ADRENOCEPTOR MEDIATED INHIBITION IN THE RAT UTERUS

A thesis presented for the degree of

Doctor of Philosophy

in the University of Glasgow

by

Sunday Edet Ohia B.Sc., M.Sc. (Ibadan)

Department of Pharmacology,
University of Glasgow,
Glasgow.

February, 1986

TABLE OF CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

PLATE

ACKNOWLEDGEMENTS

I am greatly indebted to a number of well-meaning people who made the completion of this project possible. First of all, thanks to my professorial supervisor, J.S. Gillespie for the timely criticisms, and also for giving me the opportunity to work in this prestigious Pharmacology Department. I am equally grateful to my direct supervisor, Dr Frances Boyle for initiating this project, providing the excellent laboratory facilities and above all, for the stimulating academic discussions that prevailed during the last three years.

Other members of the academic staff were also helpful: Dr D. Pollock, for the expert advice he gave me during the radioactive calcium flux experiments, and Dr E. MacIntyre for the invaluable gifts of flurbiprofen and BW 755C. All members of the technical staff were exceedingly nice to me in both personal and material terms. I thank Mr Robert Auld and Miss Julie Gardner for the technical assistance during the radioimmunoassay studies. I also acknowledge the overall interest shown in this work by my colleagues, past and present which include: Drs Bayo Laniyonu, Ade Ajayi, Khamis Dawood and Siew Lim.

I am very grateful to members of my entire family for their unreserved demonstration of support, patience and understanding throughout my stay in Glasgow. My profound gratitude to my wife, Ekanem for all she has done to make the execution and completion of this thesis

possible. I will never forget her initial encouragements which remained the main source of inspiration throughout the course of this project.

I will seize this opportunity to thank Mrs Lorna Peedle of the Anatomy Department, for the excellent secretarial assistance. Finally, I hereby acknowledge the partial financial assistance from the Overseas Research Students Award scheme and the University of Glasgow Scholarship funds.

PUBLICATIONS

Some of the work presented in this thesis has been previously published:

(a) BOYLE, F.C. & OHIA, S.E. (1984)
The effect of uptake mechanisms and alpha receptor activity on rat uterine response to catecholamines.
Abstract of the 9th IUPHAR Congress, 389P.

(b) BOYLE, F.C. & OHIA, S.E. (1985)
Changes in catecholamine inhibitory effects in the rat isolated uterus induced by ovariectomy and cyclo-oxygenase inhibition.
Br. J. Pharmac. 84, 42P.

(c) BOYLE, F.C. & OHIA, S.E. (1985)
Specificity of enhancement of salbutamol responses by cyclo-oxygenase inhibition in the rat isolated uterus.
Br. J. Pharmac. 86, 648P.

SUMMARY

1. The rat uterus varies in its response to adrenoceptor agonists throughout the oestrous cycle, and the mechanisms underlying this variation have been investigated using pharmacological and biochemical techniques.
2. Acetylcholine (ACh) and potassium chloride (KCl) were used to raise tone in isolated uterine horn preparations. Both motor agents produced the highest tension in oestrus and this effect may reflect changes in thickness of the myometrium during the oestrous cycle.
3. The three adrenoceptor agonists studied, noradrenaline (NA), adrenaline (ADR) and salbutamol (SAL) produced inhibitory responses throughout the oestrous cycle. However, the maximum degree of inhibition achieved by each agonist varied in the four phases.
4. Stimulation of α-excitatory adrenoceptors contributed to the variation seen with NA and ADR (which possess α-adrenoceptor activity), but could not explain that of SAL, a selective β_2-adrenoceptor agonist.
5. Blockade of agonist uptake processes into neuronal and extraneuronal sites, enhanced the inhibitory responses produced by NA, ADR and SAL, indicating a role for the removal mechanisms in the observed variation in response.
6. The variation in agonists responses persisted even when the contribution of both α-adrenoceptors and the uptake processes were controlled, suggesting that other factors could be involved.

7. Ovarian hormones appeared to play a major role in the variation since responses to NA, ADR and SAL were enhanced in uteri from ovariectomized rats.
8. Intramural prostaglandin generation was involved in the responses to the adrenoceptor agonists, since cyclo-oxygenase inhibition, i.e., block of prostaglandin formation, enhanced the inhibition produced by NA, ADR and SAL. The effect was specific to the adrenoceptor agonists because the inhibitory responses to histamine and papaverine were unaffected by cyclo-oxygenase inhibition.
9. The leukotrienes are not involved in the adrenoceptor agonists responses, since inhibition of both cyclo-oxygenase and lipoxygenase produced effects similar to blocking the cyclo-oxygenase pathway alone.
10. Biochemical measurements of uterine adenosine 3',5' cyclic monophosphate (cAMP) were made throughout the oestrous cycle. Basal cAMP levels were similar in proestrus, oestrus, metoestrus and dioestrus.
11. cAMP did not appear to be involved in the variation in uterine response to adrenoceptor agonists because SAL-induced increases in cAMP levels were similar in all four phases of the oestrous cycle. Intramurally generated prostaglandins have no effect on cAMP formation, since cyclo-oxygenase inhibition was without effect on cAMP levels.

12. Calcium movements were studied in uteri from both intact and ovariectomized rats using radioactive calcium ($^{45}Ca^{2+}$). $^{45}Ca^{2+}$ influx and efflux varied in the different hormonal conditions of the oestrous cycle, and in the absence of the hormones, following ovariectomy.

13. SAL had no effect on $^{45}Ca^{2+}$ influx and efflux, indicating that the adrenoceptor agonists inhibitory responses in the rat uterus could involve an increased intracellular binding of calcium.

14. The variation in uterine response to the adrenoceptor agonists during the oestrous cycle is due, therefore, to the combined effects of α-adrenoceptor activity, agonist removal processes, intramurally generated prostaglandins and the ovarian hormones. Of these, the factors of major importance are the prostaglandins and the ovarian hormones.

ABBREVIATIONS

ACh	Acetylcholine
ADR	Adrenaline
AZA	Azapetine
BW 755C	3-amino-1-(m-trifluoromethyl)-phenyl-2-pyrazolone
$^{45}Ca^{2+}$	Radioactive calcium
cAMP	Adenosine 3'5' cyclic monophosphate
DMI	Desmethylimipramine
EC_{50}	Concentration producing 50% of the maximum response
EDTA	Ethylene diamine tetra-acetic acid
FBF	Flurbiprofen
HIS	Histamine
NA	Noradrenaline
PAP	Papaverine
pD_2	Negative logarithm to the base 10 of the EC_{50}
SAL	Salbutamol
TCA	Trichloroacetic acid.

INTRODUCTION

RAT UTERUS

A. ANATOMICAL AND PHYSIOLOGICAL CONSIDERATIONS

Rats possess a bicornate uterus with the two horns maintaining separate openings into the vagina. The uterus consists of an outer myometrial layer which is lined by an inner glandular mucosa called the endometrium. The myometrial layer contains an outer longitudinal and an inner circular muscle layer, separated by a well defined band of connective tissue (Bengtsson, 1982). The cyclical production of oestrogen and progesterone by the ovaries results in an oestrous cycle lasting between four to five days (Hogarth, 1978). Yoshinaga, Hawkins and Stocker (1969) showed that the oestrogen secretion rate began to rise in late dioestrus to a maximum in proestrus. The rate remained high during oestrus, and then fell to its lowest level by the end of this phase. On the other hand, the progesterone secretion rate was low at the end of oestrus, but rose in metoestrus until it reached a maximum in proestrus (Butcher, Collins & Fugo, 1974; Brenner & West, 1975).

Both adrenergic and cholinergic autonomic neurones supply the rat uterus. Adham and Schenk (1969) demonstrated that cyclical changes occurred in the density of autonomic innervation during the oestrous cycle. Both adrenergic and cholinergic nerves were more numerous in oestrus than in dioestrus, while an intermediate number of these nerves were

present in proestrus and metoestrus. The adrenergic component of the autonomic innervation in the rat uterus has been well characterized using histochemical, biochemical and morphological techniques (Silva, 1966; Sjöberg, 1967). In an ultrastructural study, Silva (1966) showed that the density of uterine adrenergic innervation in the rat was low, when compared to other mammals. Silva (1966) found most of the nerve bundles to be associated with blood vessels although some were present in the myometrium. In both rats and humans, regional differences in the density of uterine adrenergic innervation have been shown by Owman, Rosengren and Sjöberg (1967), and Adham and Schenk (1969). In these species, there was an increasing density of adrenergic innervation from the ovarian to the cervical end of the uterus. However Norberg and Fredricsson (1966) found adrenergic nerves only in the region nearest to the tubo - uterine junction.

The blood vessels of the uterus are supplied by long adrenergic neurones, while the myometrial cells are innervated by short adrenergic neurones with ganglia located in or near the uterus.(Sjöberg 1967). The long and short adrenergic neurons have been shown to differ functionally in that with reserpine treatment, noradrenaline disappeared more slowly, but returned earlier in the latter than in the former (Owman & Sjöberg, 1967). Changes in the noradrenaline content of the short neurones have also been observed following oestrogen and progesterone treatment, and during pregnancy (Hervonen, Kanerva & Lietzen, 1973).

Of the species examined, the rat uterus has the lowest noradrenaline content, which correlates well with the sparse adrenergic innervation (Silva, 1966; Marshall, 1970). Sporrong, Clase, Owman and Sjöberg (1977) suggested that sensitivity of the short neurones to sex steroids may be the basis for the disappearance of noradrenaline selectively from these nerves during pregnancy. The ovarian hormones have been shown to affect not only the transmitter content of uterine adrenergic nerves, but also its turnover and overall neuronal activity (Marshall, 1981).

Variations in oestrogen and progesterone levels during the oestrous cycle can alter the uterine catecholamine content in neurones, and possibly in extraneuronal sites. Rudzik and Miller (1962) were the first to show that uterine adrenaline stores were subject to periodic fluctuations in the four phases. Although the amount of adrenaline determined biochemically in oestrus was twice that obtained in dioestrus, no such changes in uterine noradrenaline was observed. Oestrogen treatment was also found to increase adrenaline, but not noradrenaline content in the rat uterus (Rudzik & Miller, 1962; Spratto & Miller, 1968 a,b). Further studies by Falck, Gardmark, Nybell, Owman, Rosengren and Sjöberg (1974) revealed that neither ovariectomy nor oestrogen treatment altered noradrenaline content in the rat uterus. Ovarian hormonal influence on uterine adrenaline or noradrenaline content has also been demonstrated in humans (Dujovne, De Laborde, Carril, Cheviakoff, Pedroza & Rosner, 1976).

With reference to the catecholamine content, the rat uterus can accumulate or bind catecholamines as shown with radiolabelled compounds injected systemically (Wurtman, Chu & Axelrod, 1963; Green & Miller, 1966a). Wurtman et al. (1963) found that during the oestrous cycle, uteri in oestrus bound more ^{3}H-adrenaline than those in dioestrus while the binding of ^{3}H-noradrenaline was actually decreased in oestrus. Oestrogen treatment doubled ^{3}H-adrenaline uptake in uteri from ovariectomized animals. Since phenylethanolamine-N-methyl transferase was absent in the rat uterus Wurtman, Axelrod and Kopin (1963) suggested that the ^{3}H-adrenaline must have been taken up from the circulation rather than synthesized in the uterus. To investigate this possibility, Green and Miller (1966b) measured plasma concentrations of adrenaline and noradrenaline, and found that the former increased while the latter decreased during oestrus. Thus, cyclic variations in uterine adrenaline resulted from differences in the ratio of adrenaline to noradrenaline in the plasma, and in the altered ability of the uterus to bind these amines.

Marshall (1970) suggested that adrenaline binding and storage sites in the rat uterus may be different from those for noradrenaline on the basis of the following observations: (a) adrenaline, but not noradrenaline was shown to be subjected to periodic fluctuations during the oestrous cycle and pregnancy (Wurtman, Chu & Axelrod, 1963); (b) immunosympathectomy reduced noradrenaline, but not the

adrenaline content (Klingman, 1965); (c) cocaine prevented the uptake of noradrenaline, but not adrenaline (Spratto & Miller, 1968a). Presumably, noradrenaline is stored in the adrenergic neurones, but the adrenaline storage site is unknown. The myometrial cells may serve as one of the adrenaline storage sites (Marshall, 1970).

Despite the above evidence, the physiological significance of oestrogen effects on uterine catecholamine content or accumulation is yet to be determined. Indeed, Spaziani (1975) suggested that there was no convincing evidence that the rat uterus required catecholamines or even a nervous system for normal function on the basis of the following reports: (a) denervation of the uterus (resulting in depletion of 80% of its noradrenaline content) did not interfere with changes during the oestrous cycle, conception and pregnancy (Barnea & Shelesnyak, 1965); (b) total depletion of uterine noradrenaline content with 6-hydroxydopamine did not interfere with the ability of oestrogen to increase uterine blood flow (Brody, Edvinsson & Sjöberg, 1975).

As well as changes in uterine catecholamine content, morphological changes have also been demonstrated in the myometrium during the oestrous cycle and pregnancy (Silva, 1966; Spratto & Miller, 1968a; Digges, 1980). During the oestrous cycle, the myometrium is thinner in dioestrus than in oestrus. Naftalin, Phear and Goldberg (1973)

suggested that these morphological changes may underlie the variation in mechanical tension developed in uteri from different hormonal conditions. The increased tension developed by the rat uterus during pregnancy may be due to the formation of cell to cell contacts or gap junctions (Garfield, Kannan & Daniel, 1980). These specialized types of contacts may serve to couple cells together and allow the synchronized muscle contractility of labour (Mackenzie, Puri and Garfield, 1983).

In summary, both anatomical and physiological changes occur in the rat uterus as a consequence of the cyclical production of oestrogen and progesterone during the oestrous cycle and pregnancy. The mechanism responsible for these changes are however, not well understood.

B. RAT UTERINE ADRENOCEPTORS

Ahlquist (1948, 1962) classified mammalian uterine adrenoceptors into α-excitatory and β-inhibitory receptors but only the latter type was deemed to be present in the rat. The classification of rat uterine adrenoceptors is, however controversial since evidence from literature reveals that there may be more than one adrenoceptor subtype in this tissue.

1. Evidence for the occurrence of β-adrenoceptors

In support of Ahlquist's (1962) classification of rat uterine adrenoceptors, Levy and Tozzi (1962) showed that β-receptor antagonists, dichloroisoprenaline and pronethalol blocked catecholamine inhibitory responses in this preparation. Using the radioligand binding technique, Krall, Mori, Tuck, Le Shon and Korenman (1978) and Digges (1980) confirmed the presence of β-adrenoceptors in the uterus throughout the oestrous cycle. Indeed β-inhibitory adrenoceptors are similar in both depolarized (Schild, 1966 1967; Marshall & Kroeger, 1973) and non-depolarized (Diamond & Brody, 1966; Miller, 1967; Chow & Marshall, 1981; Boyle & Digges, 1982a,b; Acritopoulou-Fourcroy, Clabaut & Schrub, 1985) rat uterus.

Lands, Ludena and Buzzo (1967) subclassified β-adrenoceptors into β_1 and β_2 with the rat uterus possessing mainly the β_2-subtype (Olsson & Persson, 1971; Levy & Apperley, 1978). However, Richardson and Nahorski (1978) using the radioligand binding method, demonstrated the presence of both β_1- and β_2-adrenoceptors in oestrogen treated, but not in progesterone treated rat uteri. In oestrogen dominated rat uterus, the ratio of β_1-:β_2-receptors was 20%:80% while in progesterone dominated tissues, all the receptors are of the β_2-type (Nahorski, 1981).

In summary, the occurrence of β-inhibitory adrenoceptors in the uterus from both non-pregnant and pregnant rats has been generally accepted.

2. Evidence for the occurrence of α-adrenoceptors

In 1949, Mann reported that both noradrenaline and adrenaline produced excitatory effects in uteri from the oestrus phase, and in those from ovariectomized rats treated with oestrogen. Similar catecholamine-induced stimulant action was demonstrated in the oestrogen dominated rat uterus by Brooks, Schaeppi and Pincus (1965) and by Diamond and Brody (1966). Tothill (1967) showed that *in vitro* treatment of the rat uterus with isoprenaline reversed the inhibitory effects produced by catecholamines to a motor response. The motor response was blocked by α-adrenoceptor and 5-hydroxytryptamine receptor antagonists. Tothill (1967) then proposed the existence of an excitatory receptor designated the "E-receptor" since both α- and 5-hydroxytryptamine receptors appeared to be involved in the response. However, Paton (1968) failed to demonstrate the presence of the "E-receptors" but found that both the catecholamines and 5-hydroxytryptamine acted on separate excitatory receptors in the rat uterus.

The presence of distinct uterine α-excitatory receptors in animals during oestrus has since been confirmed by other workers (Butterworth & Randall, 1970; Abdel-Aziz & Bakry, 1973; Butterworth & Jarman, 1974; Boyle & Digges, 1982a). The α-adrenoceptors appeared to be more temperature sensitive than the β-inhibitory receptors (Butterworth & Jarman, 1974). In the myometrium, the existence of α-excitatory

receptors has been demonstrated in the circular, but not in the longitudinal muscle layer, in both non-pregnant and pregnant animals (Kawarabayashi & Osa, 1976; Chow & Marshall, 1981; Kishikawa, 1981). In general, evidence in favour of the presence of α-excitatory receptors in the rat uterus is consistent, and these receptors may be associated with conditions of oestrogen dominance. However, there are some reports that the α-adrenoceptor population in this tissue may subserve an inhibitory function.

Wiqvist (1959) found that both dihyroergotamine and phentolamine blocked adrenaline inhibitory action in the rat isolated uterus. Jensen and Vennerod (1961) also showed that tolazoline antagonized adrenaline inhibitory effects on 5-hydroxytryptamine-induced contraction in this tissue suggesting that the α-adrenoceptors subserved relaxation. As a result of these reports, Rudzik and Miller (1962) investigated the occurrence of α-inhibitory adrenoceptors in the rat uterus. Adrenaline, noradrenaline and phenylephrine produced inhibitory effects which were antagonized by phentolamine. Although phenylephrine and isoprenaline are known to be specific for α- and β-adrenoceptors, respectively, they both produced an inhibitory response. Thus, Rudzik and Miller (1962) suggested that both uterine α- and β-adrenoceptors may be involved in the catecholamine-induced inhibitory effects.

However, the concept of α-inhibitory adrenoceptors in the rat uterus is equivocal since other workers using the

same adrenoceptor antagonists failed to demonstrate their blocking effects on catecholamine inhibitory responses. Indeed, some workers have reported an enhanced adrenaline inhibition after α-receptor blockade (Brooks et al., 1965). The antagonists used in the studies that favoured the presence of α-inhibitory adrenoceptors (Wiqvist, 1959; Jensen & Vennerod, 1961; Rudzik & Miller, 1962) have since been shown to produce non-specific excitatory effects on uterine smooth muscle (Tothill, 1967; Paton, 1968). This non-specific action may be responsible for the observed blockade of catecholamine inhibitory responses in the rat uterus.

In summary, it appears that rat uterine α-adrenoceptors subserved an excitatory rather than an inhibitory function. The presence of α-adrenoceptors in the uterus has been confirmed by Krall et al. (1978) using the radioligand binding technique. The existence of α-adrenoceptors in the rat uterus does not conform with Ahlquist's (1962) classification of adrenoceptors in this tissue. However, the above evidence strongly supports the view that the rat uterus possessed both α- and β-adrenoceptors, and is thus similar to uteri from other mammals.

C. FACTORS INFLUENCING ADRENOCEPTOR AGONIST RESPONSES IN THE RAT UTERUS

A number of factors can alter adrenoceptor mediated responses in the uterus by actions involving receptors, membrane permeability to ions and intracellular processes. These factors are: (1) ovarian hormones, (2) catecholamine uptake processes, (3) cyclic nucleotides, (4) ions, and (5) prostaglandins.

1. OVARIAN HORMONES

The ability of the ovarian hormones, oestrogen and progesterone to modify uterine responses to catecholamines has been demonstrated in some species including the cat (Tsai & Fleming, 1964) and rabbit (Willems & De Schaepdryver, 1966). In rats, Diamond and Brody (1966) reported that ovarian hormones may alter uterine responses to catecholamines by an action on the adrenoceptors. They found that an oestrogen treated muscle was excited by the amines (an α-adrenoceptor effect), while a progesterone treated one was inhibited (a β-adrenoceptor effect). Thus, they suggested that the response of the rat uterus to catecholamines could depend on the balance between α- and β-adrenoceptor activities, which in turn was regulated by the ovarian hormones. Krall *et al.* (1978) confirmed these observations using the radioligand binding method. They found that the rank order of affinity of the catecholamines for displacement

of [^{3}H] -dihydroalprenolol binding (isoprenaline > adrenaline > noradrenaline) in the four phases of the oestrous cycle was similar to that for inhibition of myometrial motility. During the oestrous cycle, myometrial α-adrenoceptor content was increased in proestrus and oestrus while β-adrenoceptor content was raised only in proestrus. Krall et al. (1978) suggested therefore, that changing receptor number might be one way through which the hormones regulated target organ function.

Oestrogen and progesterone may also produce direct effects on myometrial motility. In vitro treatment of the myometrium with the hormones has been shown to inhibit both its electrical and mechanical activity in humans (Mossman & Conrad, 1967) and rats (Saldivar & Melton, 1966; Pharriss & Russell, 1968). The mechanism of this inhibitory effect is unclear, and its implication for the response of the rat uterus to catecholamines remains to be determined.

2. CATECHOLAMINE UPTAKE PROCESSES

The existence of mechanisms whereby the neurotransmitter, noradrenaline can be taken up both into neuronal ($Uptake_1$) and extraneuronal ($Uptake_2$) sites is well known (as reviewed by Gillespie, 1973 and Iversen, 1973). These processes are physiologically important in terminating the actions of noradrenaline at the synaptic gap, but have pharmacological relevance in explaining the mechanism of action of some adrenergic drugs (O'Donnell & Wanstall, 1976; Kenakin, 1983).

Indeed, differences in the uptake of adrenoceptor agonists may account for some of the observed variation in agonists potencies (Kenakin, 1984).

Various steroids including oestradiol-17β and progesterone, have been shown to be potent inhibitors of the $Uptake_2$ process (Iversen & Salt, 1970; Salt, 1972). Thus, Bell (1972) suggested that both the changes in uterine neuronal stores of noradrenaline and the apparent capacity to accumulate adrenaline in extraneuronal stores could depend on the oestrogen and progesterone balance during the oestrous cycle. In 1982, Boyle and Digges showed that the variations in the ovarian hormones in proestrus, oestrus, metoestrus and dioestrus were accompanied by variations in uterine uptake of the catecholamines. They also found that blocking $Uptake_2$ alone produced effects similar to blocking both $Uptake_1$ and $Uptake_2$ processes which would suggest that in the rat uterus, the $Uptake_2$ process was the more important. The physiological significance of the effects of oestrogen and progesterone on the $Uptake_2$ process is, however not clear, and merits further investigation.

3. CYCLIC NUCLEOTIDES

(a) Adenosine 3',5' cyclic monophosphate metabolism

An increase in tissue levels of adenosine 3',5' cyclic monophosphate (cAMP) is a prominent feature of the effect of β-adrenoceptor activation in the rat uterus, as in other

organs. However, controversy exists concerning the precise mechanism whereby the rise in tissue cAMP produces the inhibitory response. The evidence available supports the view that cAMP is a mediator of rat uterine response to catecholamines. There is a parallel dose-response relationship between the concentration of the catecholamines required to produce relaxation and to increase tissue cAMP content (Dobbs & Robison, 1968; Triner, Nahas, Vulliemoz, Overweg, Verosky, Habif & Ngai, 1971). Robison (1970) showed that the ability of various catecholamines to relax the uterus and to increase its cAMP content was directly related to their potency at the β-adrenoceptor site. Both the relaxation and the increase in tissue cAMP content were prevented by β-adrenoceptor antagonists (Mitznegg, Heim & Meythaler, 1970; Bhalla, Sandborn & Korenman, 1972), and potentiated by inhibitors of phosphodiesterase (Triner et al., 1971). The increase in tissue cAMP content produced by stimulation of β-adrenoceptors also correlated temporally with relaxation (Marshall & Kroeger, 1973; Johansson & Andersson, 1978). The dibutyryl derivative of cAMP has been shown to mimick the relaxant effect of the catecholamines (Mitznegg et al., 1970; Triner et al., 1971; Vesin & Harbon, 1974). Further studies have also confirmed the involvement of cAMP in the inhibition produced by the catecholamines (Krall & Korenman, 1979; Kroeger, 1979; Kishikawa, 1981). In the myometrium, the longitudinal muscle has been demonstrated to possess a higher level of adenylate cyclase than the

circular muscle, which may account for the greater sensitivity of the former layer to β-adrenoceptor activation (Fortier & Krall, 1983). Thus, many of the criteria for establishing cAMP as a "second messenger" for the effects of β-adrenoceptor stimulation have been satisfied in the rat uterus.

There is evidence, however, against the involvement of cAMP as a mediator of rat uterine response to the catecholamines. Both nitroglycerine (which has no effect on tissue cAMP content) and theophylline (an inhibitor of phosphodiesterase) potentiate the relaxant effect of the catecholamines (Levy & Wilkenfeld, 1968). Polacek and his co-workers (Polacek, Bolan & Daniel, 1971; Polacek & Daniel, 1971) could not demonstrate a temporal correlation between the increased tissue cAMP content produced by the catecholamines and relaxation. Harbon and Clauser (1971) found that the relaxant, adrenaline and the stimulant, prostaglandin E_2 both increased tissue cAMP content. Later workers have also reported a discrepancy between tissue cAMP content and the relaxation produced by catecholamines (Diamond & Holmes, 1975; Meisheri & McNeill, 1979a,b; Marshall & Fain, 1985).

The above evidence shows that under certain conditions, while cAMP can mediate relaxations in the rat uterus, the nucleotide may not be an obligatory mediator. It thus appears that β-adrenoceptor activation can affect muscle tension via cAMP-independent, as well as cAMP-dependent mechanisms.

Acute administration of the ovarian hormone, oestrogen has been shown to elevate cAMP levels in the rat uterus, an effect prevented by β-adrenoceptor antagonists (Szego & Davies, 1969; Sandborn, Bhalla & Korenman, 1973). It appears that cAMP may play an intermediary role in the action of oestrogens in the rat uterus (Marshall, 1973; Downing & Porter, 1980; Kishikawa, 1981). The precise mechanism whereby oestrogen increases uterine cAMP level is, however, not well understood.

(b) Guanosine 3', 5' cyclic monophosphate metabolism

Guanosine 3',5' cyclic monophosphate (cGMP) and cAMP have been proposed to function in a reciprocal fashion in the control of smooth muscle motility (Lee, Kuo & Greengard, 1972; Schultz, Hardman, Schultz, Davis & Sutherland, 1973). The localization of cGMP and its protein kinase in the plasma membrane and cytoplasm of rat uterine cells has been demonstrated by Flandroy, Cheung and Steiner (1983). Although ovarian hormones have been shown to affect cGMP levels in the rat uterus, the physiological significance of this action remains obscure (Flandroy & Galand, 1978, 1980; Kishikawa, 1981). However, no temporal association has been found between cGMP levels and rat uterine motility (Diamond & Hartle, 1974; Diamond & Holmes, 1975; Leiber, Vesin & Harbon, 1978; Janis & Diamond, 1979).

4. IONS

The ionic mechanisms responsible for catecholamine inhibitory responses have been studied by observing changes in electrophysiological properties of uteri exposed to different ionic media. As early as 1959, Goto and Csapo showed that the ovarian hormones, oestrogen and progesterone altered calcium movements in the rat isolated uterus. They found that in a calcium-free medium, uteri under oestrogen influence quickly depolarized and lost all excitability within 20 minutes, while those under progesterone influence required 90 minutes to depolarize to the same extent. Thus, it would appear that calcium ions in the superficial (membrane) sites may be more firmly bound under progesterone influence (Goto & Csapo, 1959; Marshall, 1962).

