. and Hieble, P. (1987) Pharmacologic differentiation between pre- and postjunctional α_2-adrenoceptors by SK&F 104078. Naunyn-Schmiedeberg's Arch Pharmacol. 336, 415-418.

Salmon, D. M. and Honeyman, T. W. (1980) Proposed mechanism of cholinergic action in smooth muscle. Nature, 284: 344-345.

Saussy, D., Goetz, A., King, H. and True, T. (1994) BMY 7378 is a selective antagonist of α_{1D}-adrenoceptors: further evidence that vascular α_1-adrenoceptors are of the α_{1D}-subtype. Can. J. Physiol. Pharmacol. 72, (suppl 1), 323.

Schmidt, G., Owman, C., Sjoberg, N. and Walles, B. (1985) Influence of adrenoceptor agonists and antagonists on ovulation in the rabbit ovary perfused in vitro. J. Auton. Pharmacol. 5, 241-250.

Schmidt, H. H., Pollock, J. S., Nakane, M., Gorsky, L. D., Forstermann, U. & Murad, F. (1991) Purification of a soluble isoform of guanylyl cyclase-activating-factor synthase. Proc. Natl. Acad. Sci. USA, 88, 365-369.

Schmitz, J. M., Graham, R. M. Sagalowsky, A. and Pettinger, W. A. (1981) Renal alpha-1 and alpha-2 adrenergic receptors: biochemical and pharmacological correlations. J. Pharmacol. Exp. Ther. 219, 400-406.

Schumann, H.-J. and Lues, I. (1983) Postjunctional α-adrenoceptors in the isolated saphenous vein of the rabbit. Characterization and influence of angiotensin. Naunyn-Schmiedeberg's Arch Pharmacol. 323, 328-334.

Schwinn, D. A., Lomasney, J. W., Lorenz, W., Szkult, P. J., Fremeau, R. T., Yang-Feng, T. L., Caron, M. G., Lefkowitz, R. J. and Cotecchia, s. (1990) Molecular cloning and expression of the cDNA for a novel α_1-adrenergic receptor subtype. J. Biol. Chem. 265, 8183-8189.

Schwinn, D. A., Page, S. O., Middleton, J. P., Lorenz, W., Liogett, S. B., Yamamoto, K., Lapefine, E. G., Caron, M. G., Lefkowitz, R. J. and Cotecchia, S. (1991) The α_{1c}-adrenergic receptor: characterization of signal transduction pathways and mammalian tissue heterogeneity. Molecular pharmacology, 40, 619-626.

Shima, H and Blaustein, M. (1992) Modulation of evoked contractions in rat arteries by ryanodine, thapsigargin, and cyclopiazonic acid. Circ. Res. 70, 968-977.

Skarby, T. V. C., Anderson, K. E. & Edvinsson, L. (1983) Pharmacological characterization of postjuctional α-adrenoceptors in isolated feline cerebral and peripheral arteries. Acta. Phsiol. Scand. 117, 63-73.

Sperti, G. and Colucci, W. (1987) Phorbol ester-stimulated bidirectional transmembrane calcium flux in A7r5 vascular smooth muscle cells. Mol. Pharmacol. 32, 37-42.

Starke, K. Montel, H. and Schumann, H. J. (1971a) Influence of cocaine and phenoxybenzamine on noradrenaline uptake and release. Naunyn-Schmeideberg's Arch. Pharmacol. 270, 210-214.

Starke, K. Montel, H. and Wagner, J. (1971b) Effect of phentolamine on noradrenaline uptake and release. Naunyn-Schmeideberg's Arch. Pharmacol. 271, 181-192.

Stice, S. L., Ford, S. P., Rosazza, J. P. and Van Orden, D. E. (1987) Interaction of 4 hydroxylated estradiol and potential-sensitive Ca^{2+} channels in altering uterine blood flow during estrous cycle and early pregnancy in gilts. Biol. Reprod. 36, 369-375.

Sulpizio, A. & Hieble, J. P. (1987) Demonstration of α_2-adrenoceptors-mediated contraction in the isolated canine saphenous artery with Bay K 8644. Eur. J. Pharmacol. 135, 107-110.

Sunano, S., Osugi, S. and Shimamura, K. (1989) Blood pressure and impairment of endothelium-dependent relaxation in SHR. Experientia, 45, 705-708.