Csapo and Kuriyama (1963) and Marshall (1968) showed that the hyperpolarization produced by catecholamines in the rat uterus was related to the potassium ion concentration in the extracellular fluid. They observed that in a potassium-free medium, the amine-induced hyperpolarization was increased while in an external potassium concentration of greater than 30 mM, the amine-induced hyperpolarization decreased. On the other hand, changes in extracellular sodium or chloride ion concentrations did not affect hyperpolarization. Thus, Marshall (1968, 1970) suggested that the inhibitory effect of catecholamine may be due to a specific increase in potassium permeability. In 1973 Marshall and Kroeger found that the amine-induced hyper-

polarization was dependent on the calcium concentration in the external medium. Ouabain and low temperature ($10^{o}C$) reduced the hyperpolarization by 50%, indicating that some metabolic process was involved. Marshall and Kroeger (1973) therefore proposed that hyperpolarization may involve an electrogenic calcium pump activated by cAMP. Indeed, an outwardly directed calcium current could underlie the relaxation produced by catecholamines in the rat uterus (Kroeger, Marshall & Bianchi, 1975; Marshall, 1977).

Adrenoceptors may also play a role in the regulation of ion permeability of myometrial cells, and in this manner control uterine response to the catecholamines. Diamond and Marshall (1969a,b) showed that membrane potential changes accompanying the stimulatory or inhibitory effects of the catecholamines were reversed in the presence of the appropriate α- or β-adrenoceptor antagonist. Based on the assumption that alterations in the ionic permeability of the membrane underlie the membrane potential changes, Marshall (1970, 1973) suggested that effects on both α- and β-adrenoceptors could be mediated through these permeability changes.

The involvement of ions in the excitatory action of catecholamines was studied in the guinea-pig uterus by Bulbring and her co-workers who showed that depolarization may be due to an increase in chloride ion permeability (Szurszewski & Bulbring, 1973; Bulbring & Szurszewski, 1974; Bulbring, Ohashi & Tomita, 1981). Lanthanum ions had no

effect on depolarization or membrane resistance, but abolished the tension response suggesting that an influx of calcium ions may be necessary to maintain tension. Cooling to 6°C abolished the excitatory effect of catecholamines on both membrane potential and tension. Thus, some intermediary metabolic process may precede the increase in chloride permeability that caused depolarization (Bulbring et al. 1981).

The above evidence highlights the importance of ions in the response of the rat uterus to catecholamines. It appears that calcium ions play a pivotal role in both the stimulatory and inhibitory effects of the catecholamines in this organ.

5. PROSTAGLANDINS

The biological importance of prostaglandins in mammalian reproduction is well known (as reviewed by Goldberg & Ramwell, 1975 and Horton & Poyser, 1976). The role of prostaglandins in the regulation of rat uterine motility can be discussed on the basis of their production throughout the oestrous cycle, and the possible interactions between them and the catecholamines.

In their classic study, Vane and Williams (1973) showed that both indomethacin and meclofenamate antagonized the stimulant effects of oxytocin in the non-pregnant rat uterus, while contractions induced by acetylcholine and prostaglandin $F_{2\alpha}$ were unaffected. Uteri from rats in late pregnancy

exhibited spontaneous activity and released prostaglandins into the bathing medium. Both the prostaglandin release and uterine activity were abolished by indomethacin. Vane and Williams (1973) suggested, therefore, that rat uterine spontaneous activity may be due to intramural prostaglandin generation, and that increased prostaglandin production contributes to the expulsion of the foetus during parturition. Similar observations have been made by other workers (Harney, Sneddon & Williams, 1974; Dubin, Ghodgaonkar & King, 1979; Gimeno, Sterin-Speziale, Landa, Bonacossa & Gimeno, 1979).

Franchi, Chaud, Borda, Gimeno, Lazzari and Gimeno (1981) measured prostaglandins in the bathing medium, and found that uteri from rats in proestrus and oestrus released more prostaglandin F, while those from metoestrus and dioestrus released more prostaglandin E. However, uterine homogenates have been shown to produce more prostaglandins E and F in proestrus and oestrus, than in metoestrus and dioestrus (Van Orden, Goodale, Baker, Barley & Bhatnagar, 1980; Poyser & Scott, 1980; Brown & Poyser, 1985). In order to explain the discrepancy in results, Franchi et al. (1981) and Gimeno and Gimeno (1984) suggested that the intrinsic capacity of the rat uterus to synthesize prostaglandins E and F may depend on the integrity of the tissue. Although both the myometrium and endometrium can synthesize prostaglandins, the endometrium has been shown to be the major source of uterine prostaglandins in the rat (Campos, Liggins & Seamark, 1980; Brown & Poyser, 1985).

Oestrogen and progesterone have been reported to regulate rat uterine prostaglandin biosynthesis and release (Castracane & Jordan, 1975; Carminati, Luzzani, Soffientini & Lerner, 1975; Kogo, Yamada & Aizawa, 1977; Sterin-Speziale, Gimeno, Bonacossa & Gimeno, 1980). Although oestrogen was regarded as the predominant ovarian steroid involved in the regulation of prostaglandin biosynthesis, a prior period of exposure to progesterone may be necessary (Castracane & Jordan, 1976; Gimeno & Gimeno, 1984). Conflicting results however, exist as to which of the prostaglandins (E or F series) was most affected by the hormones. Oestrogen treatment has been shown by some workers to increase uterine prostaglandin F synthesis alone (Ham, Cirillo, Zanetti & Kuehl, 1975; Sterin-Speziale et al., 1980), while other workers found that both uterine prostaglandins E and F syntheses were augmented (Sharma & Garg, 1977; Wilson, 1983).

Both prostaglandins E and F can produce excitatory effects on uterine motility. The evidence available suggests that prostaglandins may stimulate uterine contraction by a direct effect on intracellular binding sites for calcium leading to a decrease in calcium storage (Carsten, 1974; Reiner & Marshall, 1976; Carsten & Miller, 1977). In 1966, Clegg reported that very low concentrations of prostaglandins antagonized catecholamine-induced inhibitory responses in the rat uterus. Since then, similar interactions between prostaglandins and catecholamines have been demonstrated by Vesin and Harbon (1974), and by Krall, Barrett, Jamgotchian

& Korenman (1984).

Biochemical studies have shown that catecholamines can stimulate prostaglandin biosynthesis and release from various tissues including the kidney (Davis & Horton, 1972), heart (Wennmalm & Brundin, 1978; Weis & Malik, 1985), seminal vesicles (Egan, Humes & Kuehl, 1978), cultured cells (Levine & Moskowitz, 1979) and spleen (Bedwani & Millar, 1975; Bruckner-Schmidt, Jackish & Hertting, 1981). A direct effect of the catecholamines on cyclo-oxygenase may be responsible for the increased prostaglandin production (Egan et al. 1978; Baumann, v. Bruchhausen & Wurm, 1979).

As well as prostaglandins, non-pregnant and pregnant uteri have been shown to produce other metabolites of arachidonic acid which include the leukotrienes (Carraher, Hahn, Ritchie & McGuire, 1983) and prostacyclins (Williams, Dembinska-Kiec, Zmuda & Gryglewski, 1978; Vesin, Do Khac & Harbon, 1979; Franchi et al. 1981; Brown & Poyser, 1985). However, the involvement of leukotrienes (Carraher et al. 1983) and prostacyclins (Gimeno & Gimeno, 1984) in the regulation of rat uterine motility is less clear.

The above evidence shows that several factors including types of adrenoceptors, ovarian hormones, agonist uptake processes, cyclic nucleotides, ions and prostaglandins can modulate rat uterine response to the catecholamines. These factors affect events not only at the adrenoceptor sites but also, those relating to catecholamine-induced changes in cellular function as a whole.

Despite the fact that adrenoceptor mediated inhibition in the rat uterus has been extensively studied, controversy still exists as to the mechanisms that underlie the altered uterine responses during the oestrous cycle. The conflicting results in literature may be due to the lack of standardization of the experimental procedure employed. For instance, some workers induced a particular phase of the oestrous cycle by pretreating animals with various doses of oestrogen and progesterone (Levy & Tozzi, 1963; Diamond & Brody, 1966; Tothill, 1967; Paton, 1968; Harbon & Clauser, 1971; Bengtsson, 1978; Meisheri & McNeill, 1979a b; Kishikawa, 1981), while others did not identify the hormonal state of the animals used in their studies (Ahlquist, 1948; Brooks, et al, 1965; Paterson, 1965; Schild, 1967; Lands et al., 1967; Vane & Williams, 1973). Indeed, only a few workers used uteri from rats in the naturally occurring phases of the oestrous cycle (Butterworth & Randall, 1970; Abdel-Aziz, and Bakry, 1974; Butterworth & Jarman, 1974; Krall et al., 1978; Boyle & Digges, 1982a).

The aim of the present study was therefore, to investigate the mechanism responsible for the variation in uterine response to the catecholamines throughout the natural oestrous cycle by examining: (a) rat uterine response to adrenoceptor agonists in each of the four phases in order to establish control conditions, (b) the contribution of α-receptor activity, agonist uptake processes and ovarian hormones to the overall response, and (c) the possible role

of intramural prostaglandin generation, adenosine 3', 5' cyclic monophosphate metabolism and calcium function in the altered uterine response during the oestrous cycle.

METHODS

A. ANIMALS

Virgin female Wistar rats were used in all experiments. For experiments on the oestrous cycle, animals weighing 180 g to 250 g were used. Ovariectomized rats were purchased from Charles River U.K. Limited. The animals weighed (200-260 g), and were used 14 or more days after bilateral ovariectomy.

B. VAGINAL SMEARS

In rats, cyclical production of ovarian hormones results in an oestrous cycle which lasts 4 to 5 days, and can be divided into four phases - proestrus, oestrus, metoestrus and dioestrus. While the primary events take place in the ovaries and uterus, changes also occur in the vagina which undergoes cyclical disintegration. The debris accumulates in the lumen and can be used to identify the four phases of the cycle. The events occurring in the ovaries, and the vaginal histology of rats in proestrus, oestrus, metoestrus and dioestrus are summarized in Table 1.

Vaginal smears were taken immediately after the animal was killed. 0.5 ml of distilled water was inserted into the vagina from a pasteur pipette. The fluid containing shed vaginal cells was then withdrawn, placed on a glass slide, and air dried. The smears were stained with 0.1% methylene blue and examined under a microscope. Vaginal smears from each phase of the oestrous cycle are shown in Plate 1.

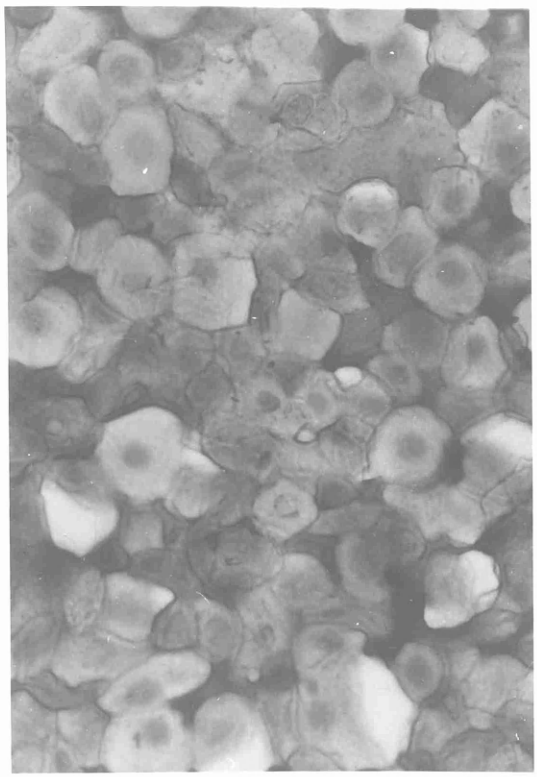
Table 1: Ovarian events, gross appearance of uterus and vaginal histology of rats during the oestrous cycle

Phase	Duration of phase	Ovarian events	Gross Appearance of Uterus	Smear Appearance
Proestrus	12 hours	Follicles grow fast and approach surface of ovary.	Uterus swollen with secretion. Blood vessels engorged.	Nucleated epithelial cells only.
Oestrus	12-18 hours	Ovulation occurs.	Uterus remains swollen. Blood vessels still engorged.	Cornified squamous cells only.
Metoestrus	10-14 hours	Corpora lutea formed.	Uterus becomes smaller.	Cornified cells, nucleated epithelial cells and polymorpho-nucleated leucocytes.
Dioestrus	48-60 hours	Regression of corpora lutea.	Uterus becomes small and thin.	Nucleated epithelial cells and leucocytes.

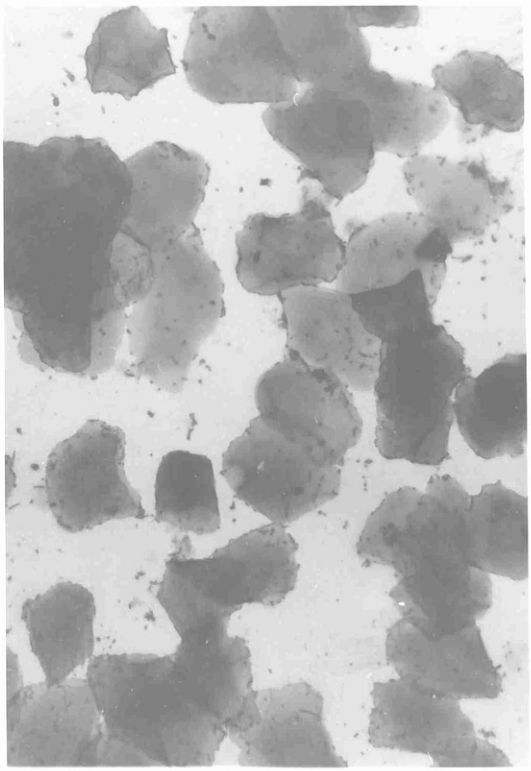
Plate 1: Vaginal smears:-

A. Proestrus	-	nucleated epithelial cells only.
B. Oestrus	-	cornified squamous cells only.
C. Metoestrus	-	cornified cells, nucleated epithelial cells and polymorpho-nucleated leucocytes.
D. Dioestrus	-	nucleated epithelial cells and leucocytes.

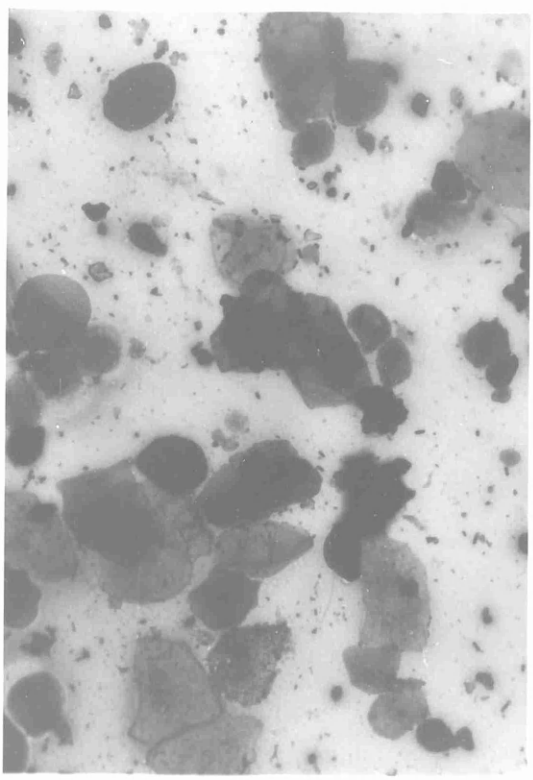
(A)



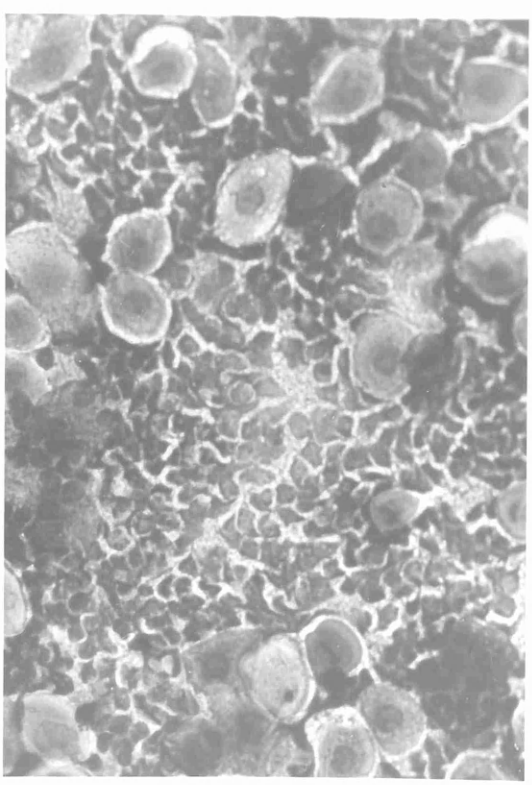
(B)



(C)



(D)



222 μm.

C. ISOLATED UTERINE HORN PREPARATIONS

Animals were killed by stunning and exsanguination. In both intact and ovariectomized rats, 2-3 cm lengths from the mid-portion of each uterine horn were used. The tissues were mounted in paired 10 ml organ baths containing Tyrode solution (see Table 2) at 37°C, and gassed with a 95% O_2:5% CO_2 mixture. Isometric tension was recorded via Grass FT03 force displacement transducers, and displayed on a Grass Model 7D polygraph. An initial tension of 0.5 g was applied to each horn, and the tissues were left to equilibrate for at least 1 hour. Uteri from animals in the metoestrus and dioestrus phases usually exhibited spontaneous activity on mounting. However, the spontaneous activity disappeared after exposure of the tissues to acetylcholine or potassium chloride.

1. EXPERIMENTS IN UTERI FROM INTACT ANIMALS

(a) Responses to motor agents

Since the rat uterus does not possess intrinsic tone, it was necessary to induce tone in order to display the relaxation produced by drugs. Various methods have been used: 5-hydroxytryptamine (Jensen & Vennerod, 1961); potassium chloride (Marshall & Kroeger, 1973); acetylcholine (Boyle & Digges, 1982a) and field stimulation (Clegg, 1966).

Table 2: Composition of Tyrode solution.

Compound	Concentration (mmol/l)
NaCl	136.90
KCl	2.68
$CaCl_2 \cdot 6H_2O$	1.80
$MgCl_2 \cdot 6H_2O$	1.05
$NaHCO_3$	11.90
$NaH_2PO_4 \cdot 2H_2O$	0.42
Glucose	5.55

In the present study, acetylcholine and potassium chloride were used to induce tone in uteri from the four phases of the oestrous cycle, and after ovariectomy.

(i) Acetylcholine

Dose-response curves were constructed to acetylcholine (ACh) in each preparation. Doses of ACh were added every four minutes, and allowed to act for 30 seconds, before being washed out. The dose that produced approximately 60-70% of the maximum response to ACh was chosen as a standard which was used throughout the remainder of the experiment. The response to the standard dose of ACh at the end of each experiment was not significantly different from that at the start of the experiment.

(ii) Potassium chloride

Preliminary experiments showed that the optimum concentration of potassium chloride (KCl) for inducing tone in the four phases of the oestrous cycle was 5×10^{-2}M. This concentration of KCl produced a biphasic contractile response with an initial transient phase, followed by a secondary sustained phase. The secondary sustained phase lasted for more than 1 hour, enabling cumulative dose-response curves to be constructed to the relaxant drugs.

(b) Adrenoceptor agonists inhibitory responses

The inhibitory responses to the adrenoceptor agonists, noradrenaline (NA), adrenaline (ADR) and salbutamol (SAL),

were examined in uteri in which tone was induced with either ACh or KCl. In each experiment, only one adrenoceptor agonist was used.

When tone was induced with ACh, the adrenoceptor agonists were added to the organ bath 30 seconds before the standard dose of ACh. The inhibitory effect of the adrenoceptor agonists was determined as percentage reduction of ACh motor response. After inhibition with the agonists, the standard ACh response was allowed to recover fully before addition of the next dose of agonist.

When KCl was used to induce tone, cumulative dose-response curves to the adrenoceptor agonists were constructed 15 minutes into the sustained phase, until maximum relaxation of the induced tone was achieved. The adrenoceptor agonist dose was added using a 2 minute cycle. The inhibitory responses to the agonists were expressed as percentage relaxation of KCl-induced tone.

In all subsequent experiments, control responses to ACh or KCl and the adrenoceptor agonist under investigation were obtained, before the addition of any antagonists. Experiments in the presence of the adrenoceptor agonists alone are referred to as the Control I studies.

(c) Adrenoceptor agonists inhibitory response in the presence of an α-receptor antagonist

The existence in the uterus of α-excitatory receptors in addition to the well characterized β-inhibitory receptors,

could influence the response to adrenoceptor agonists active at both receptors. The observed response to such agonist would then be the algebraic sum of contraction plus relaxation. In a series of experiments, α-receptor activity was abolished by using azapetine (AZA). AZA (10^{-6}M) was added to the Tyrode solution, and was present throughout the remainder of the experiment. Tissues were exposed to AZA for at least 30 minutes before addition of the adrenoceptor agonists.

(d) Adrenoceptor agonists inhibitory responses in the presence of inhibitors of neuronal and extraneuronal uptake

Agonist concentration at the receptor could be affected by amine removal mechanisms, i.e. uptake into neuronal (Uptake $_1$) and non-neuronal (Uptake $_2$) tissues. The contribution of the uptake processes to adrenoceptor agonists inhibitory responses was studied by using desmethylimipramine (DMI) to block Uptake$_1$ and normetanephrine (NMN) to block Uptake$_2$. When used, both DMI (10^{-6}M) and NMN (10^{-6}M) were present in the Tyrode solution throughout the remainder of the experiment. Tissues were exposed to DMI and NMN for at least 30 minutes before addition of the adrenoceptor agonists.

(e) Adrenoceptor agonists inhibitory responses in the presence of an α-receptor antagonist and inhibitors of neuronal and extraneuronal uptake

Since both α-excitatory receptors and amine removal mechanisms affected adrenoceptor agonist responses, a series of experiments was performed in which AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M) were present in the Tyrode solution. Such experiments are referred to as Control II studies.

(f) Adrenoceptor agonists inhibitory responses in the presence of a cyclo-oxygenase inhibitor

Intramurally generated prostaglandins produce excitatory effects on uterine motility. The possibility that prostaglandins may influence the inhibitory responses of adrenoceptor agonists was investigated in two series of experiments in which their biosynthesis was prevented by inhibiting cyclo-oxygenase with flurbiprofen (FBF). In the first series, the controls were of the Control I type i.e. agonists alone (See Section C.1(b)), while in the second series, controls were of the Control II type i.e. all three antagonists were present (See Section C.1(e)). FBF (10^{-6}M) was present in the Tyrode solution for the duration of these experiments. Tissues were exposed to FBF for at least 1 hour before addition of the adrenoceptor agonists.

(g) Adrenoceptor agonists inhibitory responses in the presence of an inhibitor of both cyclo-oxygenase and lipoxygenase

The prostaglandin precursor, arachidonic acid, is a substrate for lipoxygenase as well as for cyclo-oxygenase.

Thus inhibition of cyclo-oxygenase alone, may lead to augmented formation of leukotrienes from arachidonic acid. The leukotrienes, may also influence adrenoceptor agonists inhibitory responses in the rat uterus. A series of experiments was therefore performed in the presence of BW 755C (3-amino-1-(m-trifluoromethyl)-phenyl-2-pyrazolone), an inhibitor of both cyclo-oxygenase and lipoxygenase. The controls in these experiments were of the Control II type. BW 755 C (10^{-5}M) was present in the Tyrode solution throughout the experiment. Tissues were exposed to BW 755C for at least 1 hour before addition of the adrenoceptor agonists.

(h) Histamine inhibitory responses

In the rat uterus, the inhibitory response to histamine is due to activation of H_2-receptors. In a series of experiments, the effects of histamine were examined in uteri in which tone was induced with ACh. Mepyramine (5×10^{-8}M) was added to the Tyrode solution to block H_1-excitatory receptor activity. Histamine was added to the organ bath 2 minutes before the standard dose of ACh. The inhibitory effect produced by histamine was measured as the percentage reduction of ACh motor response, which was allowed to recover fully before addition of subsequent doses of histamine.

(i) Histamine inhibitory responses in the presence of a cyclo-oxygenase inhibitor

The excitatory effect of prostaglandins on uterine motility could also influence histamine inhibitory responses in the rat uterus. This possibility was investigated in a series of experiments in which prostaglandin biosynthesis was blocked with the cyclo-oxygenase inhibitor FBF. FBF (10^{-6}M) was present in the Tyrode solution throughout the experiments. Tissues were exposed to FBF for at least 1 hour before addition of histamine.

(j) Papaverine inhibitory responses

Uterine responses to the non-specific smooth muscle relaxant papaverine were examined in uteri in which tone was induced with ACh. Papaverine was added to the organ bath 1 minute before the standard dose of ACh. The inhibitory effect produced by papaverine was measured as the percentage reduction of the ACh motor response, which was allowed to recover fully before addition of the next dose of papaverine.

(k) Papaverine inhibitory responses in the presence of a cyclo-oxygenase inhibitor

Involvement of prostaglandins in the inhibitory responses to papaverine was investigated in a series of experiments in which their biosynthesis was inhibited with FBF. As described above, FBF (10^{-6}M) was present in the Tyrode solution throughout the experiment. Tissues were exposed to FBF for at least 1 hour before addition of papaverine.

2. EXPERIMENTS IN UTERI FROM OVARIECTOMIZED ANIMALS

(a) Adrenoceptor agonists inhibitory responses

The inhibitory responses to the adrenoceptor agonists NA, ADR and SAL were studied in uteri in which tone was induced with ACh, as in those from intact animals. Experiments in the presence of the adrenoceptor agonists alone are again referred to as Control I studies.

(b) Adrenoceptor agonists inhibitory responses in the presence of an α-receptor antagonist

The presence of functional α-receptors in uterus from ovariectomized rats was investigated in a series of experiments in which α-receptor antagonist, AZA was used. As with experiments in intact animals, AZA (10^{-6}M) was present in the Tyrode solution throughout the experiment.

(c) Adrenoceptor agonists inhibitory responses in the presence of inhibitors of neuronal and extraneuronal uptake

The effect of amine removal mechanisms on adrenoceptor agonists inhibitory response was also studied in uteri from ovariectomized rats. In these experiments, both $Uptake_1$ and $Uptake_2$ processes were blocked by DMI and NMN, respectively. DMI (10^{-6}M) and NMN (10^{-6}M) were both present in the Tyrode solution throughout the experiment.

(d) Adrenoceptor agonist inhibitory responses in the presence of an α-receptor antagonist and inhibitors of neuronal and extraneuronal uptake

As in experiments in uteri from intact rats, a series of Control II experiments was performed in the presence of all three antagonists, AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M).

(e) Adrenoceptor agonists inhibitory responses in the presence of a cyclo-oxygenase inhibitor

The possibility that intramural prostaglandin production may influence adrenoceptor against inhibitory responses in uteri from ovariectomized rats was investigated in experiments in which FBF (10^{-6}M) was present in the Tyrode solution. As in the study with intact rats, Control I and Control II series of experiments were carried out.

D. MEASUREMENT OF ADENOSINE 3'5' CYCLIC MONOPHOSPHATE CONTENT IN THE UTERUS

Stimulation of β-adrenoceptors in the rat uterus increases tissue adenosine 3'5' cyclic monophosphate (cAMP) content. The effect of the β-receptor agonist, SAL on cAMP levels, was investigated in the four phases of the oestrous cycle.