Sutko, J. L., Ito, K. and Kenyon, J. L. (1985) Ryanodine: a modifier of sarcoplasmic reticulum calcium release in striated muscle. fed. Proc. 44, 2984-2988.

Taylor, S. G. & Weston, A. H. (1988) Endothelium derived hyperpolarizing factor: an endogenous inhibitor released from the vascular endothelium. TIPS. 9, 272-274.

Templeton, A. G. B., MacMillan, J., McGrath, J. C., Storey, N. D. & Wilson, V. G. (1989) Evidence for prazosin-resistant, rauwolscine-sensitive α-adrenoceptors mediating contractions in the isolated vascular bed of the rat tail. Br. J. Pharmacol. 97, 563-571.

Tesfamariam, B. and Halpern, W. (1987) Modulation of adrenergic reponses in pressurized resistance arteries by flow. Am. J. Physiol. 253, H1112-H1119.

Testa, R., Taddei, C., Poggesi, E., Destefani, C., Cotecchia, S., Hieble, J., Naselsky, D., Bergsma, D., Swift, A., Ganguly, S. and Leonardi, A. (1995) Rec 15/2739 (SB 216469) A novel prostate selective α_1-adrenoceptor antagonist. Pharmacol. Communic. 6, 79-86.

Thulesius, O., Ugaily-Thulesius, L., Neglen, P. and Shuhaiber, P. (1988) The role of endothelium in the control of venous tone: studies on isolated human veins. Clin. Physiol. 8, 359-366.

Timmermans, P., Kwa, H. and Van Zwieten, P. (1979) Possible subdivision of postsynaptic α-adrenoceptors mediating pressor responses in the pithed rat. Naunyn-Schmeideberg's Arch. Pharmacol. 310, 189-193.

Toma, C., Jensen, P. E., Prieto, D., Hughes, A. D. and Aalkjaer, C. (1995) Effects of tyrosine kinase inhibitors on the contractility of rat mesenteric resistance arteries. Br. J. Pharmacol. 114, 1266-1272.

Tyson, C. A., Zande, H. V. and Green, D. E. (1976) Phospholipids as ionophores. J. biol. Chem. 251: 1326-1332.

Uchida, E., Bohr, D., and Hoobler, S. (1967) A method for studying isolated resistance vessels from rabbit mesentery and brain and their responses to drugs. Circ. Res. vol. XXI, 525-536.

Ullrich, A. and Schlessinger, J. (1990) Signal transduction by receptors with tyrosine kinase activity. Cell, 61, 203-212.

Ushiro, H. and Cohen, S. (1980) Identification of phosphotyrosine as a product of epidermal growth factor-activated protein kinase in A-431 cell membranes. J. Biol. Chem. 255, 8363-8365.

Van Breemen, C., Cauvin, C., Johns, A., Leijten, P. and Yamomoto, H. (1986) Ca^{2+} regulation of vascular smooth muscle. Fed. Proc. 45, 2746-2751.

van Meel, J. G. , de Jonge, A., Kalkman, H. O., Wilffert, B., Timmermans, P. B. M. and van Zwieten, P. A. (1981) Organic and inorganic antagonists reduce vasoconstriction in vivo mediated by postsynaptic α_2-adrenoceptors. Naunyn-Schmiedeberg's Arch Pharmacol. 316: 288-293.

Vanhoutte, P. M. (1987) Endothelium-dependent contractions in arteries and veins. Bld. vessels. 24,141-144.

Vanhoutte, P. M. (1988) Endothelium-dependent contractions in veins and arteries. In Relaxing and contracting factors, ed. Vanhoutte, P. M. pp, 27-39, Humana Press, New York.

Vanhoutte, P. M., Luscher, T. and Graser, T. (1991) Endothelium-dependent contractions. Blood Vessels, 28, 74-83.

Varga, B., Zsolnai, B. and Bernard, A. (1979) Stimulation of the alpha and beta-adrenergic receptors in human ovarian vasculature in vitro. Gynecol. Obstet. Invest. 10, 81-87.

Vargas, R., Delaney, M., Farhat, M., Wolfe, R., Rego, A. and Ramwell, P. (1995) Effect of estradiol 17ß on pressor responses of rat mesenteric bed to noradrenaline, K^+, and U-46619. J. Cardiov. Pharmac. 25, 200-206.

Vargas, R., Thomas, G., Worblewska, B. and Ramwell, P. (1989) Differential effects of 17α and 17ß estradiol on $PGF2_{\alpha}$ mediated contraction of the porcine coronary artery. Adv. Prostaglandin Thromboxane Leukotriene Res. 19, 227-280.

Villalobos, M. R., Horng, E. and Garcia-Sainz, J. A. (1982) Correlation between phosphoinositol labelling and contraction in rabbit aorta, effect of α_1-adrenergic activation. J. Pharmac. exp. Ther. 222: 258-261.

Virutamasen, P., Hickok, R. L. and Wallach, E. E. (1971) Local ovarian effects of catecholamines on human chorionic gonadotrophin-induced ovulation in the rabbit. Fertil. Steril. 22, 235-43.

Wallace, M. R. (1971) Oral contraceptives and severe hypertension. Aust. N.Z. J. Med. 1, 49-52.

Wallach, E. E., Wright, K. H. and Hamada, Y. (1978) Investigation of mammalian ovulation with an in vitro perfused rabbit ovary preparation. Am. J. Obstet. Gynecol. 132, 728-738.

Walles, B., Edvinsson, L., Owman, C., Sjoberg, N. O. and Swensson, K. G. (1975) Mechanical response in the wall of ovarian follicles mediated by adrenergic receptors. J. Pharmacol. Exp. Ther. 193, 460-473.

Waterfall, J., Rhodes, K. and Lattimer, N. (1985) Studies of α_2-adrenoceptor antagonist potency in vitro: comparisons in tissues from rats, rabbits, dogs and humans. Clin. Sci. 68(suppl 10), 21S-24S.

Watson, S. and Girdlestone, D. (1996) Receptor and ion channel nomenclature supplement. TIPS, 7th ed., p.11.

Wilson, K. M. and Minneman, K. P. (1990) Pertusis toxin inhibits norepinephrine-stimulated inositol phosphate formation in primary brain cell cultures. Mol. Pharmacol. 38, 274-281.

Wingerd, B. D. (1985) Rabbit dissection manual, The Johns Hopkins University Press, London, pp. 65-70.

Wolff, D. W., Gesek, F. A. and Strandhoy, J. W. (1987) In vivo assessment of rat renal vascular alpha adrenoceptors. J. Pharmacol. Exp. Ther. 241, 472-476.

Wurtman, R. J. (1964) An effect of luteinizing hormone on the fractional perfusion of the rat ovary. Endocrinology; 75, 927-933.

Wurtman, R. J. (1973) Biogenic amines and endocrine function. Introduction: neuroendocrine transducers and monoamines. Fed. Proc. 32, 1769-1771.

Yamada, S., Kashiwabara, T., Yamazawa, T., Harada, Y. and Nakayama, K. (1988) Demonstration of β_1-adrenoceptor mediating relaxation of porcine coronary artery by radioligand binding and pharmacological methods. Life Sci. 43, 1999-2006.

Yui, Y., Kaoru, M., Sase, K., Kawamoto, T., Toda, K., Doi, Y., Ogoshi, S., Hattori, R., Aoyama, T., Yamamoto, Y., Hashimoto, K., Yang, R., Kawai, C., Sasayama, S. and Shizuta, Y. (1994) Molecular biology of nitric oxide synthase and structure of endothelial nitric oxide synthase gene. In. Endothelium-derived factors and vascular functions. ed. T. Masaki. pp. 21-27, Elsevier Science B. V. Amsterdam.

Zachariae, F. (1958) Studies on the mechanism of ovulation permeability of the blood-liquor barrier. Acta. Endocrin. 27, 339-342.

List of publications

1. Yousif, M. H., Williams, K. I and Oriowo, M. A. (1996) Characterization of α-adrenoceptor subtype(s) mediating vasoconstriction in the perfused rabbit ovarian vascular bed. FASEB J. 10, A424.

2. Yousif, M. H., Williams, K. I and Oriowo, M. A. (1996) Characterization of α-adrenoceptor subtype(s) mediating vasoconstriction in the perfused rabbit ovarian vascular bed. J. Aut. Pharmac. (Revised).

3. Yousif, M. H., Oriowo, M. A. and Williams, K. I (1996) Role of tyrosine kinase in NA-induced vasoconstriction in the perfused rabbit ovarian vascular bed. BPS, Dec. (In preparation).