(a) Extraction of tissue cAMP

Animals were killed, and the phase of the oestrous cycle identified from vaginal smears. The uterus was isolated, and 1 cm lengths of each horn were blotted and weighed. Tissues were incubated in Tyrode solution containing a phosphodiesterase inhibitor, theophylline (10^{-3}M), at 37°C for 1 hour. After incubation, the tissues were blotted dry and quickly transferred to small tubes containing 1 ml of ice-cold 5% trichloroacetic acid (T.C.A.), which were left in a cold room (2-4°C) for 24 hours.

0.5 ml of the T.C.A. extract was transferred from each sample tube to a correspondingly marked tube, and 2 ml of water saturated diethyl ether were added and vortexed for 5 seconds. The ether phase was removed using a pasteur pipette and discarded. The process of adding ether, vortexing and removing the ether layer was repeated twice. Any remaining ether was evaporated off in a water bath at 80°C.

Some crystals of calcium carbonate were added to each tube to neutralize any remaining T.C.A. and to maintain a constant sample pH.

(b) Assay of cAMP

The cAMP assay was performed using a radioimmunoassay kit purchased from Amersham International PLC. The assay is based on competition between unlabelled cAMP and a fixed quantity of tritium labelled compound for binding to a protein which has a high affinity and specificity for cAMP. Measurement of protein-bound radioactivity enables the amount of unlabelled cAMP in the sample to be calculated. The amount of labelled protein - cAMP complex formed is related inversely to the amount of unlabelled cAMP present in the assay sample.

Separation of the protein bound cAMP from unbound nucleotide is achieved by adsorption of the free nucleotide on to coated charcoal, followed by centrifugation. An aliquot of the supernatant containing the bound nucleotide is then removed for liquid scintillation counting. The concentration of unlabelled cAMP is then determined from a linear standard curve.

(i) Preparation of standard cAMP solution

0.5 ml Tris EDTA buffer (50 mM Tris/HCl solution containing 4 mM EDTA at pH 7.5) was added to each of four small glass tubes. 0.5 ml of adenosine 3'5' cyclic phosphate

standard (320 pmol) was added to the first tube and mixed. 0.5 ml of this dilution was transferred to the second tube and mixed again. This procedure was repeated successively with each remaining tube. Together with the original solution, five concentrations of standard cAMP were prepared. 50 µl from each solution gave 16, 8, 4, 2 and 1 pmol and were used for preparation of the calibration curve.

(ii) Assay procedure

(1) 14 assay tubes for standards, and additional tubes for unknowns in duplicate, were maintained at 0°C in an ice bath. The tubes were labelled consecutively.

(2) 150 µl of Tris EDTA buffer were pipetted into tubes 1 and 2, which were used for the determination of blank counts per minute.

(3) 50 µl of Tris EDTA buffer were pipetted into tubes 3 and 4 for determination of binding in the absence of unlabelled cAMP.

(4) Beginning with the lowest concentration of standard cAMP, 50 µl of each dilution were added into each successive pair of assay tubes (5-14).

(5) 50 µl of each unknown sample, in duplicate, were added into the remaining assay tubes as required.

(6) 50 µl of the labelled compound [8 - ^{3}H] adenosine 3'5' cyclic phosphate (5µCi) were added to each assay tube.

(7) 100 µl of the binding protein, purified from bovine muscle, were added to all tubes with the exception of the blanks.

(8) All tubes were vortexed for 5 seconds.

(9) The tubes, contained in the ice bath, were left in the cold room (2-4°C) for 2 hours.

(10) Fifteen minutes before the end of incubation time, 20 ml of ice-cold distilled water were added to the charcoal reagent in a beaker, which was placed in an ice bath and stirred continuously with a magnetic stirrer.

(11) 100 µl of the charcoal suspension were added to all assay tubes and vortexed for 5 seconds. Charcoal was added only to the number of tubes which could be centrifuged in one batch.

(12) The tubes were centrifuged at 12,000g for 5 minutes in a refrigerated centrifuge to sediment the charcoal.

(13) Without disturbing the sediment, a 200 µl sample from each tube were removed and placed in vials containing 10 ml of scintillant ES 299.

(14) Samples were counted for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer (Model 3390).

(iii) Preparation of standard curve for cAMP assay

(1) The blank counts per minute (c.p.m.) for the assay were determined from the mean c.p.m. for tubes 1 and 2.

(2) The c.p.m. bound in the absence of unlabelled cAMP (C_o) were obtained from the mean c.p.m. for tubes 3 and 4, and then subtracted from the blank c.p.m.

(3) The c.p.m. bound in the presence of unlabelled cAMP (C_x) were determined by first averaging the c.p.m. for each pair of duplicates in tubes 5-14 for the standard, and the additional pairs of tubes for the unknowns. The results were then subtracted from the blank c.p.m. to give C_x.

(4) C_o/C_x was calculated for each concentration of standard cAMP and the unknowns.

(5) C_o/C_x was plotted against pmoles of standard cAMP/tube to give the standard curve as shown in Figure 1. A straight line was obtained with an intercept of 1.0 on the ordinate.

(6) From the C_o/C_x value for the unknowns, the number of pmoles of cAMP was read from the standard curve. The cAMP concentration was expressed as pmol/g wet weight of tissue.

(c) Measurement of basal tissue levels of cAMP in the four phases of the oestrous cycle

Since variation in the ovarian hormones may affect cAMP generation, basal cAMP levels were measured in uteri from

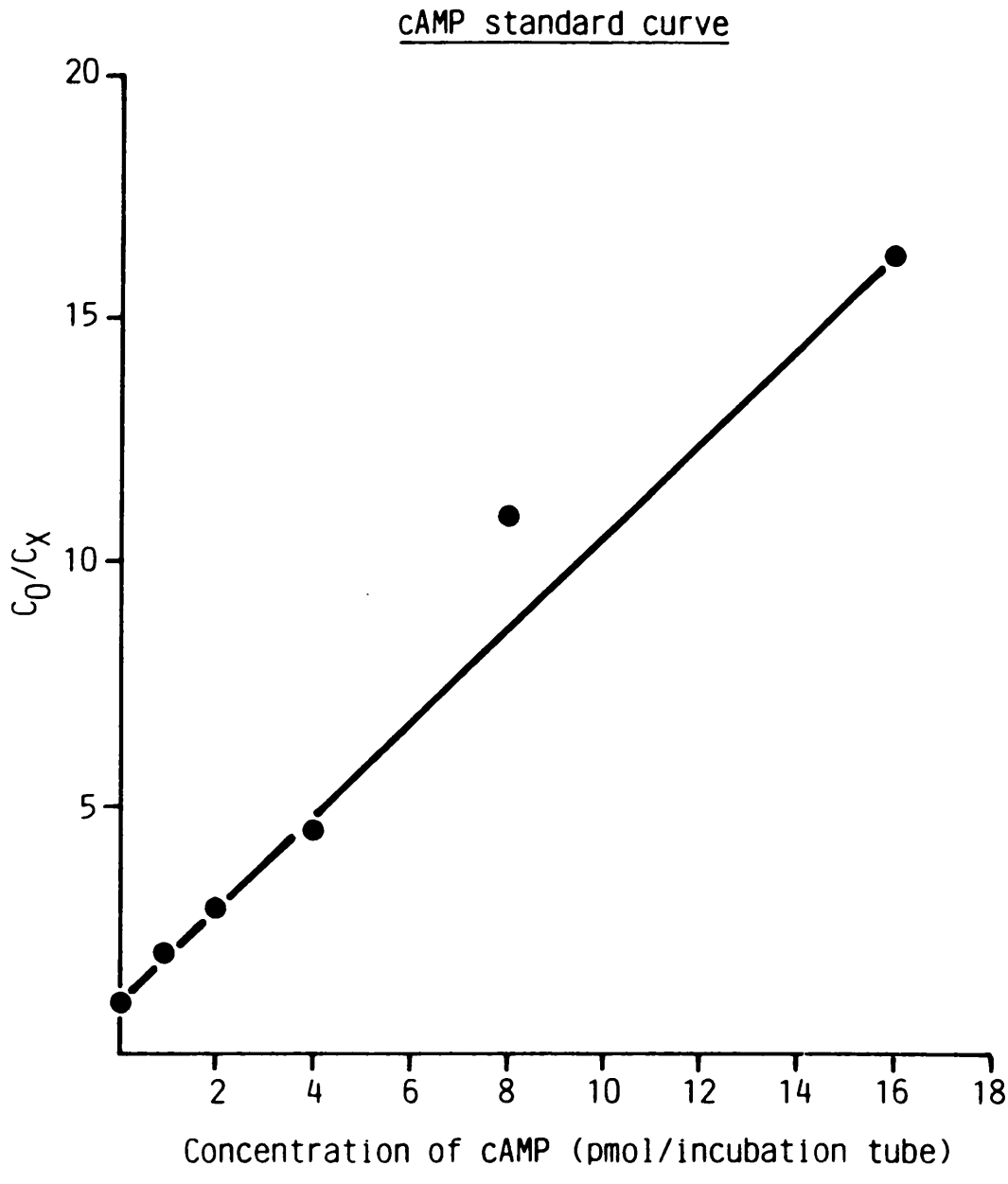


Fig. 1: Standard curve for determination of unknown cAMP concentration in samples. C_o = counts per minute (c.p.m.) of labelled compound bound in the absence of unlabelled cAMP; C_x = c.p.m. of labelled compound bound in the presence of standard unlabelled cAMP.

(f) Effect of a β-adrenoceptor agonist on cAMP levels in the presence of a cyclo-oxygenase inhibitor

The effect of SAL (5×10^{-6}M) on cAMP levels was determined in uteri from the four phases of the oestrous cycle, after treatment with the cyclo-oxygenase inhibitor, FBF. Tissues were incubated in Tyrode solution containing FBF (10^{-6}M) at 37°C for 1 hour. After incubation, the tissues were transferred to Tyrode solution containing FBF and SAL for 1 minute, and then immediately subjected to cAMP analysis.

E. MEASUREMENT OF CALCIUM FLUXES IN THE UTERUS

The final determinant of smooth muscle motility is the level of intracellular free calcium (Ca^{2+}), which is regulated by three processes - influx, efflux and intracellular binding. The influence of ovarian hormones on Ca^{2+} movement was investigated by studying the influx and efflux of tracer calcium ($^{45}Ca^{2+}$) in uteri from four phases of the oestrous cycle and from ovariectomized rats. The effect of an adrenoceptor agonist on $^{45}Ca^{2+}$ fluxes was also examined.

(a) Determination of optimum tracer concentration for $^{45}Ca^{2+}$ flux experiments

Animals in the oestrus (oestrogen dominated) and metoestrus (progesterone dominated) phases were used. 1 cm lengths of each uterine horn were blotted, weighed and impaled on stainless steel rods. Tissues were incubated in Tyrode solution at 37°C for 1 hour. After incubation, tissues were transferred to beakers containing the following dilutions of $^{45}Ca^{2+}$ - 0.0005, 0.005, 0.05, and 5 μCi/ml in Tyrode solution at 37°C for 2 minutes. Tissues were then removed, blotted and rinsed for 5 seconds in each of five successive beakers containing 50 ml of Tyrode solution at 37°C. Tissues were blotted dry and digested in 1 ml of 4M potassium hydroxide (KOH) at 60°C for 2 hours. 10 ml of scintillant ES 299 were added to each sample which was

counted for radioactivity in a Packard Tri-Carb liquid scintillation spectrometer. Quenching in each sample was estimated by using the internal standard method (Peng, 1977). Tissue $^{45}Ca^{2+}$ was expressed as disintegrations per minute (d.p.m.) per wet weight of tissue.

(b) Measurement of $^{45}Ca^{2+}$ efflux in uteri from four phases of the oestrous cycle and in ovariectomized animals

Animals were killed, and the phases of the oestrous cycle identified. Samples were prepared as described above. After incubation, tissues were transferred to a conical flask containing $^{45}Ca^{2+}$ (2 μCi/ml) in 25 ml of Tyrode solution at 37°C for 2 hours. The $^{45}Ca^{2+}$ concentration was chosen on the basis of results from Section E.(a). After loading with $^{45}Ca^{2+}$, tissues were rinsed for 5 seconds in a large volume of Tyrode solution (25 ml/tissue) at 37°C. At 5 minute intervals, tissues were sequentially passed through a series of vials containing 2 ml of Tyrode solution at 37°C. 10 ml of scintillant ES 299 were added to each efflux sample and counted for radioactivity.

At the end of the efflux experiment, each tissue was blotted dry and digested in 1 ml of 4M KOH at 60°C for 2 hours. 10 ml of scintillant were added to each sample and counted for radioactivity. Quenching in each sample was estimated by the internal standard method.

Calculations:

Decline of tissue $^{45}Ca^{2+}$ was expressed as disintegrations per minute per milligramme wet weight of tissue (d.p.m./mg wet wt.), and plotted against time.

The rate constant of $^{45}Ca^{2+}$ loss was calculated according to Deth (1978).

$$\text{Rate Constant} = \frac{^{45}Ca^{2+} \text{ leaving tissue during collection interval}}{^{45}Ca^{2+} \text{ in tissue before collection interval.}}$$

(c) Effect of a β-adrenoceptor agonist on $^{45}Ca^{2+}$ efflux

The effect of β-receptor agonist, SAL on $^{45}Ca^{2+}$ efflux was investigated in uteri from the oestrus (oestrogen dominated) and metoestrus (progesterone dominated) phases of the oestrous cycle. SAL, at a concentration of 5×10^{-6}M, which had produced maximum inhibition in isolated uterine horn preparations, was present in the Tyrode solution during the 65-70 minutes period of efflux.

The effect of SAL was also examined in preparations in which KCl (5×10^{-2}M) was present in the Tyrode solution during the 50-80 minutes period of efflux.

(d) Measurement of $^{45}Ca^{2+}$ influx in uteri from four phases of the oestrous cycle and in ovariectomized animals

Animals were killed, and the phase of the oestrous cycle identified. Samples were prepared as described above. After incubation, tissues were transferred to a beaker containing $^{45}Ca^{2+}$ (0.25 μCi/ml) in 100 ml of Tyrode solution

at 37°C. The $^{45}Ca^{2+}$ concentration was chosen on the basis of results from Section E.(a). At 1/2, 1, 5, 10, 20, 40, 80 and 160 minute intervals, tissues were removed from the radioactive solution, blotted and rinsed (5 seconds) in each of five successive beakers containing 50 ml of Tyrode solution at 37°C. Tissues were then blotted dry and digested in 1 ml of 4M KOH at 60°C for 2 hours. 10 ml of scintillant were added to each sample and counted for radioactivity. Quenching in each sample was estimated by the internal standard method.

Calculations

The uptake of $^{45}Ca^{2+}$ by tissue is expressed in terms of space values or distribution volumes (Kroeger, Marshall & Bianchi, 1975).

$$^{45}Ca^{2+} \text{ space (ml/g tissue)} = \frac{\text{tissue } ^{45}Ca^{2+} \text{ (c.p.m./g)}}{\text{bathing solution } ^{45}Ca^{2+} \text{(c.p.m./ml)}}$$

c.p.m. = counts per minute.

This calculation is based on the assumption that the ratio of $^{45}Ca^{2+}$ to non-tracer Ca^{2+} ions entering the tissue is identical with the ratio of the same ions in solution. This is equivalent to assuming homogeneity of distribution of tracer to non-tracer Ca^{2+} ions in interstitial fluid, and no difference in penetration of the two ions into the tissue.

The $^{45}Ca^{2+}$ space values were then converted to Uptake units (μmol/g wet weight of tissue).

$^{45}Ca^{2+}$ estimate of total tissue Ca^{2+} (μmol/g) = $^{45}Ca^{2+}$ space (ml/g) x concentration of non-tracer Ca^{2+} (μmol/ml).

(e) Effect of a β-adrenoceptor agonist on $^{45}Ca^{2+}$ influx

The effect of β-receptor agonist SAL on $^{45}Ca^{2+}$ influx was investigated in uteri from animals in the metoestrus and dioestrus phases. SAL (5×10^{-6}M) was present in the Tyrode solution containing $^{45}Ca^{2+}$ (0.25 μCi/ml) for the duration of the influx experiments.

F. CHEMICALS AND DRUGS

Acetylcholine chloride	- Sigma
DL-Adrenaline bitartrate	- Sigma
Ascorbic acid	- B.D.H.
Azapetine phosphate	- Roche
BW 755C 3-amino-1-(m-trifluoromethyl)-phenyl-2-pyrazolone	- I.C.I.
Calcium chloride	- B.D.H.
Desmethylimipramine hydrochloride	- Geigy
Glucose	- Formachem
Histamine acid phosphate	- B.D.H.
Magnesium chloride	- M & B
Mepyramine maleate	- M & B
Methylene blue	- B.D.H.
L-Noradrenaline bitartrate	- Sigma
DL-Normetanephrine hydrochloride	- Sigma
Papaverine sulphate	- B.D.H.
Potassium chloride	- Koch-Light
Potassium hydroxide	- Analar
DL-Propanolol hydrochloride	- I.C.I.
Salbutamol sulphate	- Glaxo
Scintillant ES 299	- Amersham
Sodium bicarbonate	- Koch-Light
Sodium chloride	- Koch-Light
Sodium dihydrogen orthophosphate	- Analar
Sodium flurbiprofen dihydrate	- Boots
Theophylline	- Sigma

^{45}Calcium (1 mCi as calcium chloride solution) - Amersham

Cyclic AMP radioimmunoassay kit (TRK 432) containing Tris/EDTA buffer, [8 - ^{3}H] adenosine 3'5'-cyclic phosphate (5 µCi), adenosine 3'5'-cyclic phosphate standard, binding protein purified from bovine muscle and charcoal adsorbent - Amersham

G. STATISTICS

All values given are mean $\pm$ standard error of the mean. Students t-tests were carried out to compare results. In diagrams and tables * represents $p < 0.05$; ** represents $p < 0.01$; and *** represents $p < 0.001$.

EC_{50} values were derived from individual dose-response curves. pD_2 values = negative $\log_{10}$ EC_{50}.

RESULTS

I. ISOLATED UTERINE HORN PREPARATIONS

A. Responses to acetylcholine

Acetylcholine (ACh), in the concentration range 10^{-8}M to 3 x 10^{-4}M, produced a dose-related contraction of the rat uterus in all phases of the oestrous cycle, and in ovariectomized animals. The mean maximum tension developed to ACh under these conditions varied (Table 3). Within the oestrous cycle, the highest maximum tension was achieved in oestrus and the lowest in dioestrus , while in ovariectomized rats, the maximum tension was less than in dioestrus . Despite the marked variation in the maximum tension developed to ACh, the sensitivity of the preparations to ACh was similar in all phases of the oestrous cycle, and after bilateral ovariectomy. There was no variation in potency as measured by pD_2 values (Table 3).

Since the tension induced by ACh varied, a dose-response curve was required in each experiment and the dose which produced approximately 60%-70% of the maximum was then used as the standard. Thus, agonist inhibitory effects could be measured as a reduction of this standard ACh response. The response to the standard dose of ACh at the end of each experiment was not significantly different from that at the beginning.

Table 3: Maximum tension developed to acetylcholine (ACh) and potency in rat uteri in the four phases of the oestrous cycle and after ovariectomy.

State	Maximum tension (g)	Potency (pD_2 values)	n
Proestrus	5.31 ± 0.22	7.92 ± 0.17	14
Oestrus	6.39 ± 0.19	7.95 ± 0.18	19
Metoestrus	4.32 ± 0.20	8.10 ± 0.21	14
Dioestrus	2.95 ± 0.09	8.29 ± 0.18	18
Ovariectomized	1.30 ± 0.10	7.85 ± 0.23	10

Values are mean ± S.E.M.; n = number of observations.

B. Responses to adrenoceptor agonists in the four phases of the oestrous cycle

The criteria used for the choice of the agonists were based on their physiological and pharmacological importance. The endogenous catecholamines - noradrenaline (NA) and adrenaline (ADR) are the adrenergic neurotransmitter and the adrenal hormone respectively, and both activate α- and β-adrenoceptors. The non-catecholamine salbutamol (SAL) is, however, selective for β-adrenoceptors of the β_2-subtype.

Dose-response curves and pD_2 values for the agonists in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 2 to 5, and Table 4 respectively.

(a) Proestrus (Figure 2)

NA (10^{-9}M - 10^{-5}M), ADR (10^{-10}M - 3×10^{-7}M) and SAL (1.5×10^{-9}M - 1.5×10^{-6}M) produced dose-related inhibition of the standard ACh-induced contraction. The maximum degrees of inhibition produced by NA was 20%, while for both ADR and SAL, it was approximately 30%. ADR had the highest pD_2 value, whilst NA had the lowest value (Table 4).

(b) Oestrus (Figure 3)

NA (10^{-9}M - 3×10^{-6}M), ADR (10^{-10}M - 10^{-7}M) and SAL (1.5×10^{-9}M - 5×10^{-6}M) produced dose-related inhibition of the standard ACh-induced contraction. The maximum degrees of inhibition produced by NA and ADR (20% and 30%, respectively)

Fig. 2: Log. dose-response curves to noradrenaline, (A), adrenaline, (B) and salbutamol, (C) in the rat isolated uterus in proestrus: controls; in presence of azapetine (AZA, 10^{-6}M); in presence of desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M); in presence of AZA with DMI and NMN (all at 10^{-6}M). Number of observations in brackets.

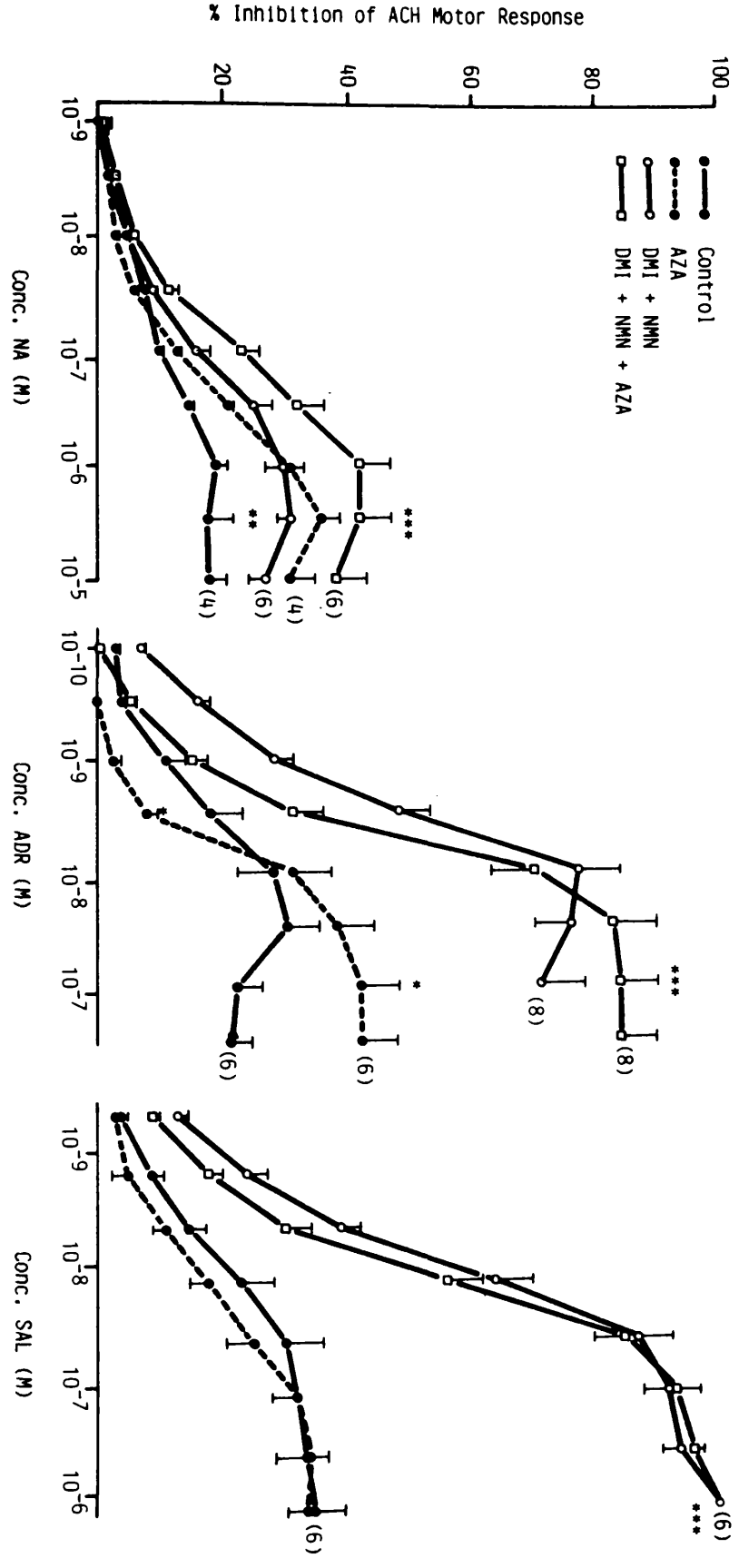

% Inhibition of ACH Motor Response
Control
AZA
DMI + NMN
DMI + NMN + AZA
(A)
(B)
(C)
Proestrus Phase
Conc. NA (M)
Conc. ADR (M)
Conc. SAL (M)

Fig. 3: Log. dose-response curves to noradrenaline, (A), adrenaline, (B) and salbutamol, (C) in the rat isolated uterus in oestrus: controls; in presence of azapetine (AZA, 10^{-6}M); in presence of desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M); in presence of AZA with DMI and NMN (all at 10^{-6}M). Number of observations in brackets.

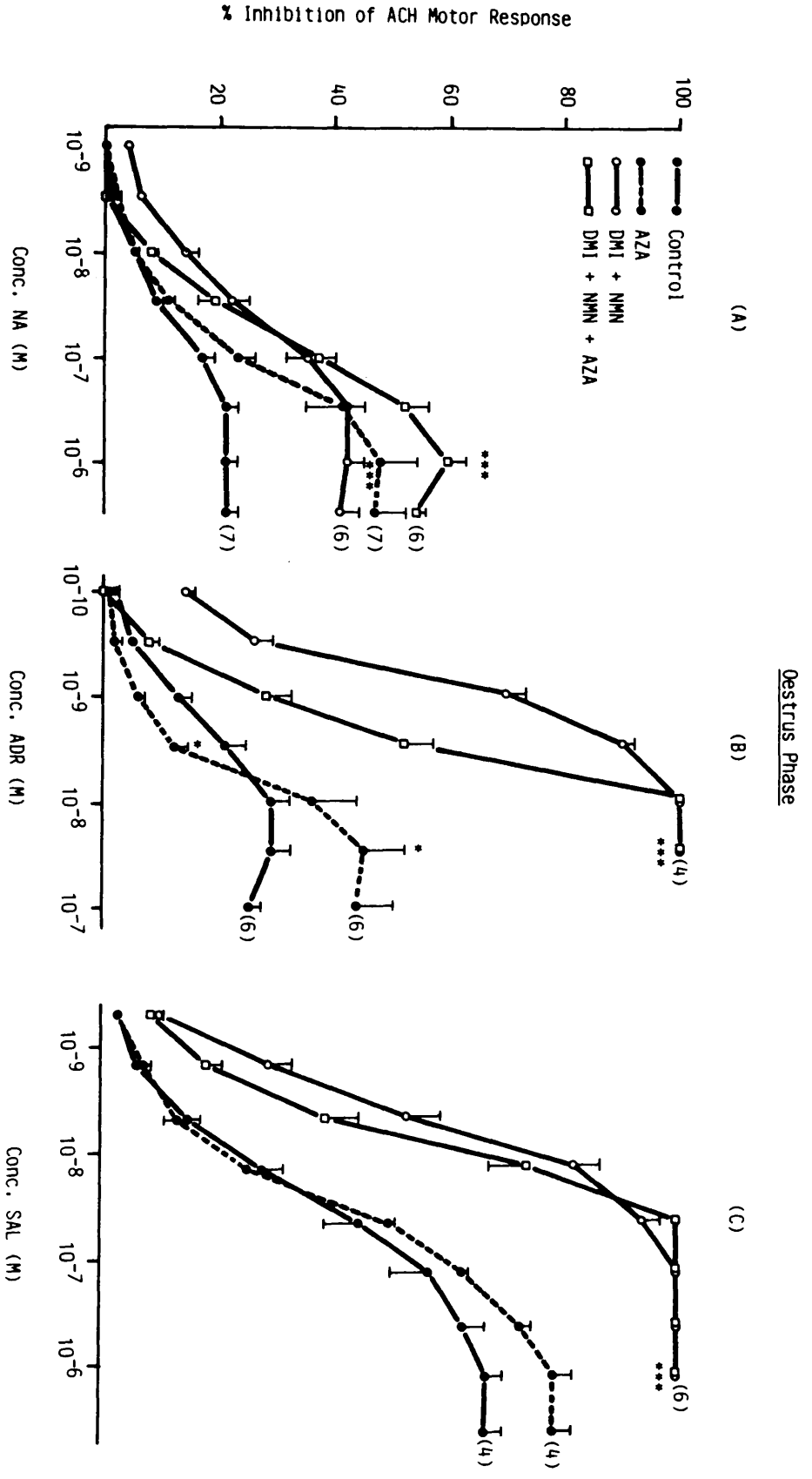

% Inhibition of ACH Motor Response
Oestrus Phase
Control
AZA
DMI + NMN
DMI + NMN + AZA
(A)
(B)
(C)
Conc. NA (M)
Conc. ADR (M)
Conc. SAL (M)

Fig. 4: Log. dose-response curves to noradrenaline, (A), adrenaline, (B) and salbutamol, (C) in the rat isolated uterus in metoestrus: controls; in presence of azapetine (AZA, 10^{-6}M); in presence of desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M); in presence of AZA with DMI and NMN (all at 10^{-6}M). Number of observations in brackets.

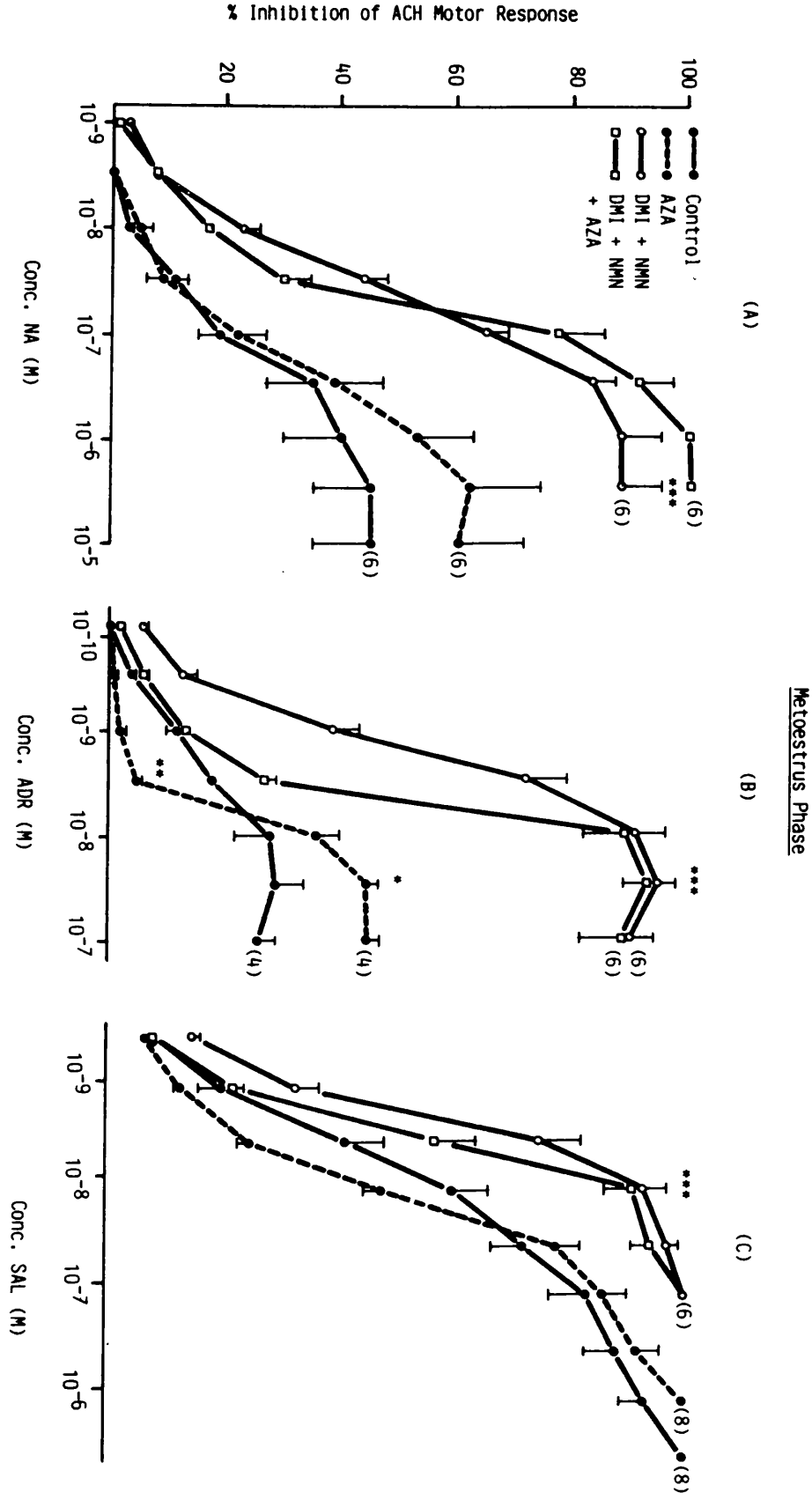

Metoestrus Phase
(A)
(B)
(C)
% Inhibition of ACH Motor Response
Control
AZA
DMI + NMN
DMI + NMN + AZA
Conc. NA (M)
Conc. ADR (M)
Conc. SAL (M)

Fig. 5: Log. dose-response curves to noradrenaline, (A), adrenaline, (B) and salbutamol, (C) in the rat isolated uterus in dioestrus: controls; in presence of azapetine (AZA, 10^{-6}M); in presence of desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M); in presence of AZA with DMI and NMN (all at 10^{-6}M). Number of observations in brackets.

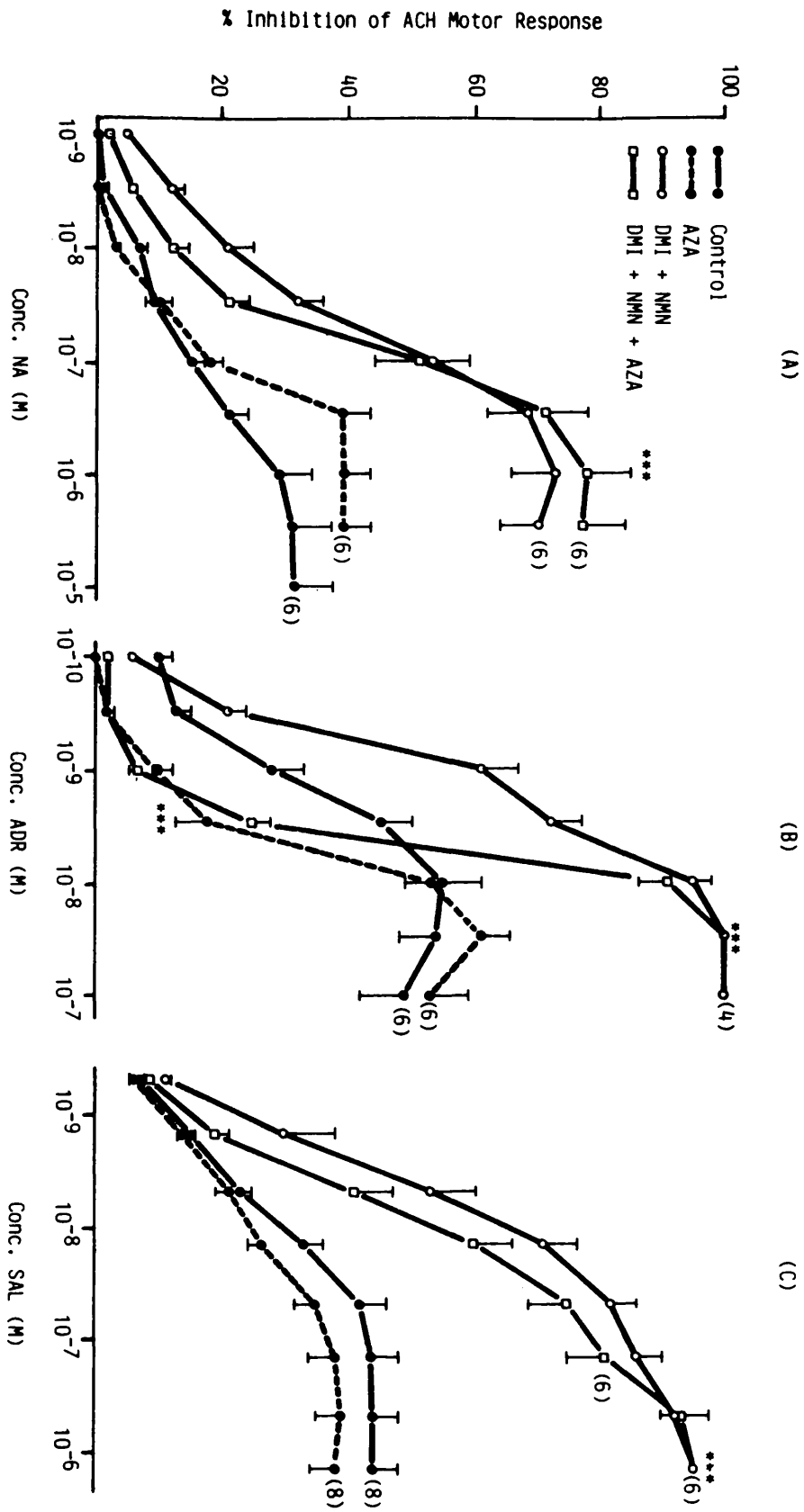

% Inhibition of ACH Motor Response
Control
AZA
DMI + NMN
DMI + NMN + AZA
(A)
(B)
(C)
Conc. NA (M)
Conc. ADR (M)
Conc. SAL (M)
Dioestrus Phase

Table 4: The potency of adrenoceptor agonists - noradrenaline (NA), adrenaline (ADR) and salbutamol (SAL) in the rat uterus (pD_2 values).

Phase	pD_2 values		
	NA	ADR	SAL
Proestrus	7.95 ± 0.46 (4)	9.33 ± 0.09 (6)	9.14 ± 0.28 (6)
Oestrus	8.79 ± 0.04 (7)	9.53 ± 0.29 (6)	8.39 ± 0.07 (4)
Metoestrus	7.28 ± 0.04 (6)	9.24 ± 0.09 (4)	9.00 ± 0.26 (8)
Dioestrus	7.95 ± 0.43 (6)	10.31 ± 0.34 (6)	9.58 ± 0.08 (8)

Values are mean ± S.E.M.; number of observations in brackets.

were similar to those achieved in proestrus, but for SAL, a greater degree of inhibition was obtained (approximately 70%). ADR had the highest pD_2 value, whilst SAL had the lowest value (Table 4).

(c) Metoestrus (Figure 4)

NA (3×10^{-9}M - 10^{-5}M), ADR(10^{-10}M - 10^{-7}M) and SAL (1.5×10^{-9}M - 5×10^{-6}M) produced dose-related inhibition of the standard ACh-induced contraction. The maximum degrees of inhibition produced by NA and ADR were approximately 40% and 30%, respectively. In contrast, a complete inhibition of the ACh contraction was observed with SAL. ADR had the highest pD_2 value, whilst NA had the lowest value (Table 4).

(d) Dioestrus (Figure 5)

NA(3×10^{-9}M - 10^{-5}M), ADR (10^{-10}M - 10^{-7}M) and SAL (1.5×10^{-9}M - 1.5×10^{-6}M) produced dose-related inhibition of the standard ACh-induced contraction. The maximum degrees of inhibition produced by NA, ADR and SAL, were approximately 30%, 60% and 40% respectively. ADR had the highest pD_2 value, whilst NA had the lowest value (Table 4).

The degrees of inhibition produced by NA, ADR and SAL, and their corresponding pD_2 values varied throughout the oestrous cycle. ADR had the highest, and NA the lowest pD_2 value in all phases except oestrus, where SAL was the least potent.

C. Responses to adrenoceptor agonists in the presence of an α-receptor antagonist and uptake inhibitors in the four phases of the oestrous cycle

The observed differences in both potency and degree of inhibition produced by the agonists during the oestrous cycle might have been due to:

(i) the presence of α-excitatory receptors, which could oppose the inhibition produced by NA and ADR. Indeed, there was some evidence for this effect as these drugs produced motor responses in some phases of the oestrous cycle.

(ii) the presence of avid amine removal mechanisms into neuronal and/or non-neuronal tissues, which could affect the responses to all the agonists.

Therefore, three series of experiments were performed in the presence of: (a) an α-receptor antagonist (b) inhibitors of both amine removal mechanisms, and (c) an α-receptor antagonist, and inhibitors of both amine removal mechanisms.

1. Motor response

In proestrus and oestrus, both NA and ADR in the concentration range 10^{-6}M to 3 x 10^{-5}M, produced small contractile responses. Unlike the ACh motor responses which were rapid in onset and sustained, the NA and ADR motor responses had a slow onset, with a latency of 10 seconds

and a duration of only 10 seconds. Generally, the motor responses were not dose-related, and were abolished by the α-receptor antagonist, azapetine (10^{-6}M) (Fig. 6).

2. Inhibitory responses to NA, ADR and SAL in the presence of an α-receptor antagonist

In the first series of experiments, the inhibitory responses were re-examined in the presence of azapetine (AZA, 10^{-6}M) which was chosen since it had no effect on the ACh-induced contraction (Digges, 1980).

Dose-response curves and pD_2 values for the agonists in the presence of AZA, in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 2 to 5, and Table 5 respectively.

(a) Proestrus (Figure 2)

AZA enhanced significantly the maximum degree of inhibition produced by both NA ($p < 0.01$) and ADR ($p < 0.05$). For ADR only, there was in addition, a significant ($p < 0.05$) shift in the dose-response curves to the right, at the lower concentrations. In contrast, AZA had no significant effect on SAL dose-response curves. Compared with the control value, there was a significant increase ($p < 0.001$) in the pD_2 value for ADR in the presence of AZA, but there were no significant differences in the pD_2 values for NA and SAL (Table 5).

Fig. 6: Isometric responses to acetylcholine (ACh) in the rat isolated uterus in oestrus, and inhibition of these responses by noradrenaline (NA). ACh (3 x 10^{-6}M) added at A; NA added at arrows; Wash out ●
Upper trace (a), control; lower trace (b), in presence of azapetine (10^{-6}M).

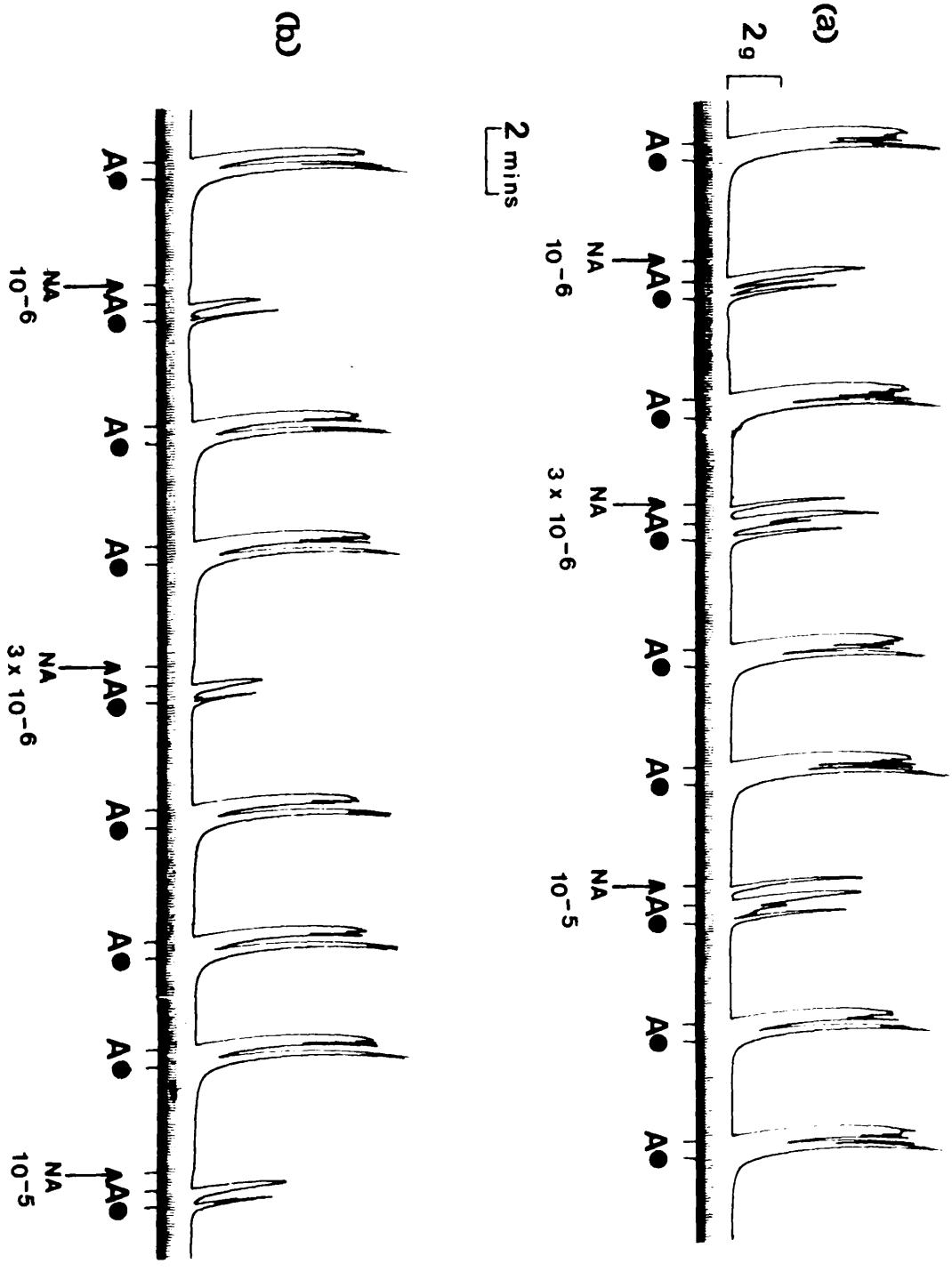

(a)
2g
2 mins
A
NA
10⁻⁶
3 x 10⁻⁶
10⁻⁵
(b)
A
NA
10⁻⁶
3 x 10⁻⁶
NA
10⁻⁵

Table 5: Effect of azapetine (AZA, 10^{-6}M) on the potency of adrenoceptor agonists in the rat uterus (pD_2 values).

Phase	Drug	Control	After AZA	n
Proestrus	NA	7.95 ± 0.46	7.28 ± 0.05	4
	ADR	9.33 ± 0.09	9.79 ± 0.02***	6
	SAL	9.14 ± 0.28	8.77 ± 0.25	6
Oestrus	NA	8.79 ± 0.04	7.09 ± 0.04***	7
	ADR	9.53 ± 0.29	9.80 ± 0.05	6
	SAL	8.39 ± 0.07	8.47 ± 0.03	4
Metoestrus	NA	7.28 ± 0.04	7.28 ± 0.04	6
	ADR	9.24 ± 0.09	9.83 ± 0.05***	4
	SAL	9.00 ± 0.26	8.41 ± 0.21	8
Dioestrus	NA	7.95 ± 0.43	7.75 ± 0.30	6
	ADR	10.31 ± 0.34	9.67 ± 0.04	6
	SAL	9.58 ± 0.08	9.13 ± 0.20	8

Values are mean ± S.E.M.; n = number of observations.

(b) Oestrus (Figure 3)

AZA enhanced significantly the maximum degree of inhibition produced by both NA ($p < 0.001$) and ADR ($p < 0.05$). As in proestrus, there was a significant ($p < 0.05$) shift in the ADR dose-response curves to the right, at the lower concentrations. AZA had no significant effect on the dose-response curves to SAL. Compared with the control value, there was a significant decrease ($p < 0.001$) in the pD_2 value for NA in the presence of AZA, but there were no significant differences in the pD_2 values for ADR and SAL (Table 5).

(c) Metoestrus (Figure 4)

AZA enhanced significantly ($p < 0.05$) the maximum degree of inhibition produced by ADR. As in proestrus and oestrus, there was a significant ($p < 0.01$) shift in the ADR dose-response curves to the right, at the lower concentrations. In contrast, AZA had no significant effect on NA and SAL dose-response curves. Compared with the control value, there was a significant increase ($p < 0.001$) in the pD_2 values for ADR in the presence of AZA, but there were no significant differences in the pD_2 values for NA and SAL (Table 5).

(d) Dioestrus (Figure 5)

In the presence of AZA, the ADR dose-response curve was shifted significantly ($p < 0.001$) to the right, at the lower concentrations. However, the maximum degree of inhibition produced by all three agonists was unaffected by AZA treatment. As in metoestrus, AZA had no significant effect on NA and SAL dose-response curves. There were no significant differences in the pD_2 values for the agonists, in the presence of AZA (Table 5).

α-receptor antagonism increased the maximum degree of inhibition produced by NA and ADR, but had no effect on SAL responses. Paradoxical shifts in the ADR dose-response curve to the right, at the lower concentrations, were observed in all phases of the oestrous cycle. α-receptor antagonism also produced changes in pD_2 values which reached significance for ADR (proestrus and oestrus) and NA (oestrus).

3. Inhibitory responses to NA, ADR and SAL in the presence of inhibitors of neuronal and extraneuronal uptake mechanisms

In the second series of experiments, desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M), were used to inhibit neuronal and extraneuronal uptake, respectively.

Dose-response curves and pD_2 values for the agonists in the presence of DMI and NMN, in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 2 to 5, and Table 6 respectively.

(a) Proestrus (Figure 2)

DMI and NMN shifted ADR and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. SAL, now produced complete inhibition of the ACh-induced contraction. For NA, only the maximum degree of inhibition was enhanced significantly ($p < 0.01$). There were no significant differences in the pD_2 values for the agonists, in the presence of DMI and NMN (Table 6).

(b) Oestrus (Figure 3)

DMI and NMN shifted NA, ADR and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. For ADR and SAL, complete inhibition of the ACh-induced contraction was achieved. Compared with control values, there were significant increases ($p < 0.001$) in the pD_2 values for ADR and SAL in the presence of DMI and NMN, but there was no significant difference in the pD_2 value for NA (Table 6).

(c) Metoestrus (Figure 4)

DMI and NMN shifted NA, ADR and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. SAL, now produced complete inhibition of the ACh-induced contraction. Compared with the control value, there was a significant increase ($p < 0.001$)

Table 6: Effect of desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M) on the potency of adrenoceptor agonists in the rat uterus (pD_2 values).

Phase	Drug	Control	n	After DMI+NMN	n
Proestrus	NA	7.95 ± 0.46	(4)	7.71 ± 0.35	(6)
	ADR	9.33 ± 0.09	(6)	9.29 ± 0.04	(8)
	SAL	9.14 ± 0.28	(6)	9.30 ± 0.27	(6)
Oestrus	NA	8.79 ± 0.04	(7)	8.46 ± 0.30	(6)
	ADR	9.53 ± 0.29	(6)	10.76 ± 0.04***	(4)
	SAL	8.39 ± 0.07	(4)	9.66 ± 0.10***	(6)
Metoestrus	NA	7.28 ± 0.04	(6)	8.51 ± 0.13***	(6)
	ADR	9.24 ± 0.09	(4)	9.18 ± 0.07	(6)
	SAL	9.00 ± 0.26	(8)	9.44 ± 0.08	(6)
Dioestrus	NA	7.95 ± 0.43	(6)	8.66 ± 0.07	(6)
	ADR	10.31 ± 0.34	(6)	10.09 ± 0.31	(4)
	SAL	9.58 ± 0.08	(8)	9.08 ± 0.26	(6)

Values are mean ± S.E.M.; n = number of observations.

in the pD_2 value for NA in the presence of DMI and NMN, but there were no significant differences in the pD_2 values for ADR and SAL (Table 6).

(d) Dioestrus (Figure 5)

DMI and NMN shifted NA, ADR and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. For ADR and SAL, complete inhibition of the ACh-induced contraction was achieved. There were no significant differences in the pD_2 values for the agonists, in the presence of DMI and NMN (Table 6).

Blockade of agonist removal mechanisms increased the maximum degree of inhibition produced by the three agonists in all phases of the cycle. The dose-response curves to these agonists were also shifted to the left, except for NA in proestrus. Shifts in the dose-response curves were, however, not reflected in corresponding changes in agonist pD_2 values since significant increases were achieved in only oestrus and metoestrus.

4. Inhibitory responses to NA, ADR and SAL in the combined presence of both an α-receptor antagonist and inhibitors of neuronal and extraneuronal uptake mechanisms

In the third series of experiments, both α-receptor activity, and neuronal and extraneuronal uptake mechanisms

were prevented with AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M), respectively.

Dose-response curves and pD_2 values for the agonists in the presence of these antagonists, in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 2 to 5, and Table 7 respectively.

(a) Proestrus (Figure 2)

The combined antagonists shifted NA, ADR and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. SAL gave a complete inhibition of the ACh-induced contraction. Compared with the control value, there was a significant increase ($p < 0.001$) in the pD_2 value for ADR in the presence of the combined antagonists, but there were no significant differences in the pD_2 values for NA and SAL (Table 7).

(b) Oestrus (Figure 3)

The combined antagonists shifted NA, ADR and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. ADR and SAL gave complete inhibition of the ACh-induced contraction. There were no significant differences in the pD_2 values for the agonists in the presence of the combined antagonists (Table 7).

Table 7: Effect of azapetine (AZA, 10^{-6}M), desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M) on the potency of adrenoceptor agonists in the rat uterus (pD_2 values).

Phase	Drug	Control	n	After AZA+DMI+NMN	n
Proestrus	NA	7.95 ± 0.46	(4)	8.30 ± 0.33	(6)
	ADR	9.33 ± 0.09	(6)	9.66 ± 0.03***	(8)
	SAL	9.14 ± 0.28	(6)	8.77 ± 0.27	(6)
Oestrus	NA	8.79 ± 0.04	(7)	8.78 ± 0.05	(6)
	ADR	9.53 ± 0.29	(6)	9.41 ± 0.10	(4)
	SAL	8.39 ± 0.07	(4)	8.86 ± 0.28	(6)
Metoestrus	NA	7.28 ± 0.04	(6)	8.73 ± 0.07***	(6)
	ADR	9.24 ± 0.09	(4)	9.65 ± 0.03***	(6)
	SAL	9.00 ± 0.26	(8)	9.64 ± 0.07***	(6)
Dioestrus	NA	7.95 ± 0.43	(6)	8.82 ± 0.03	(6)
	ADR	10.31 ± 0.34	(6)	9.66 ± 0.06	(4)
	SAL	9.58 ± 0.08	(8)	8.75 ± 0.40	(6)

Values are mean ± S.E.M.; n = number of observations.

(c) Metoestrus (Figure 4)

The combined antagonists shifted NA, ADR and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. NA and SAL gave complete inhibition of the ACh-induced contraction. Compared with control values, there were significant increases ($p < 0.001$) in the pD_2 values for all three agonists, in the presence of the combined antagonists (Table 7).

(d) Dioestrus (Figure 5)

The combined antagonists shifted NA and SAL dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. ADR dose-response curve was shifted to the right at the lower concentrations, and like NA and SAL, the maximum degree was enhanced significantly ($p < 0.001$). ADR and SAL gave complete inhibition of the ACh-induced contraction. There were no significant differences in the pD_2 values for the agonists in the presence of the combined antagonists (Table 7).

In the presence of combined α-receptor and $uptake_1$ and $uptake_2$ antagonists, the maximum degrees of inhibition produced by NA, ADR and SAL were increased in all phases of the cycle. Agonists dose-response curves were also shifted to the left, except for ADR in dioestrus, where an opposite shift was observed. Generally, the effects produced by the combined antagonists were no greater than those achieved in the presence of both $Uptake_1$ and $Uptake_2$ blockade.

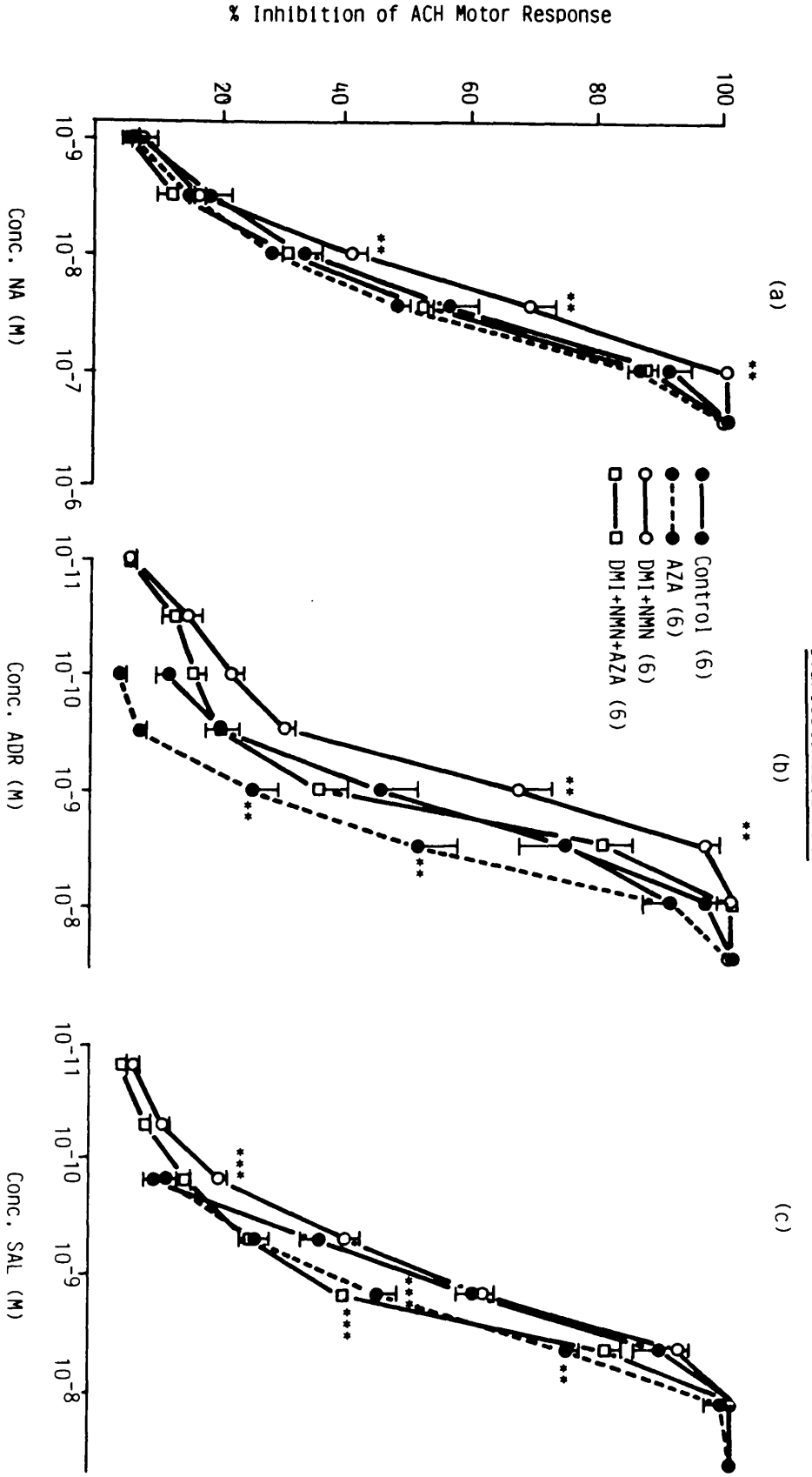
D. Responses to adrenoceptor agonists in uteri from ovariectomized animals

Variations in the degree of inhibition produced by NA and ADR persisted, even when α-receptors were blocked and/or uptake mechanisms were prevented. Since the uterus is under different ovarian hormonal influence in the four phases of the oestrous cycle, the possibility that the presence of the hormones could underlie the differences in adrenoceptor agonists responses was investigated, therefore, in rats after bilateral ovariectomy. As in intact animals, the same three series of experiments were carried out, viz: in the presence of (a), an α-receptor antagonist (b), inhibitors of both amine removal mechanisms, and (c), an α-receptor antagonist, and inhibitors of both amine removal mechanisms.

1. Inhibitory responses to NA, ADR and SAL

NA (10^{-9}M - 3 x 10^{-7}M), ADR (10^{-11}M - 3 x 10^{-8}M) and SAL (1.5 x 10^{-11}M - 5 x 10^{-8}M) produced dose-related inhibition of the standard ACh-induced contraction (Fig. 7). In contrast with the experiments in uteri from intact animals, all three agonists produced complete inhibition of the ACh motor response. The mean pD_2 values ($\pm$ S.E.M.) for NA, ADR and SAL were 8.34 $\pm$ 0.06 (n = 6), 9.81 $\pm$ 0.32 (n = 6) and 10.34 $\pm$ 0.36 (n = 6) respectively. SAL pD_2 values in

Fig. 7: Log. dose-response curves to noradrenaline, (a), adrenaline, (b) and salbutamol, (c) in uteri from ovariectomized rats: controls; in presence of azapetine (AZA, 10^{-6}M); in presence of desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M); in presence of AZA with DMI and NMN (all at 10^{-6}M). Number of observations in brackets.


% Inhibition of ACH Motor Response
Ovariectomized Rats
(a)
(b)
(c)
Control (6)
AZA (6)
DMI+NMN (6)
DMI+NMN+AZA (6)
Conc. NA (M)
Conc. ADR (M)
Conc. SAL (M)

ovariectomized animals were in general, higher than in the four phases of the oestrous cycle (see Table 4).

2. Inhibitory responses to NA, ADR and SAL in the presence of an α-receptor antagonist

In the first series of experiments, AZA (10^{-6}M) shifted significantly ADR ($p < 0.01$) and SAL ($p < 0.001$) dose-response curves to the right (Fig. 7). Compared with the control value, there was a significant decrease ($p < 0.01$) in the pD_2 value for SAL in the presence of AZA, but there were no significant differences in the pD_2 values for NA and ADR (Table 8).

3. Inhibitory responses to NA, ADR and SAL in the presence of inhibitors of neuronal and extraneuronal uptake mechanisms

In the second series of experiments, DMI (10^{-6}M) and NMN (10^{-6}M) shifted significantly ($p < 0.01$) NA and ADR dose-response curves to the left (Fig. 7). For SAL, only the responses to the lower concentrations were enhanced significantly ($p < 0.001$) in the presence of DMI and NMN. Compared with the control value, there was a significant increase ($p < 0.001$) in the pD_2 value for ADR in the presence of DMI and NMN, but there were no significant differences in the pD_2 values for NA and SAL (Table 9).

Table 8: Effect of azapetine (AZA, 10^{-6}M) on the potency of adrenoceptor agonists in uteri from ovariectomized rats (pD_2 values).

Drug	Control	After AZA	n
NA	8.34 ± 0.06	8.50 ± 0.03	6
ADR	9.81 ± 0.32	9.41 ± 0.07	6
SAL	10.34 ± 0.36	9.27 ± 0.04**	6

Values are mean ± S.E.M.; n = number of observations.

Table 9: Effect of desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M) on the potency of adrenoceptor agonists in uteri from ovariectomized rats (pD_2 values).

Drug	Control	n	After DMI + NMN	n
NA	8.34 ± 0.06	(6)	8.18 ± 0.04	(6)
ADR	9.81 ± 0.32	(6)	10.78 ± 0.05**	(4)
SAL	10.34 ± 0.36	(6)	10.29 ± 0.38	(6)

Values are mean ± S.E.M.; n = number of observations.

4. Inhibitory responses to NA, ADR and SAL in the combined presence of both an α-receptor antagonist and inhibitors of neuronal and extraneuronal uptake mechanisms

In the third series of experiments, the combined antagonists, i.e. AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M), shifted significantly ($p < 0.001$) the SAL dose-response curve to the right (Fig. 7). In contrast, the dose-response curves to NA and ADR were unaffected. Compared with the control value, there was a significant decrease ($p < 0.05$) in the pD_2 value for SAL in the presence of the combined antagonists, but there were no significant differences in the pD_2 values for NA and ADR (Table 10).

All three adrenoceptor agonists produced complete inhibition of the ACh-induced contraction in uteri from ovariectomized animals suggesting that hormonal influences underlie the differences in responses to NA, ADR and SAL.

One possible explanation was that the hormones were affecting the adrenoceptors, and thus the variation in the degrees of inhibition produced by the agonists would be reflected by corresponding changes in their pD_2 values. When the degrees of inhibition and pD_2 values were compared in the different hormonal states (Figs. 8, 9, 10), no such correlation was found. This would suggest that the events responsible for the altered agonist responses lay beyond the level of receptor activation.

Table 10: Effect of azapetine (AZA, 10^{-6}M), desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M) on the potency of adrenoceptor agonists in uteri from ovariectomized rats (pD_2 values).

Drug	Control	n	After AZA+DMI+NMN	n
NA	8.34 ± 0.06	(6)	8.42 ± 0.03	(6)
ADR	9.81 ± 0.32	(6)	9.16 ± 0.04	(4)
SAL	10.34 ± 0.36	(6)	9.31 ± 0.21*	(6)

Values are mean ± S.E.M.; n = number of observations.

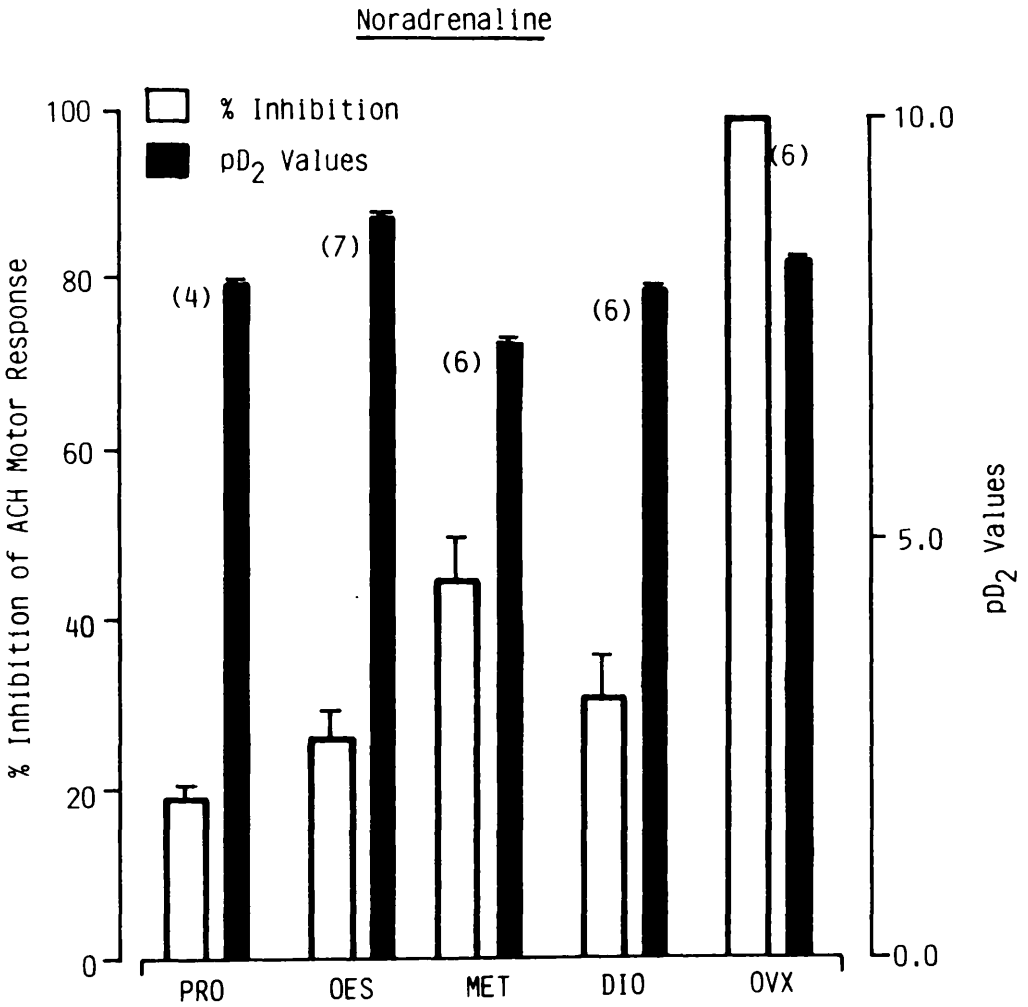


Fig. 8: Maximum degrees of inhibition of ACh-induced contraction produced by noradrenaline in uteri in the four phases of the oestrous cycle and after ovariectomy, and corresponding pD_2 values. Open columns = maximum degree of inhibition; filled columns = pD_2 values. PRO = proestrus; OES = oestrus; MET = metoestrus; DIO = dioestrus. Number of observations in brackets.

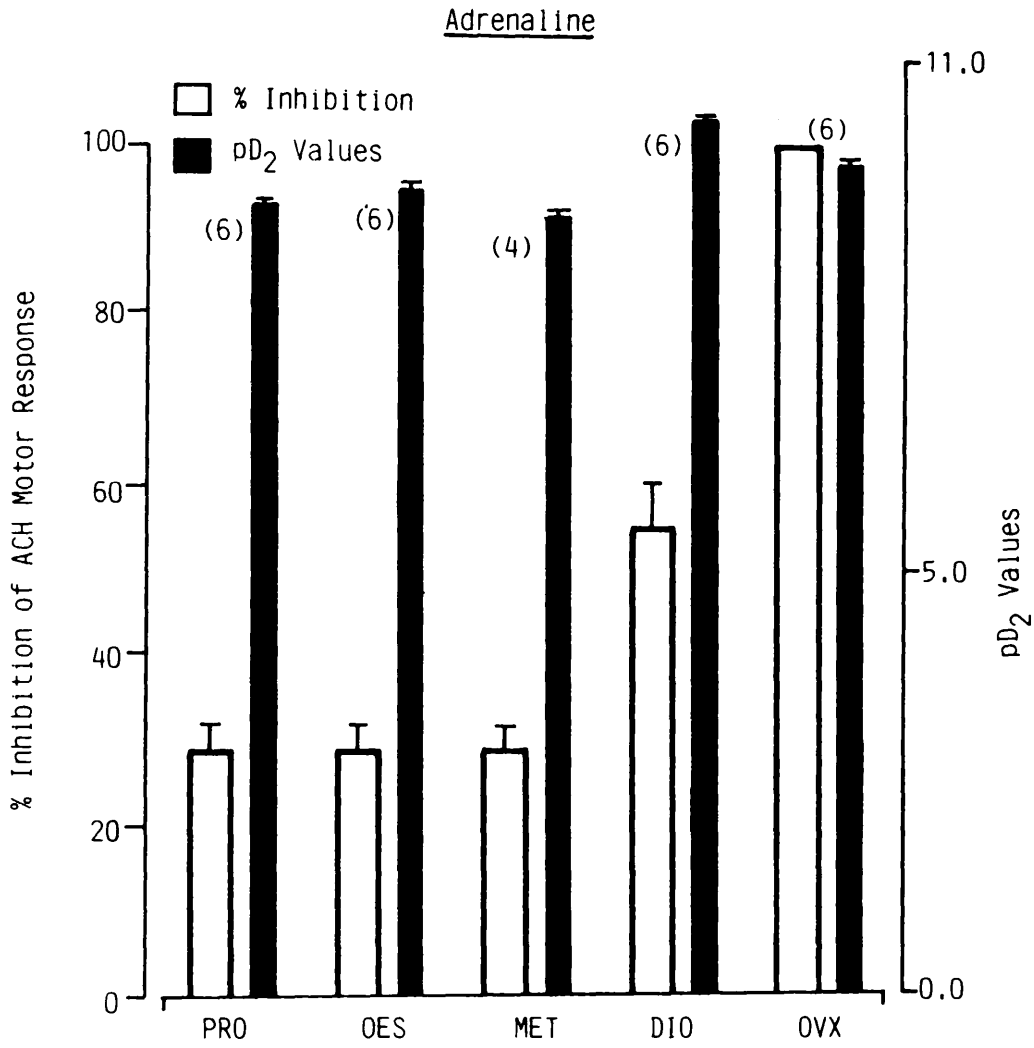


Fig. 9: Maximum degrees of inhibition of ACh-induced contraction produced by adrenaline in uteri in the four phases of the oestrous cycle and after ovariectomy, and corresponding pD_2 values. Open columns = maximum degree of inhibition; filled columns = pD_2 values. PRO = proestrus; OES = oestrus; MET = metoestrus; DIO = dioestrus. Number of observations in brackets.

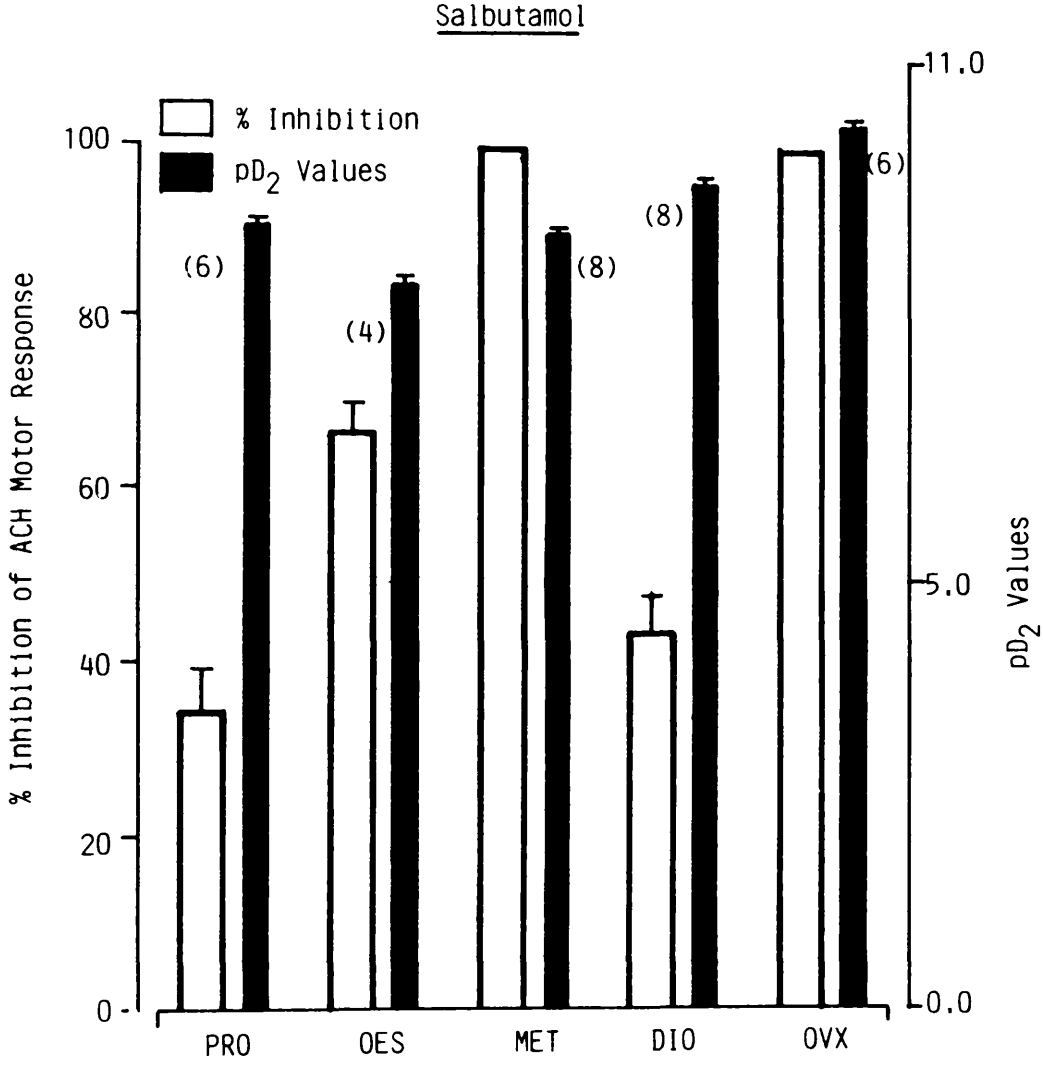


Fig. 10: Maximum degrees of inhibition of ACh-induced contraction produced by salbutamol in uteri in the four phases of the oestrous cycle and after ovariectomy, and corresponding pD_2 values. Open columns = maximum degree of inhibition; filled columns = pD_2 values. PRO = proestrus; OES = oestrus; MET = metoestrus; DIO = dioestrus. Number of observations in brackets.

E. Responses to adrenoceptor agonists in the presence of a cyclo-oxygenase inhibitor in the four phases of the oestrous cycle

The possibility that the adrenoceptor agonists were causing intramural generation of prostaglandins, which then opposed their inhibitory actions was investigated. Prostaglandin generation was blocked with the irreversible inhibitor of cyclo-oxygenase, flurbiprofen (FBF). FBF (10^{-6}M) had no effect on the motor responses to ACh. Dose-response curves were constructed to NA, ADR and SAL alone, i.e. Control I experiments (See Methods, C.1(b)), and to the three agonists in the presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M), i.e. Control II experiments (see Methods, C.1(e)).

(a) Proestrus

Dose-response curves and pD_2 values for the agonists in the absence, and presence of FBF are shown in Figures 11 to 13, and Tables 11 and 12, respectively.

Noradrenaline (Figure 11)

FBF shifted both Control I and Control II dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. In Control II experiments, complete inhibition of the ACh-induced contraction was achieved. There were no significant differences in both Control I and Control II pD_2 values for NA, in the presence of FBF (Tables 11 and 12).

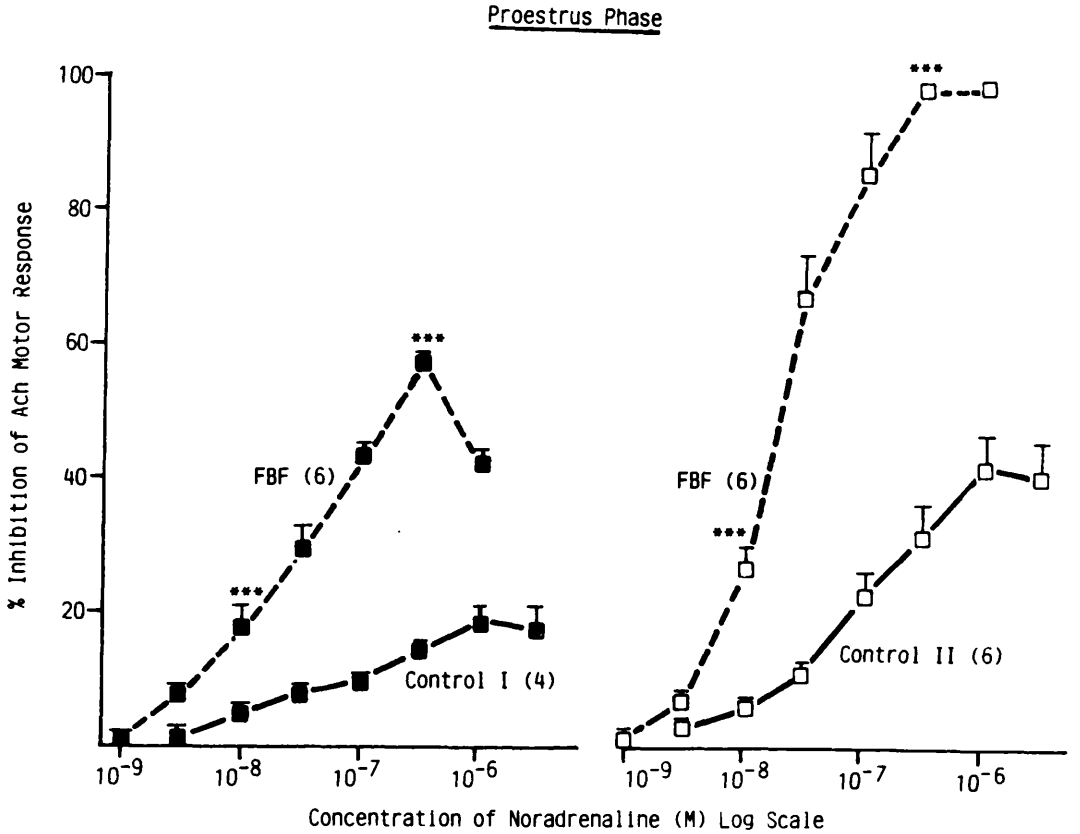


Fig. 11: Log. dose-response curves to noradrenaline (NA), in the rat isolated uterus in proestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = NA alone; Control II = NA, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

Table 11: Effect of flurbiprofen (FBF, 10^{-6}M) on the potency of adrenoceptor agonists in the rat uterus (pD_2 values).

Phase	Drug	Control I	n	After FBF	n
Proestrus	NA	7.95 ± 0.46	(4)	8.76 ± 0.20	(6)
	ADR	9.33 ± 0.09	(6)	10.90 ± 0.01***	(6)
	SAL	9.14 ± 0.28	(6)	9.63 ± 0.09	(6)
Oestrus	NA	8.79 ± 0.04	(7)	8.69 ± 0.02	(6)
	ADR	9.53 ± 0.29	(6)	10.90 ± 0.03***	(6)
	SAL	8.39 ± 0.07	(4)	9.53 ± 0.11***	(6)
Metoestrus	NA	7.28 ± 0.04	(6)	8.46 ± 0.06**	(6)
	ADR	9.24 ± 0.09	(4)	10.21 ± 0.30*	(6)
	SAL	9.00 ± 0.26	(8)	9.73 ± 0.25	(6)
Dioestrus	NA	7.95 ± 0.43	(6)	8.83 ± 0.05	(6)
	ADR	10.31 ± 0.34	(6)	10.70 ± 0.01	(6)
	SAL	9.58 ± 0.08	(8)	9.59 ± 0.05	(6)

Values are mean ± S.E.M.; n = number of observations.

Table 12: Effect of flurbiprofen (FBF, 10^{-6}M) on the potency of adrenoceptor agonists in the rat uterus (pD_2 values).

Phase	Drug	Control II	n	After FBF	n
Proestrus	NA	8.30 ± 0.33	(6)	8.37 ± 0.09	(6)
	ADR	9.66 ± 0.03	(8)	9.51 ± 0.15	(6)
	SAL	8.77 ± 0.27	(6)	9.74 ± 0.02***	(4)
Oestrus	NA	8.78 ± 0.05	(6)	8.61 ± 0.03	(6)
	ADR	9.41 ± 0.10	(4)	9.40 ± 0.10	(6)
	SAL	8.86 ± 0.28	(6)	9.80 ± 0.03***	(6)
Metoestrus	NA	8.73 ± 0.07	(6)	8.53 ± 0.02	(6)
	ADR	9.65 ± 0.03	(6)	9.43 ± 0.10	(6)
	SAL	9.64 ± 0.07	(6)	9.95 ± 0.30	(6)
Dioestrus	NA	8.82 ± 0.03	(6)	8.77 ± 0.04	(6)
	ADR	9.66 ± 0.06	(4)	9.94 ± 0.23	(6)
	SAL	8.75 ± 0.19	(6)	9.92 ± 0.03***	(6)

Values are mean ± S.E.M.; Control II = agonists in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M); n = number of observations.

Adrenaline (Figure 12)

FBF shifted both Control I and Control II dose-response curves to the left, and enhanced significantly ($p < 0.001$ and $p < 0.05$, respectively) their maximum degree of inhibition. In both control experimental conditions, complete inhibition of the ACh-induced contraction was achieved. In Control I experiments, there was a significant increase ($p < 0.001$) in the pD_2 values for ADR, in the presence of FBF (Table 11). In contrast, there was no significant difference in the Control II pD_2 value for ADR (Table 12).

Salbutamol (Figure 13)

FBF shifted significantly ($p < 0.001$) both Control I and Control II dose-response curves to the left. In Control I experiments, FBF also enhanced significantly ($p < 0.001$) the maximum degree of inhibition. In Control II experiments, there was a significant increase ($p < 0.001$) in the pD_2 value for SAL, in the presence of FBF (Table 12). In contrast, there was no significant difference in the Control I pD_2 value for SAL (Table 11).

(b) Oestrus

Dose-response curves and pD_2 values for the agonists in the absence, and presence of FBF are shown in Figures 14 to 16, and Tables 11 and 12, respectively.

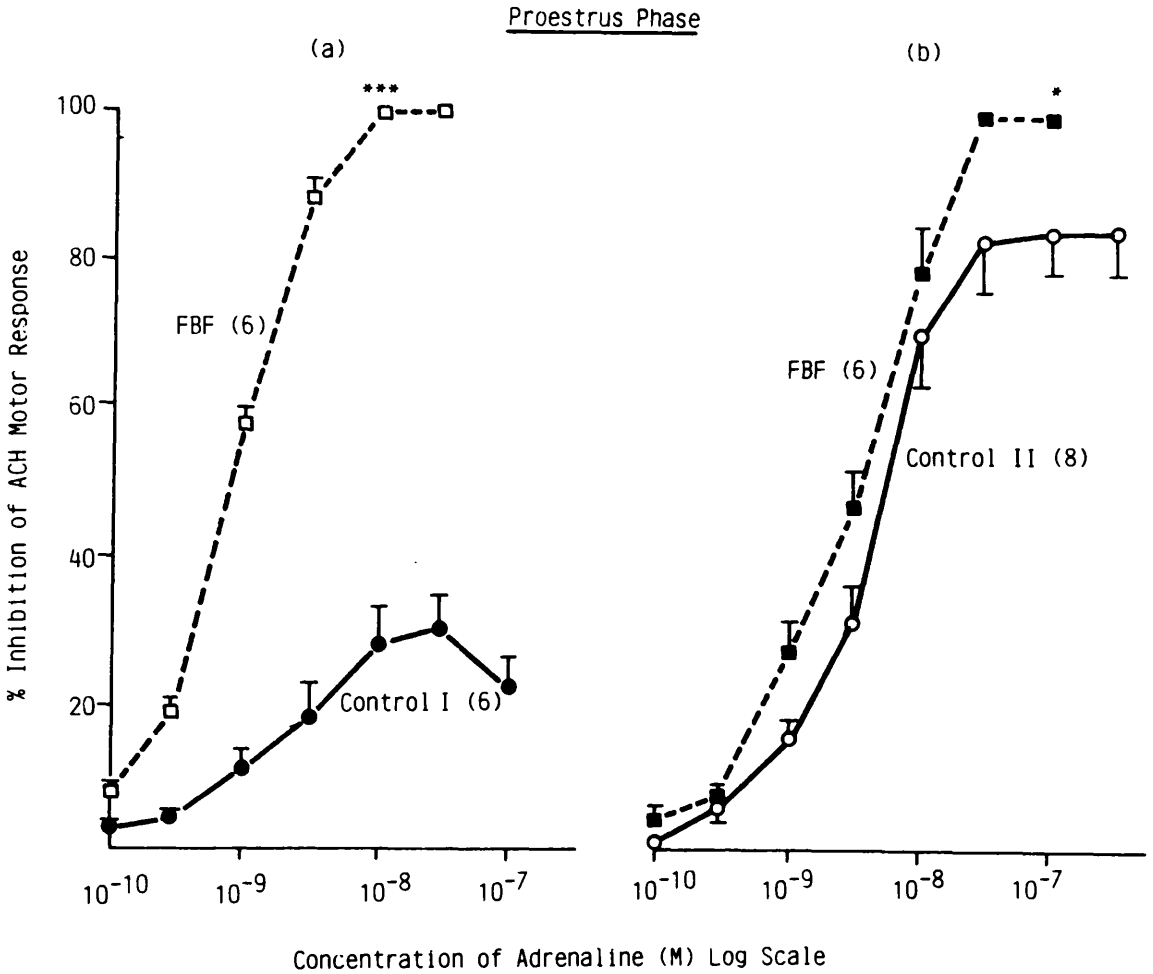


Fig. 12: Log. dose-response curves to adrenaline (ADR), in the rat isolated uterus in proestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = ADR alone; Control II = ADR, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

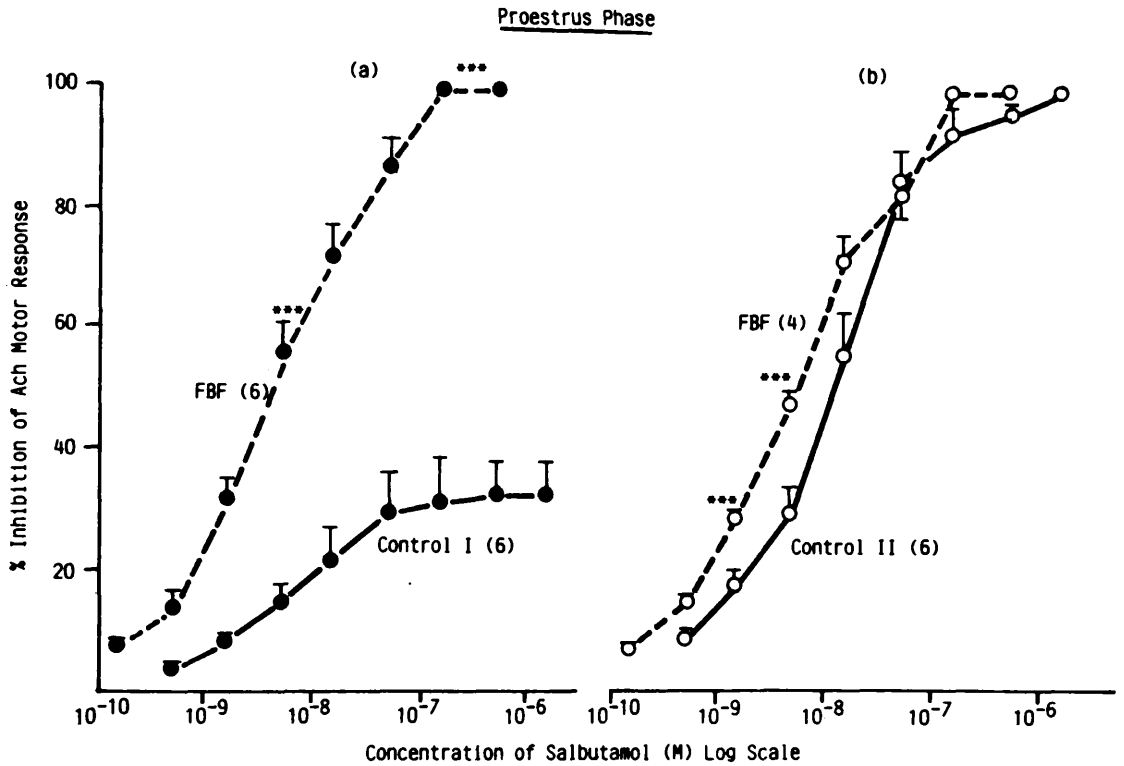


Fig. 13: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in proestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

Noradrenaline (Figure 14)

FBF shifted both Control I and Control II dose-response curves to the left, and enhanced significantly ($p < 0.001$) their maximum degree of inhibition. In Control II experiments, complete inhibition of the ACh-induced contraction was achieved. There were no significant differences in both Control I and Control II pD_2 values for NA, in the presence of FBF (Tables 11 and 12).

Adrenaline (Figure 15)

FBF shifted the Control I dose-response curve to the left, and enhanced significantly ($p < 0.001$) the maximum degree of inhibition. However, the Control II dose-response curve was unaffected by FBF treatment. In Control I experiments there was a significant increase ($p < 0.001$) in the pD_2 values for ADR, in the presence of FBF (Table 11). In contrast, there was no significant difference in the Control II pD_2 value for ADR (Table 12).

Salbutamol (Figure 16)

FBF shifted the Control I dose-response curve to the left, and enhanced significantly ($p < 0.001$) the maximum degree of inhibition. In Control II experiments, only a slight but non-significant leftward shift in the dose-response curve was achieved at the lower concentrations. In both Control I and Control II experiments, there was a significant

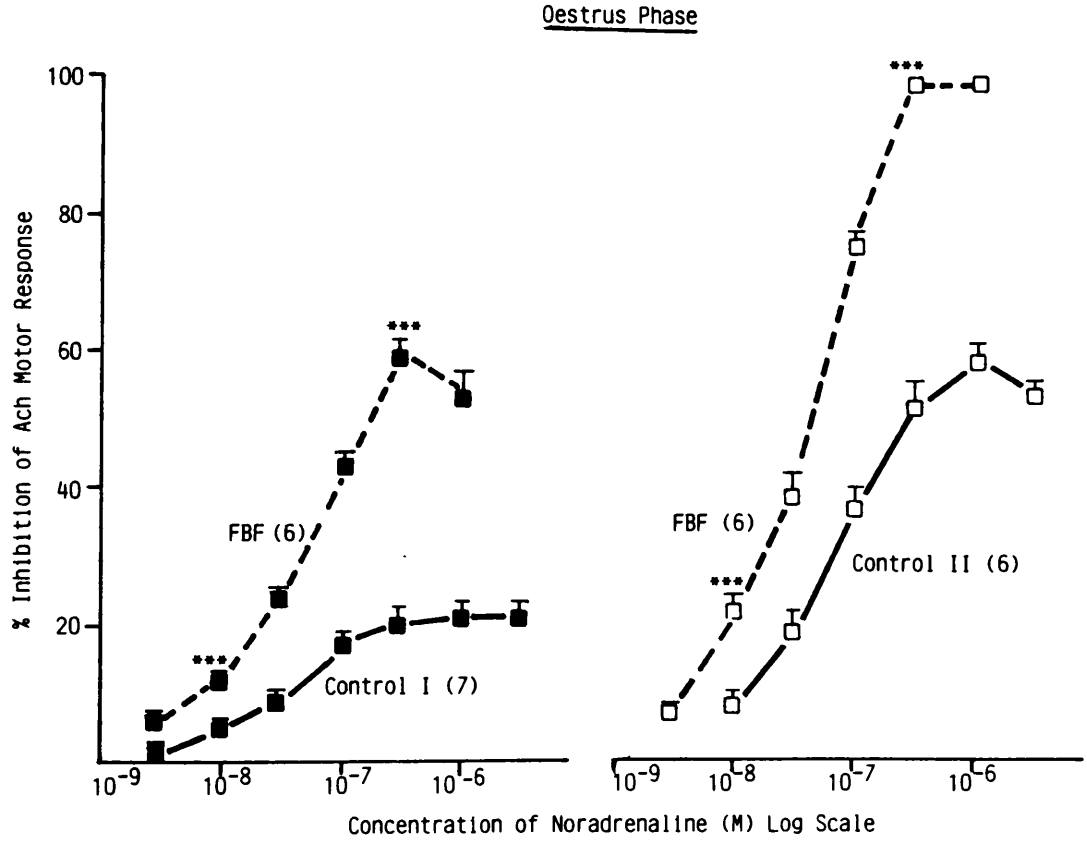


Fig. 14: Log. dose-response curves to noradrenaline (NA), in the rat isolated uterus in oestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = NA alone; Control II = NA, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

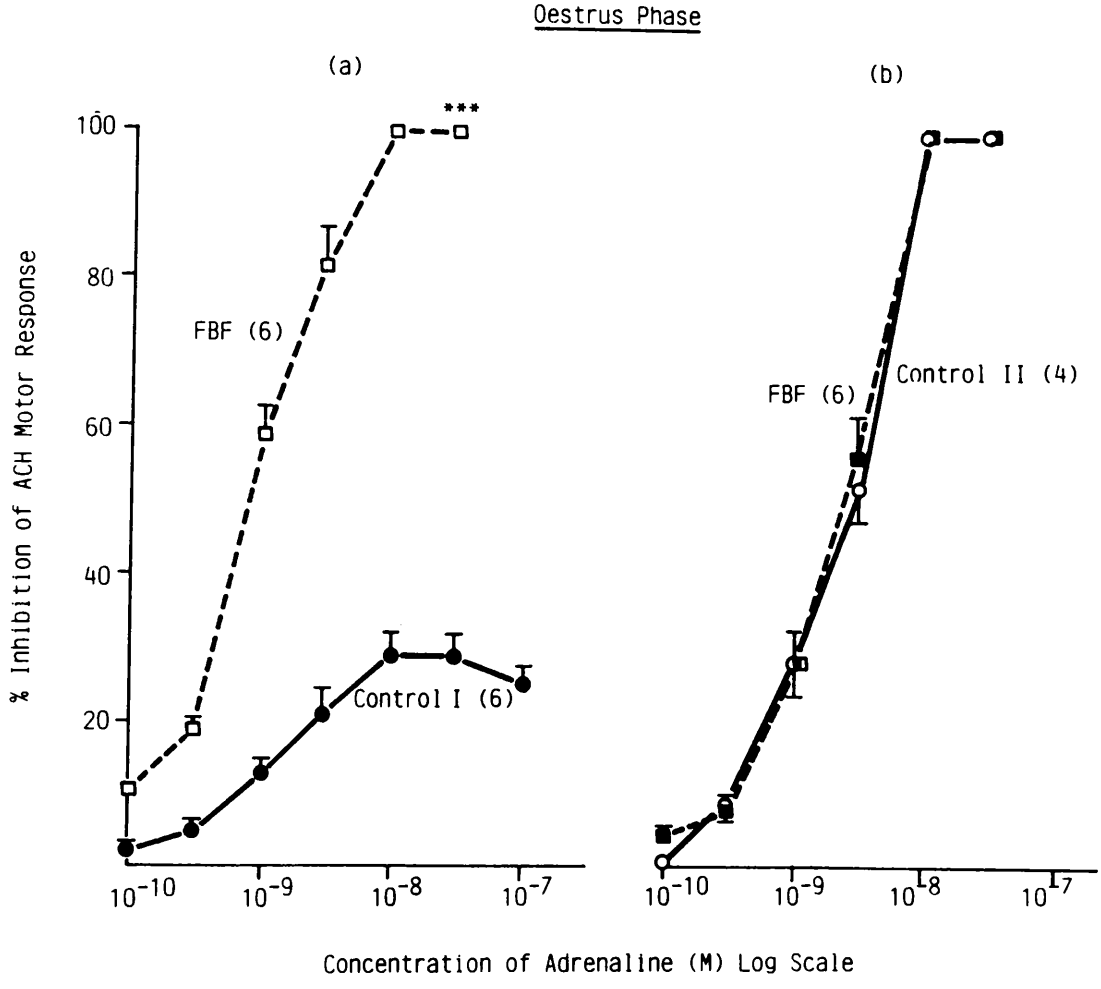


Fig. 15: Log. dose-response curves to adrenaline (ADR), in the rat isolated uterus in oestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = ADR alone; Control II = ADR, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

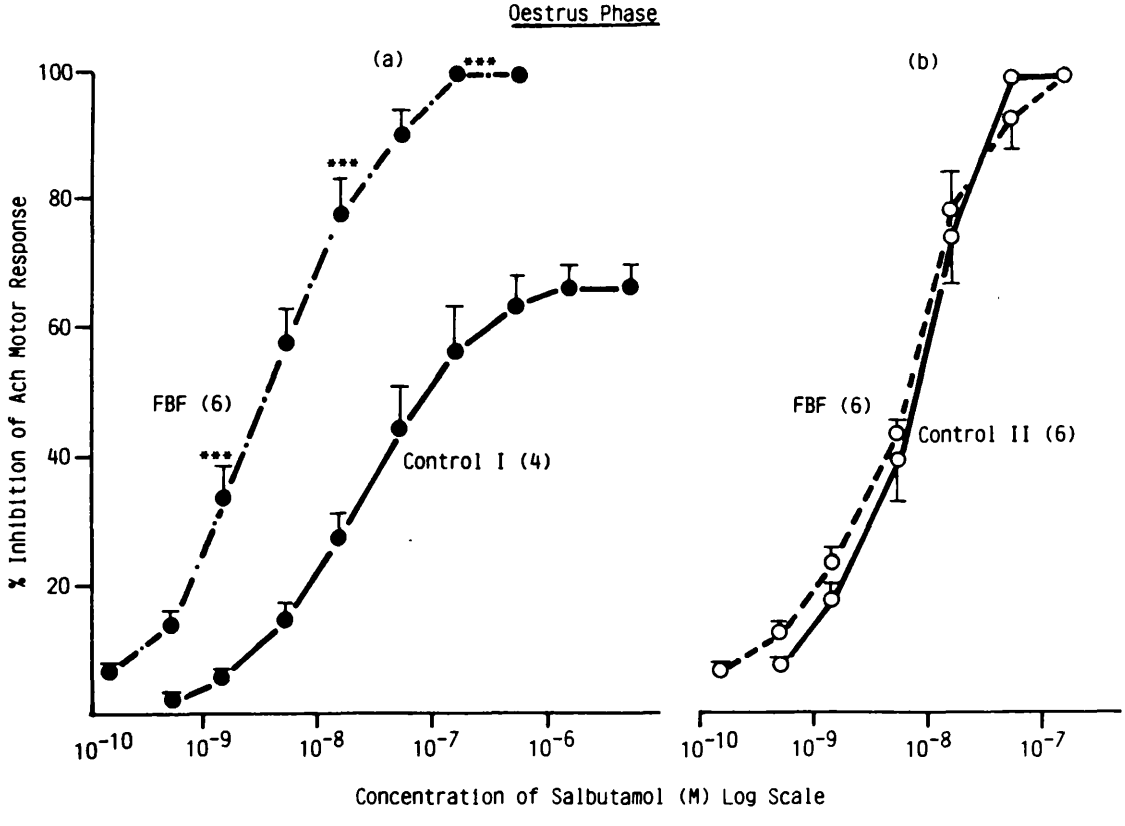


Fig. 16: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in oestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

increase ($p < 0.001$) in the pD_2 values for SAL, in the presence of FBF (Tables 11 and 12).

(c) Metoestrus

Dose-response curves and pD_2 values for the agonists in the absence, and presence of FBF are shown in Figures 17 to 19, and Tables 11 and 12, respectively.

Noradrenaline (Figure 17)

FBF shifted the Control I dose-response curve to the left, and enhanced significantly ($p < 0.001$) the maximum degree of inhibition. In Control II experiments, only a slight but non-significant leftward shift in the dose-response curve was achieved. In Control I experiments, there was a significant increase ($p < 0.01$) in the pD_2 value for NA, in the presence of FBF (Table 11). In contrast, there was no significant difference in the Control II pD_2 value for NA (Table 12).

Adrenaline (Figure 18)

FBF shifted both Control I and Control II dose-response curves to the left, and enhanced significantly ($p < 0.001$ and $p < 0.01$, respectively) their maximum degree of inhibition. In both control experimental conditions, complete inhibition of the ACh-induced contraction was achieved. In Control I experiments, there was a significant increase ($p < 0.05$) in the pD_2 values for ADR, in the presence of FBF (Table 11).

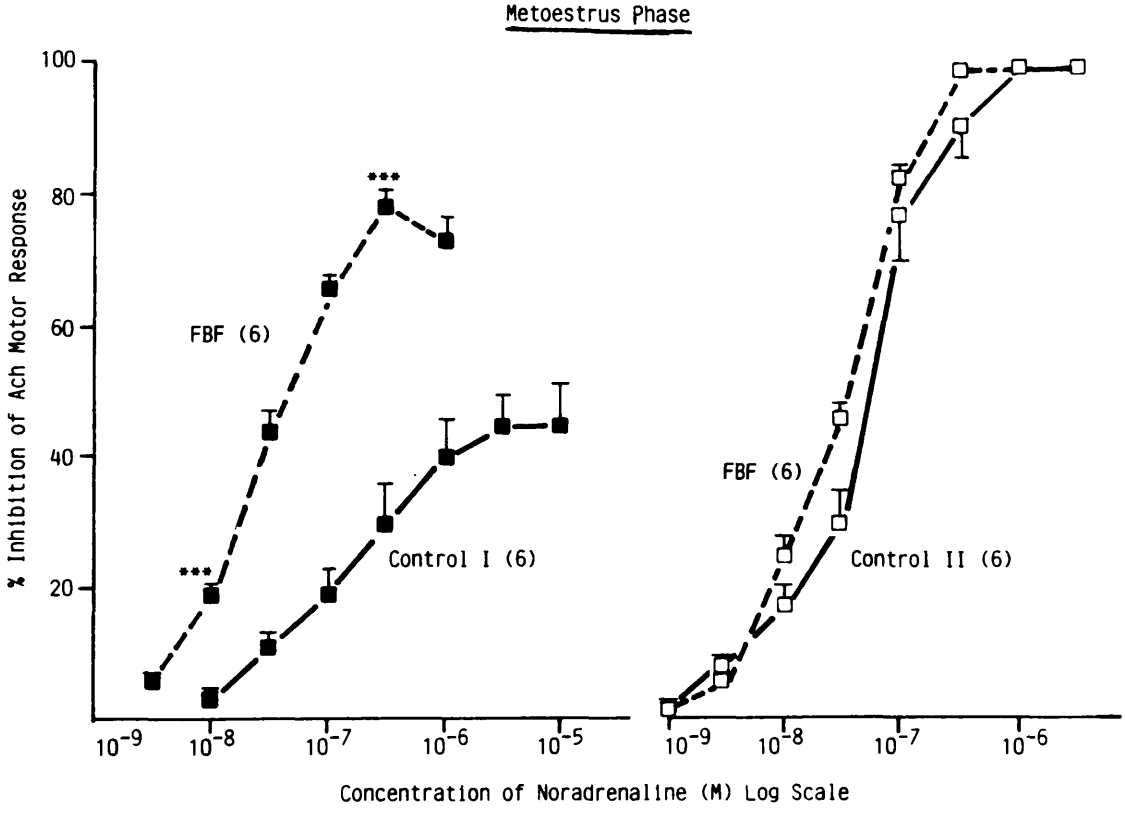


Fig. 17: Log. dose-response curves to noradrenaline (NA), in the rat isolated uterus in metoestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = NA alone; Control II = NA, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

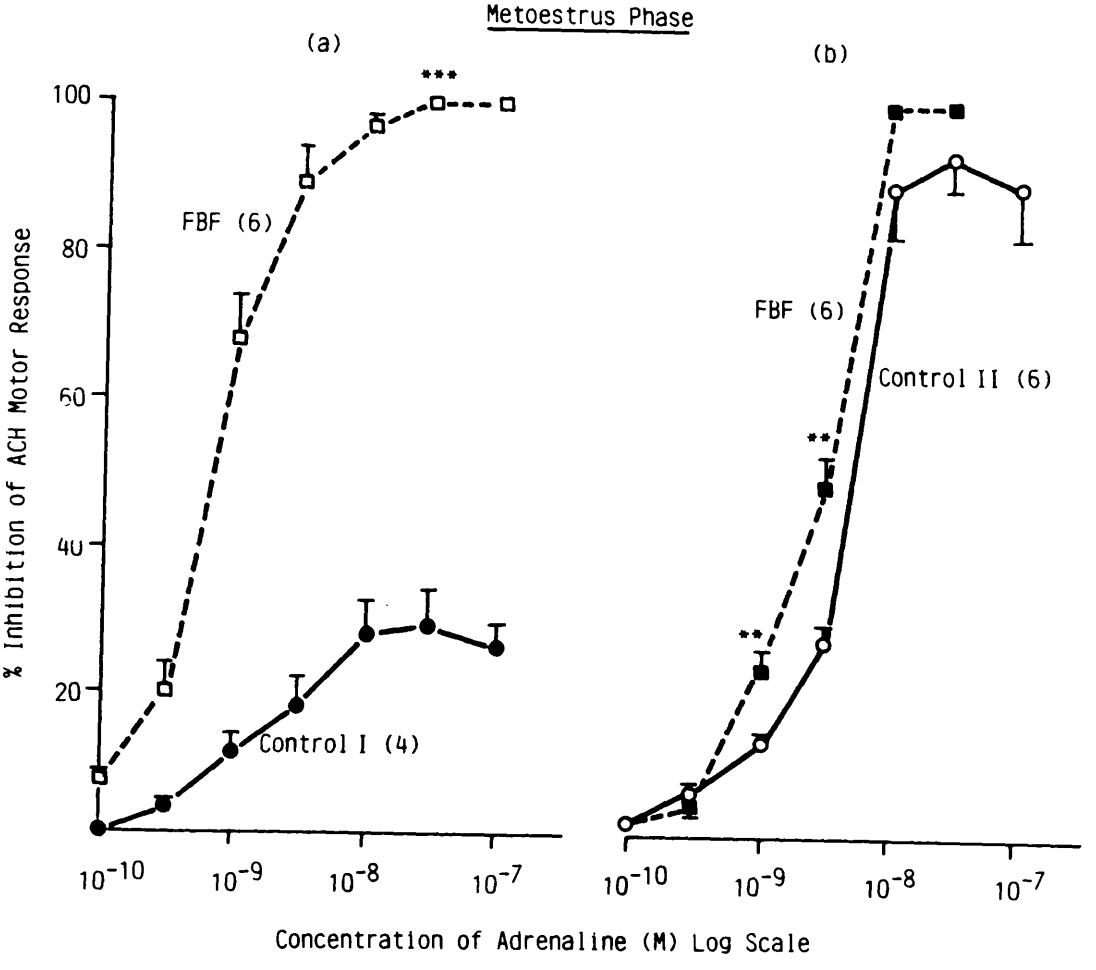


Fig. 18: Log. dose-response curves to adrenaline (ADR), in the rat isolated uterus in metoestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = ADR alone; Control II = ADR, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

In contrast, there was no significant difference in the Control II pD_2 value for ADR (Table 12).

Salbutamol (Figure 19)

FBF shifted significantly ($p < 0.001$) both Control I and Control II dose-response curves to the left. There were no significant differences in both Control I and Control II pD_2 values for SAL, in the presence of FBF (Tables 11 and 12).

(d) Dioestrus

Dose-response curves and pD_2 values for the agonists in the absence, and presence of FBF are shown in Figures 20 to 22, and in Tables 11 and 12, respectively.

Noradrenaline (Figure 20)

FBF shifted both Control I and Control II dose-response curves to the left, and enhanced significantly ($p < 0.001$ and $p < 0.01$, respectively) their maximum degree of inhibition. In Control II experiments, complete inhibition of the ACh-induced contraction was achieved. There were no significant differences in both Control I and Control II pD_2 values for NA, in the presence of FBF (Tables 11 and 12).

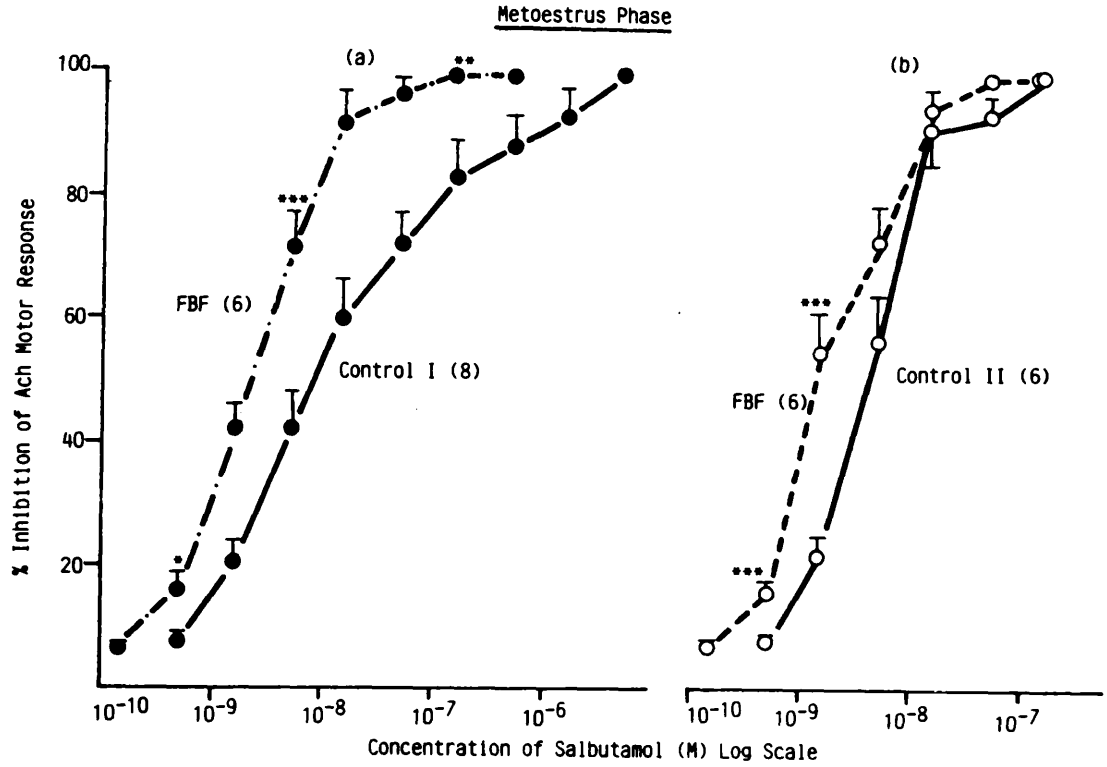


Fig. 19: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in metoestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

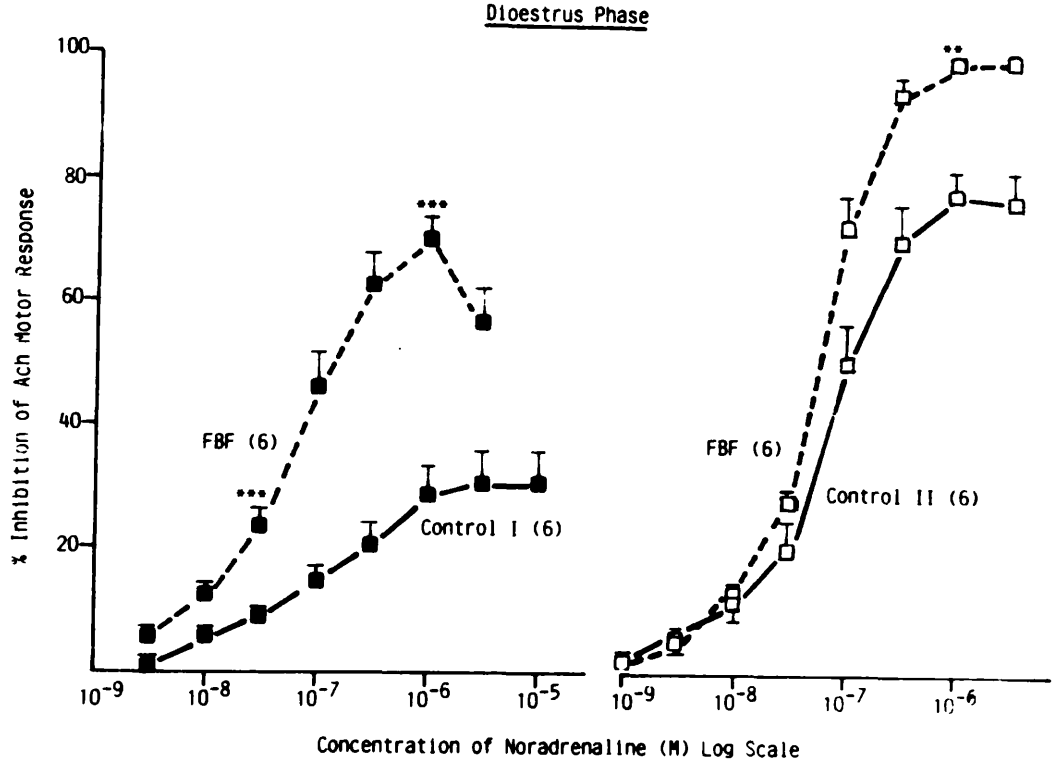


Fig. 20: Log. dose-response curves to noradrenaline (NA), in the rat isolated uterus in dioestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = NA alone; Control II = NA, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

Adrenaline (Figure 21)

FBF shifted the Control I dose-response curve to the left, and enhanced significantly ($p < 0.001$) the maximum degree of inhibition. In Control II experiments, FBF only shifted significantly ($p < 0.001$) the dose-response curve to the left. There were no significant differences in both Control I and Control II pD_2 values for ADR, in the presence of FBF (Tables 11 and 12).

Salbutamol (Figure 22)

FBF shifted the Control I dose-response curve to the left, and enhanced significantly ($p < 0.001$) the maximum degree of inhibition. In Control II experiments only responses to the higher concentrations of SAL were enhanced significantly ($p < 0.01$). In Control II experiments, there was a significant increase ($p < 0.001$) in the pD_2 value for SAL, in the presence of FBF (Table 12). In contrast, there was no significant difference in the Control I pD_2 value for SAL (Table 11).

Cyclo-oxygenase inhibition shifted the agonist dose-response curves to the left, and increased their maximum degree of inhibition when no antagonists were present, i.e. in Control I experiments. Since the combined antagonists (Control II experiments) had already produced leftward shifts in the dose-response curves, and increased inhibition, cyclo-oxygenase inhibition produced lesser effects in the Control II, than in Control I experiments.

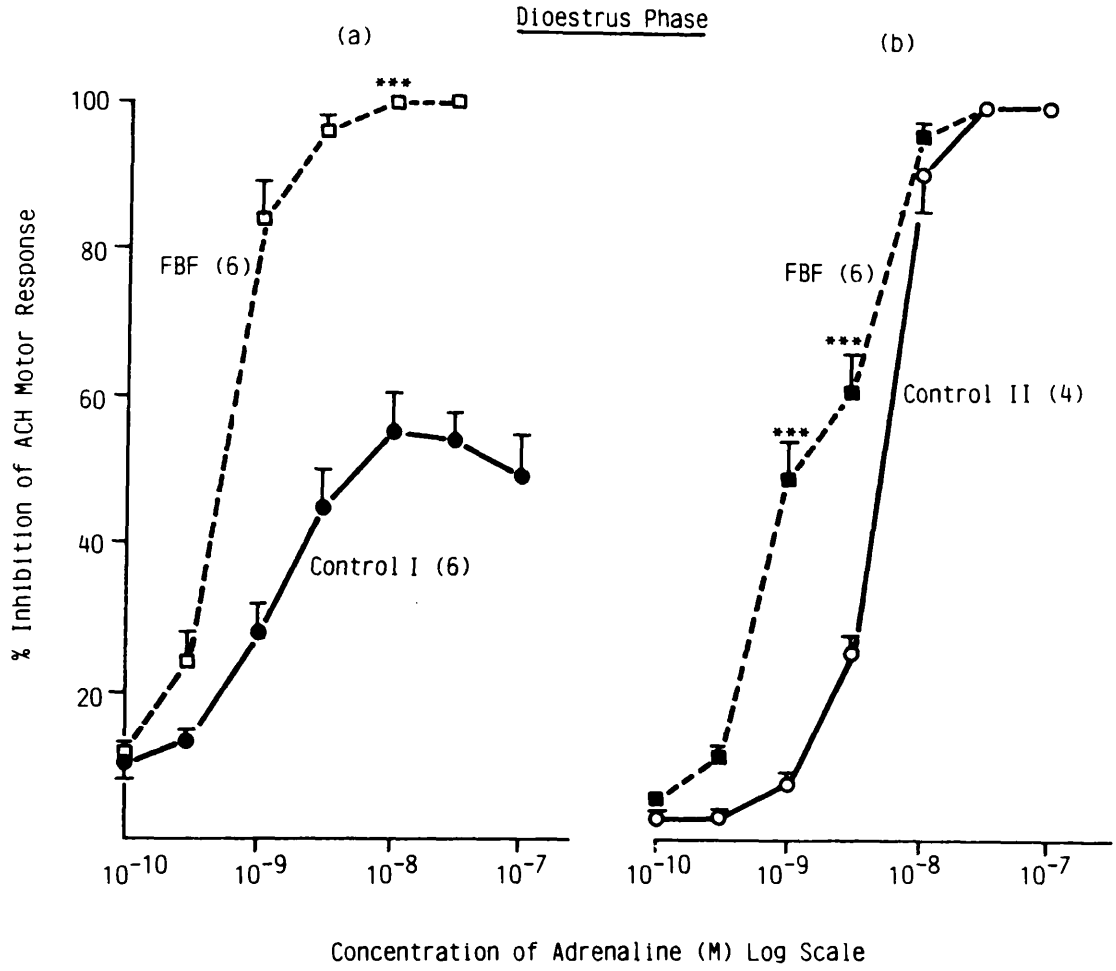


Fig. 21: Log. dose-response curves to adrenaline (ADR), in the rat isolated uterus in dioestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = ADR alone; Control II = ADR, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

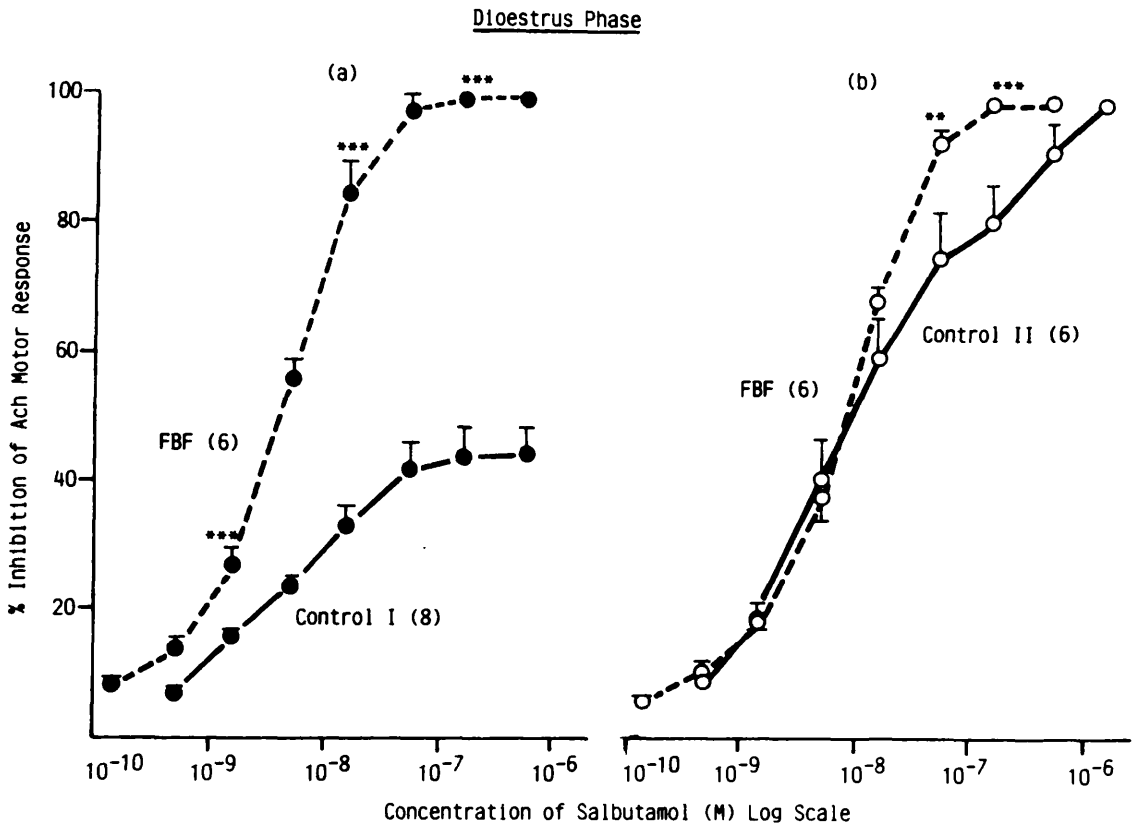


Fig. 22: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in dioestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

F. Responses to adrenoceptor agonists in the presence of a cyclo-oxygenase inhibitor in uteri from ovariectomized animals

Since the ovarian hormones affected the degree of inhibition produced by the adrenoceptor agonists, the possible role of the prostaglandins was examined in uteri from ovariectomized animals. Dose-response curves to the agonists were obtained in the absence (Control I experiments), and presence (Control II experiments) of the combined antagonists and cyclo-oxygenase activity was inhibited with FBF (10^{-6}M).

Dose-response curves and pD_2 values for the agonists in the absence, and presence of FBF are shown in Figures 23 to 25, and Table 13, respectively.

Noradrenaline (Figure 23)

FBF shifted significantly ($p < 0.001$) the Control II dose-response curve to the left, but had no effect on the Control I curve. There were no significant differences in both Control I and Control II pD_2 values for NA, in the presence of FBF (Table 13).

Adrenaline (Figure 24)

FBF shifted significantly both Control I ($p < 0.001$) and Control II ($p < 0.05$, higher concentrations only)

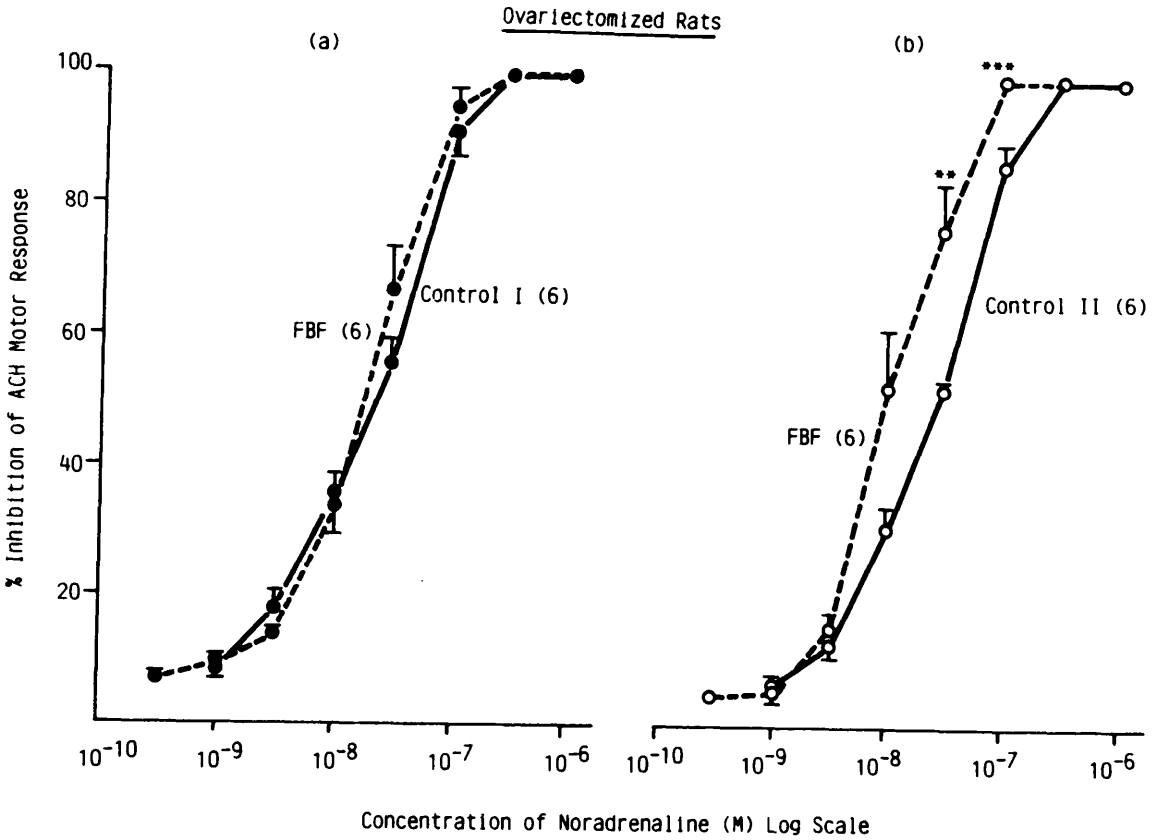


Fig. 23: Log. dose-response curves to noradrenaline (NA) in uteri from ovariectomized rats: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = NA alone; Control II = NA, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

Table 13: Effect of flurbiprofen (FBF, 10^{-6}M) on the potency of adrenoceptor agonists in uteri from ovariectomized rats (pD_2 values).

Experiment	Drug		
	NA	ADR	SAL
Control I	8.34 ± 0.06 (6)	9.81 ± 0.32 (6)	10.34 ± 0.36 (6)
After FBF	8.28 ± 0.07 (6)	10.73 ± 0.04**(6)	10.88 ± 0.04 (6)
Control II	8.42 ± 0.03 (6)	9.16 ± 0.04 (4)	9.31 ± 0.01 (6)
After FBF	8.72 ± 0.30 (6)	10.29 ± 0.34**(6)	9.95 ± 0.37 (6)

Values are mean ± S.E.M.; Control I = agonists alone; Control II = agonists in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M); number of observations in brackets.

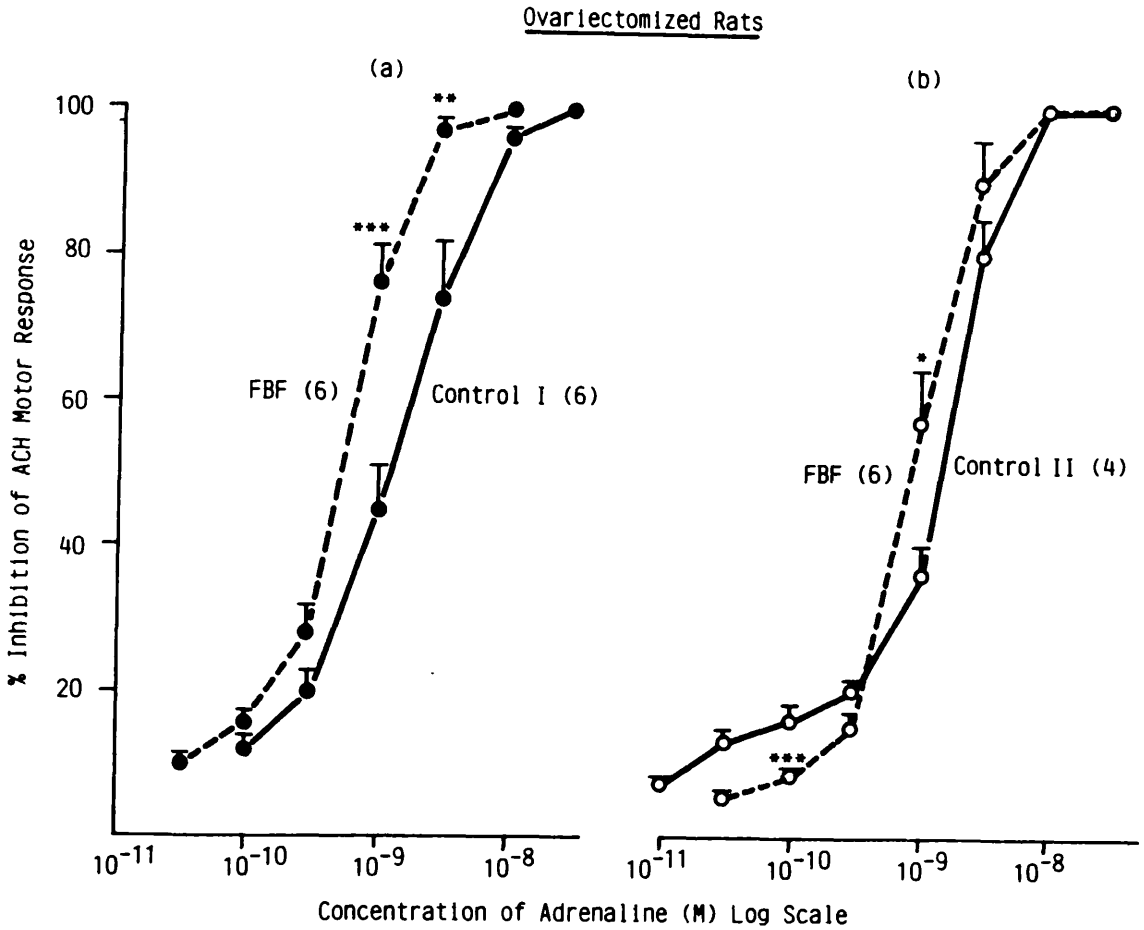


Fig. 24: Log. dose-response curves to adrenaline (ADR) in uteri from ovariectomized rats: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = ADR alone, Control II = ADR, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

dose-response curves to the left. However, the Control II dose-response curve was also shifted significantly ($p < 0.001$) to the right, at the lower concentrations. In both Control I and Control II experiments, there was a significant increase ($p < 0.01$) in the pD_2 values for ADR, in the presence of FBF (Table 13).

Salbutamol (Figure 25)

FBF shifted significantly both Control I ($p<0.05$) and Control II ($p < 0.001$) dose-response curves to the left. However, the increases in both Control I and Control II pD_2 values for SAL in the presence of FBF, were not statistically significant (Table 13).

Since the adrenoceptor agonists produced 100% inhibition of the ACh-induced contraction in uteri from ovariectomized animals, the effects of FBF could be seen only in terms of shifts in the dose-response curves, and changes in pD_2 values. Cyclo-oxygenase inhibition enhanced agonist responses in these uteri suggesting that prostaglandin production induced by NA, ADR and SAL was independent of the hormonal status of the animal.

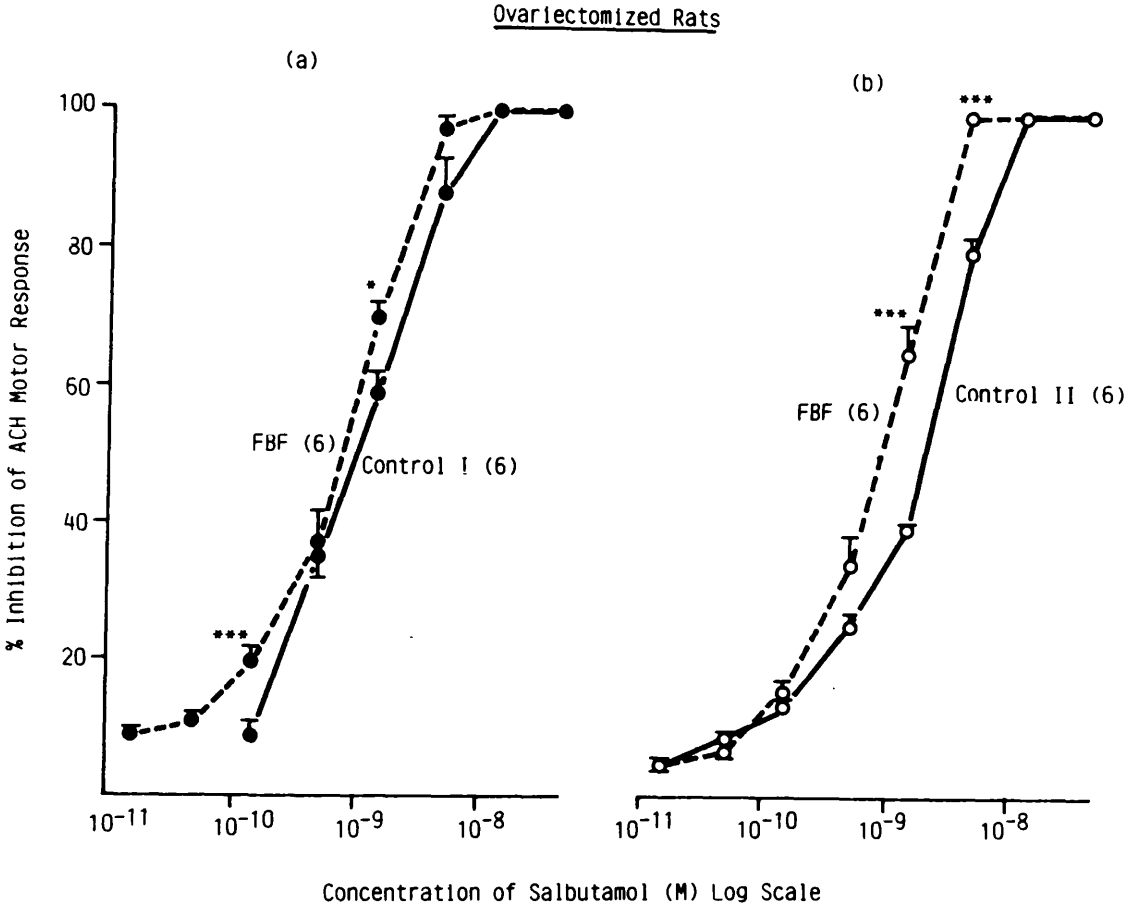


Fig. 25: Log. dose-response curves to salbutamol (SAL) in uteri from ovariectomized rats: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

G. Responses to histamine in the presence of a cyclo-oxygenase inhibitor in the four phases of the oestrous cycle

Uterine relaxants other than adrenoceptor agonists may affect intramural prostaglandin production. Hence, a series of experiments was carried out using histamine (HIS) which acts via H_2-receptors. The inhibitory effect produced by HIS was examined in the presence of H_1-receptor antagonist, mepyramine (5×10^{-8}M).

1. Inhibitory responses to histamine

Dose-response curves and pD_2 values for histamine are shown in Figures 26 and 27, and Table 14, respectively.

(a) Proestrus (Figure 26A)

HIS (10^{-7}M - 3×10^{-4}M) produced dose-related inhibition of the standard ACh-induced contraction with the maximum degree of inhibition being approximately 40%.

(b) Oestrus (Figure 26B)

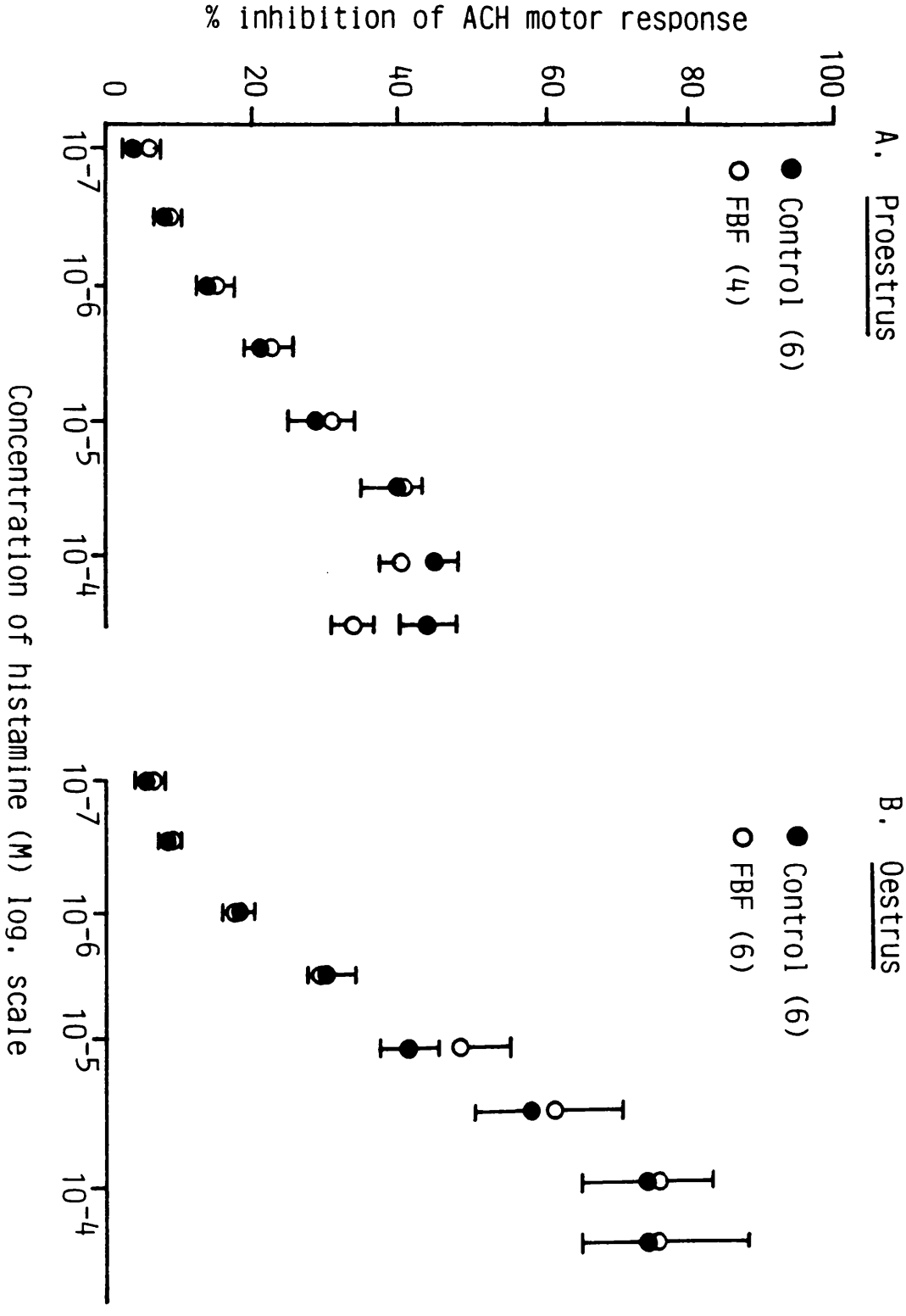
HIS (10^{-7}M - 3×10^{-4}M) produced dose-related inhibition of the standard ACh-induced contraction with the maximum degree of inhibition being approximately 70%.

Table 14: Effect of flurbiprofen (FBF, 10^{-6}M) on histamine (HIS) potency in the rat uterus (pD_2 values).

Phase	Control	After FBF	n
Proestrus	6.32 ± 0.23	6.32 ± 0.07	6
Oestrus	6.41 ± 0.31	6.05 ± 0.25	6
Metoestrus	6.58 ± 0.13	6.04 ± 0.26	8
Dioestrus	6.42 ± 0.22	6.47 ± 0.23	6

Values are mean ± S.E.M.; n = number of observations.

Fig. 26: Log. dose-response curves to histamine, in the rat isolated uterus in proestrus (A) and oestrus (B): controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Number of observations in brackets.


% inhibition of ACH motor response
0
20
40
60
80
100
A. Proestrus
● Control (6)
○ FBF (4)
B. Oestrus
● Control (6)
○ FBF (6)
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Concentration of histamine (M) log. scale

(c) Metoestrus (Figure 27A)

HIS (10^{-7}M - 3 x 10^{-4}M) produced dose-related inhibition of the standard ACh-induced contraction with the maximum degree of inhibition being approximately 80%.

(d) Dioestrus (Figure 27B)

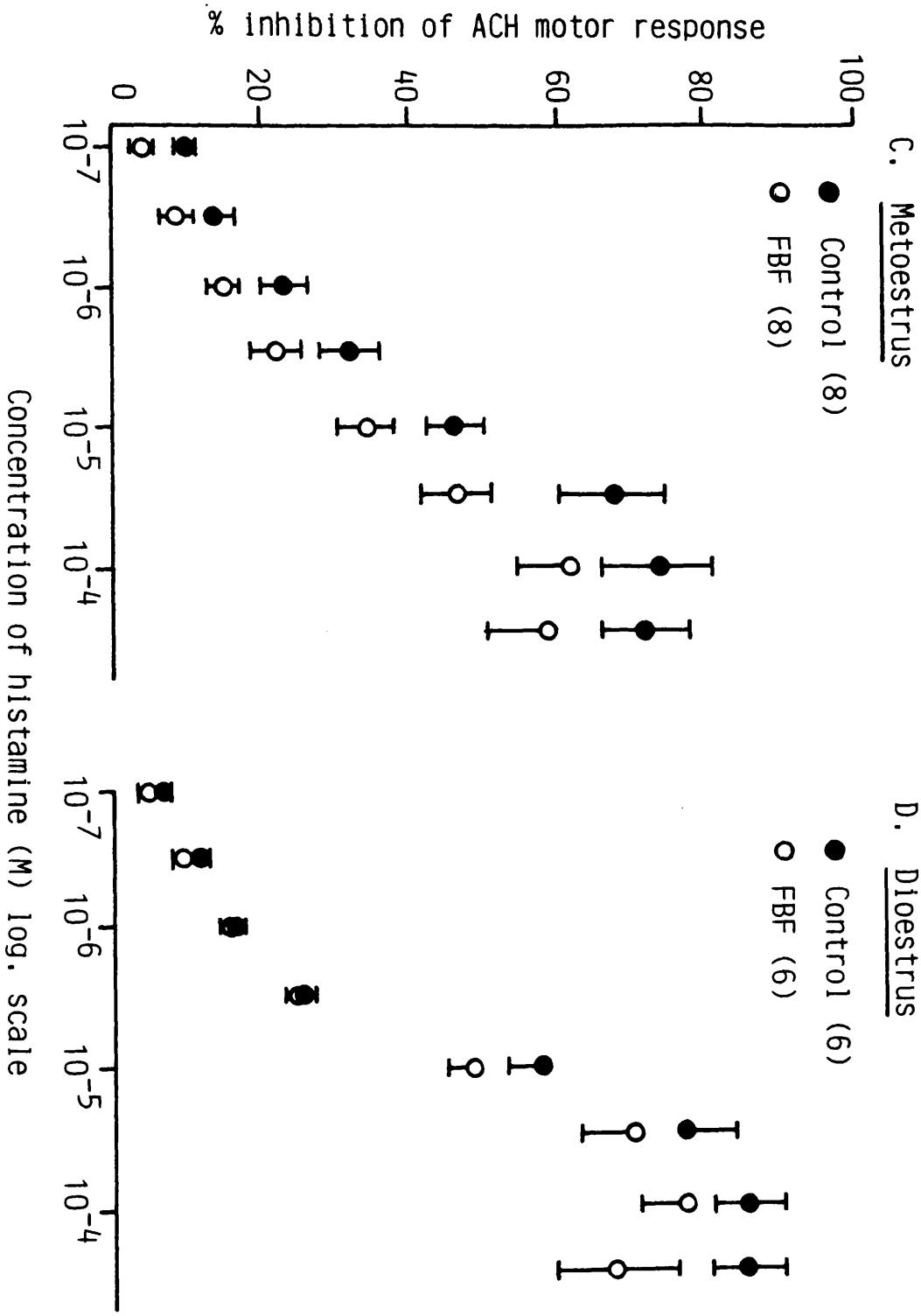
HIS (10^{-7}M - 3 x 10^{-4}M) produced dose-related inhibition of the standard ACh-induced contraction. As in metoestrus, the maximum degree of inhibition achieved was approximately 80%.

2. Inhibitory responses to histamine in the presence of a cyclo-oxygenase inhibitor

Dose-response curves to HIS in proestrus, oestrus (Fig. 26), metoestrus and dioestrus (Fig. 27) were unaffected by FBF treatment. There were no significant differences in the pD_2 values for HIS in the presence of FBF, in the four phases of the oestrous cycle.

Although the maximum degree of inhibition produced by histamine varied throughout the oestrous cycle, cyclo-oxygenase inhibition did not affect these responses.

Fig. 27: Log. dose-response curves to histamine, in the rat isolated uterus in metoestrus (C) and dioestrus (D): controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Number of observations in brackets.


% inhibition of ACH motor response
0
20
40
60
80
100
C. Metoestrus
Control (8)
FBF (8)
D. Dioestrus
Control (6)
FBF (6)
10^{-7}
10^{-6}
10^{-5}
10^{-4}
Concentration of histamine (M) log. scale

H. Responses to papaverine in the presence of a cyclo-oxygenase inhibitor in the four phases of the oestrous cycle

Since both the adrenoceptor agonists and histamine acted via receptors, the effect of papaverine (PAP), a non-specific smooth muscle relaxant, was examined.

1. Inhibitory responses to papaverine

PAP (3×10^{-7}M - 10^{-4}M) produced dose-related inhibition of the standard ACh motor response in all four phases. The dose-response curves and pD_2 values for PAP are shown in Figure 28, and Table 15, respectively. Unlike the adrenoceptor agonists and histamine, PAP produced complete inhibition of the ACh-induced contraction in each phase.

2. Inhibitory responses to papaverine in the presence of a cyclo-oxygenase inhibitor

Dose-response curves to PAP in proestrus, oestrus, metoestrus and dioestrus were not affected by FBF treatment (Fig. 28). There were no significant differences in the pD_2 values for PAP in the presence of FBF (Table 15).

As with HIS , cyclo-oxygenase inhibition did not affect PAP responses in the four phases of the oestrous cycle. The inability of FBF to modify the responses to HIS and PAP, unlike those to the adrenoceptor agonists, suggested that an effect on intramural prostaglandin production was not initiated by all uterine relaxants.

Fig. 28: Log. dose-response curves to papaverine, in the rat isolated uterus in proestrus (a), oestrus (b), metoestrus (c) and dioestrus (d): controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Number of observations in brackets.

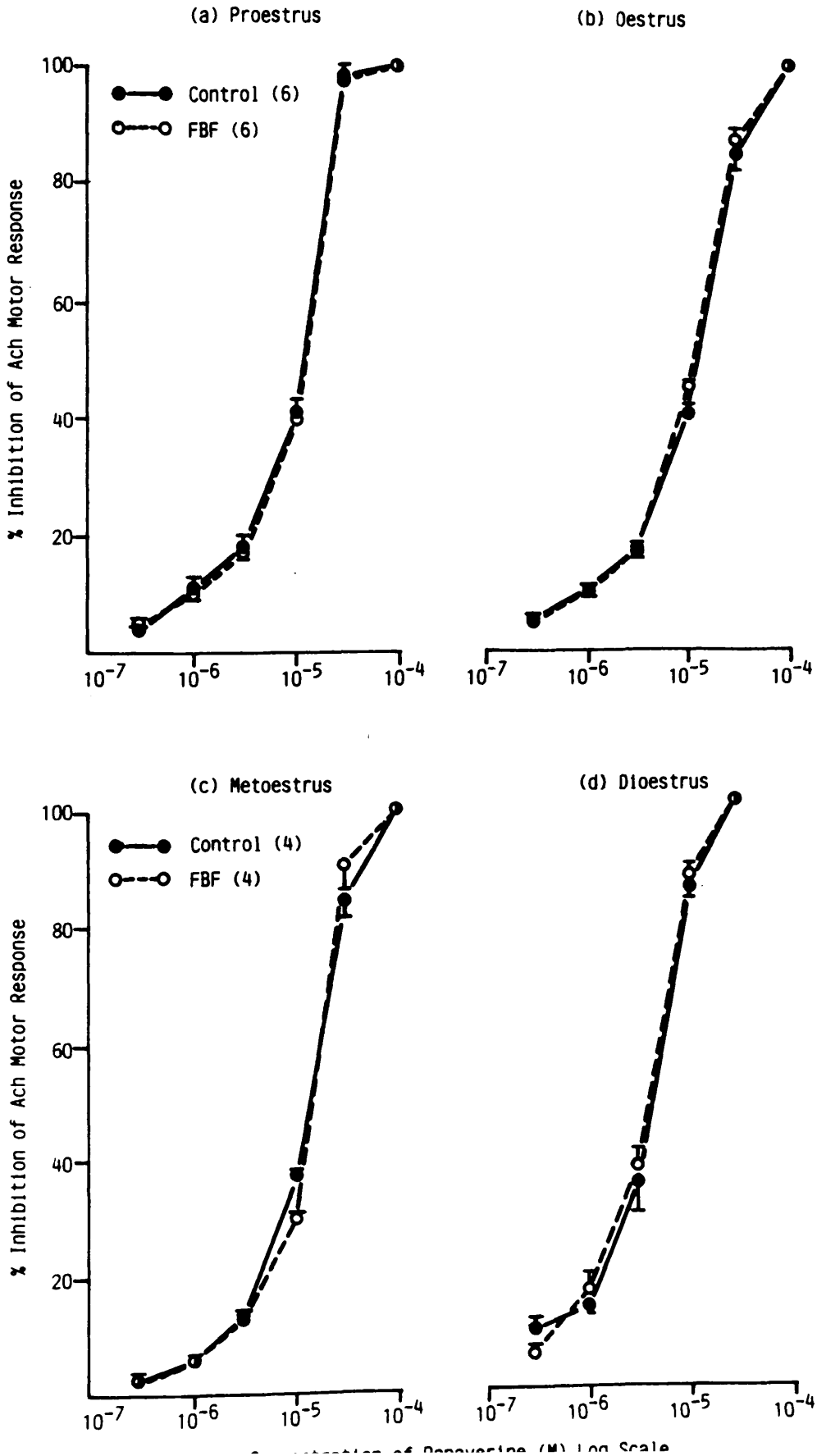

(a) Proestrus
(b) Oestrus
(c) Metoestrus
(d) Dioestrus
Control (6)
FBF (6)
Control (4)
FBF (4)
% Inhibition of Ach Motor Response
100
80
60
40
20
10^{-7}
10^{-6}
10^{-5}
10^{-4}

Table 15: Effect of flurbiprofen (FBF, 10^{-6}M) on papaverine (PAP) potency in the rat uterus (pD_2 values).

Phase	Control	After FBF	n
Proestrus	5.08 ± 0.01	5.09 ± 0.01	6
Oestrus	5.11 ± 0.02	5.06 ± 0.01	6
Metoestrus	5.13 ± 0.01	5.16 ± 0.03	4
Dioestrus	6.64 ± 0.04	6.60 ± 0.04	4

Values are mean ± S.E.M.; n = number of observations.

I. Responses to potassium chloride

In order to demonstrate the relaxant effects of drugs in the uterus, tone must be induced. Several methods of inducing tone are available, and it is possible that the means chosen may affect the observed relaxation. Since in all experiments described so far tone was induced with ACh, this possibility was examined in a series of corresponding experiments in which tone was induced with potassium chloride (KCl).

In preliminary experiments, the effects of KCl concentrations between 3×10^{-3}M and 10^{-1}M were examined in uteri from the four phases of the oestrous cycle. The standard concentration chosen was 5×10^{-2}M, which produced a biphasic contractile response with an initial transient phase, and a secondary sustained phase lasting for more than 1 hour. The mean maximum tension developed to KCl during the secondary sustained phase is shown in Table 16. The highest tension was developed in oestrus, and the lowest in metoestrus.

Cumulative dose-response curves to the adrenoceptor agonist SAL were constructed 15 minutes into the sustained phase, until a maximum relaxation of the KCl-induced tone was achieved. Of the three adrenoceptor agonists, SAL was chosen for the next series of experiments because it is selective for β_2-adrenoceptors.

Table 16: Maximum tension developed to potassium chloride (KCl, 5 x 10^{-2}M) in the rat uterus.

Phase	Maximum tension (g)	n
Proestrus	2.10 ± 0.35	16
Oestrus	2.52 ± 0.37	16
Metoestrus	1.84 ± 0.09	15
Dioestrus	1.90 ± 0.24	16

Values are mean ± S.E.M.; n = number of observations.

1. Inhibitory responses to SAL in potassium chloride depolarized uteri

SAL (1.5×10^{-11}M - 5×10^{-6}M) produced dose-related relaxation of the KCl-induced tone in proestrus, oestrus, metoestrus and dioestrus. Mean pD_2 values for SAL in KCl-depolarized uteri are shown in Table 17. Corresponding values for SAL in experiments using ACh are also shown. Compared with the pD_2 values in uteri in which tone was induced with ACh, there were no significant differences in the pD_2 value for SAL in KCl-depolarized uteri except in dioestrus. These experiments represent the Control I situation as described earlier.

2. Inhibitory responses to SAL in the combined presence of both an α-receptor antagonist and inhibitors of neuronal and extraneuronal uptake mechanisms

Responses to SAL were then examined in the presence of all three antagonists - AZA, DMI and NMN, i.e. the experiments represented the Control II situation as described earlier. Mean pD_2 values for SAL in KCl-depolarized uteri, in the presence of the combined antagonists are shown in Table 18. In comparison with the Control I values, there were significant increases ($p < 0.001$) in the pD_2 values for SAL in metoestrus and dioestrus, in the presence of the combined antagonists.

Table 17: Effect of the method of inducing tone on salbutamol potency (pD_2 values) in the rat uterus.

Phase	pD_2 values			
	ACh-induced tone	n	KCl-induced tone	n
Proestrus	9.14 ± 0.28	(6)	9.17 ± 0.31	(6)
Oestrus	8.39 ± 0.07	(4)	9.12 ± 0.33	(6)
Metoestrus	9.00 ± 0.26	(8)	9.38 ± 0.10	(5)
Dioestrus	9.58 ± 0.08	(8)	9.33 ± 0.06*	(6)

Values are mean ± S.E.M.; n = number of observations.

Table 18: Effect of azapetine (AZA, 10^{-6}M), desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M) on salbutamol (SAL) potency (pD_2 values) in depolarized rat uterus.

Phase	Control	After AZA + DMI + NMN	n
Proestrus	9.17 ± 0.31	9.77 ± 0.30	6
Oestrus	9.12 ± 0.33	9.88 ± 0.30	6
Metoestrus	9.38 ± 0.10	10.65 ± 0.05***	5
Dioestrus	9.33 ± 0.06	10.25 ± 0.03***	6

Values are mean ± S.E.M.; n = number of observations.

J. Responses to SAL in the presence of a cyclo-oxygenase inhibitor in potassium chloride depolarized uteri

Both Control I and Control II experiments were repeated in the presence of the cyclo-oxygenase inhibitor, FBF (10^{-6}M). Figure 29 illustrates a typical experiment obtained under the Control II condition, in dioestrus.

Dose-response curves and pD_2 values for SAL in the absence and presence of FBF in the four phases are shown in Figures 30 to 33, and Table 19, respectively.

(a) Proestrus (Figure 30)

FBF shifted significantly ($p < 0.001$) both Control I and Control II dose-response curves to the left. In Control I experiments, FBF also enhanced significantly ($p < 0.001$) the maximum degree of relaxation. In Control II experiments, there was a significant increase ($p < 0.01$) in the pD_2 value for SAL, in the presence of FBF. However, the increase in the Control I pD_2 value for SAL was not statistically significant (Table 19).

(b) Oestrus (Figure 31)

FBF shifted significantly ($p < 0.01$) both Control I and Control II dose-response curves to the left. In Control I experiments, the maximum degree of inhibition was enhanced significantly ($p < 0.001$), as in proestrus. In Control II

Fig. 29: Isometric responses to potassium chloride (KCl, 5 x 10^{-2}M), in the rat isolated uterus in dioestrus, and relaxation of the KCl-induced tone by salbutamol (added at arrows). AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M) was present in the Tyrode solution throughout the experiment. Drugs washed out ● Upper trace (a), control; lower trace (b), in presence of flurbiprofen (10^{-6}M).

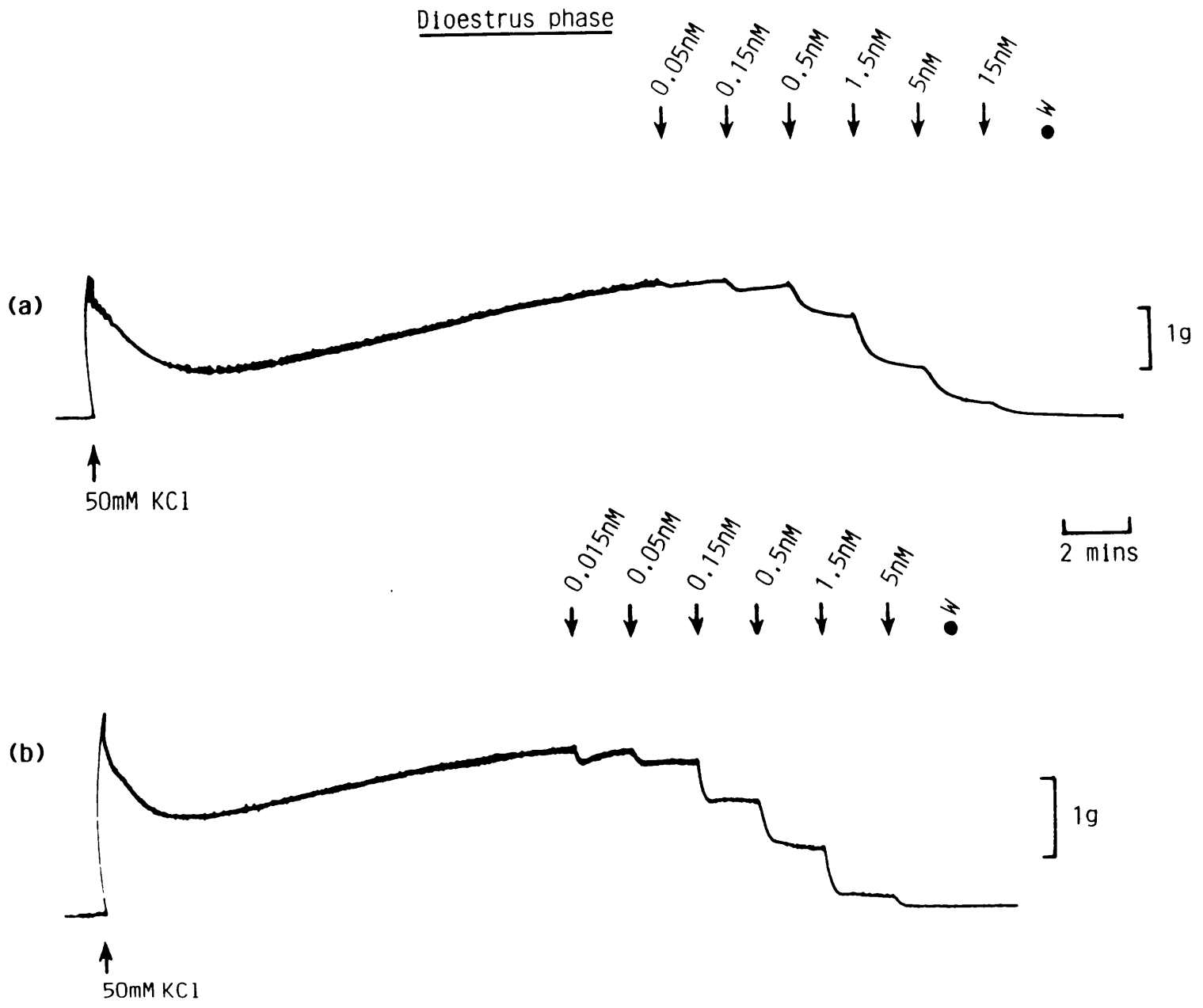

Dioestrus phase
0.05nM
0.15nM
0.5nM
1.5nM
5nM
15nM
W
(a)
1g
50mM KCl
0.015nM
0.05nM
0.15nM
0.5nM
1.5nM
5nM
W
2 mins
(b)
1g
50mM KCl

Fig. 30: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in proestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets. Note reversal of the ordinate scale.

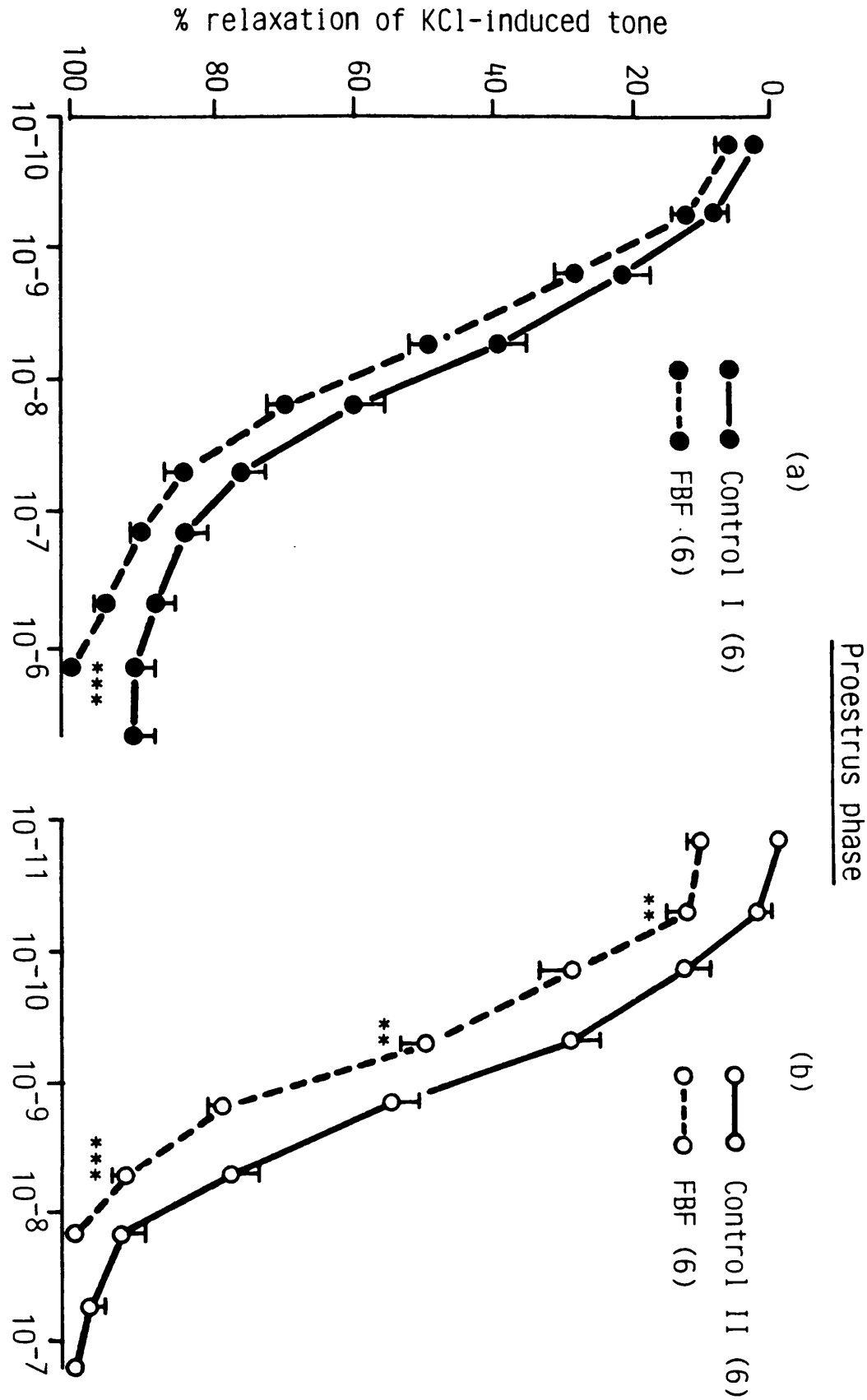
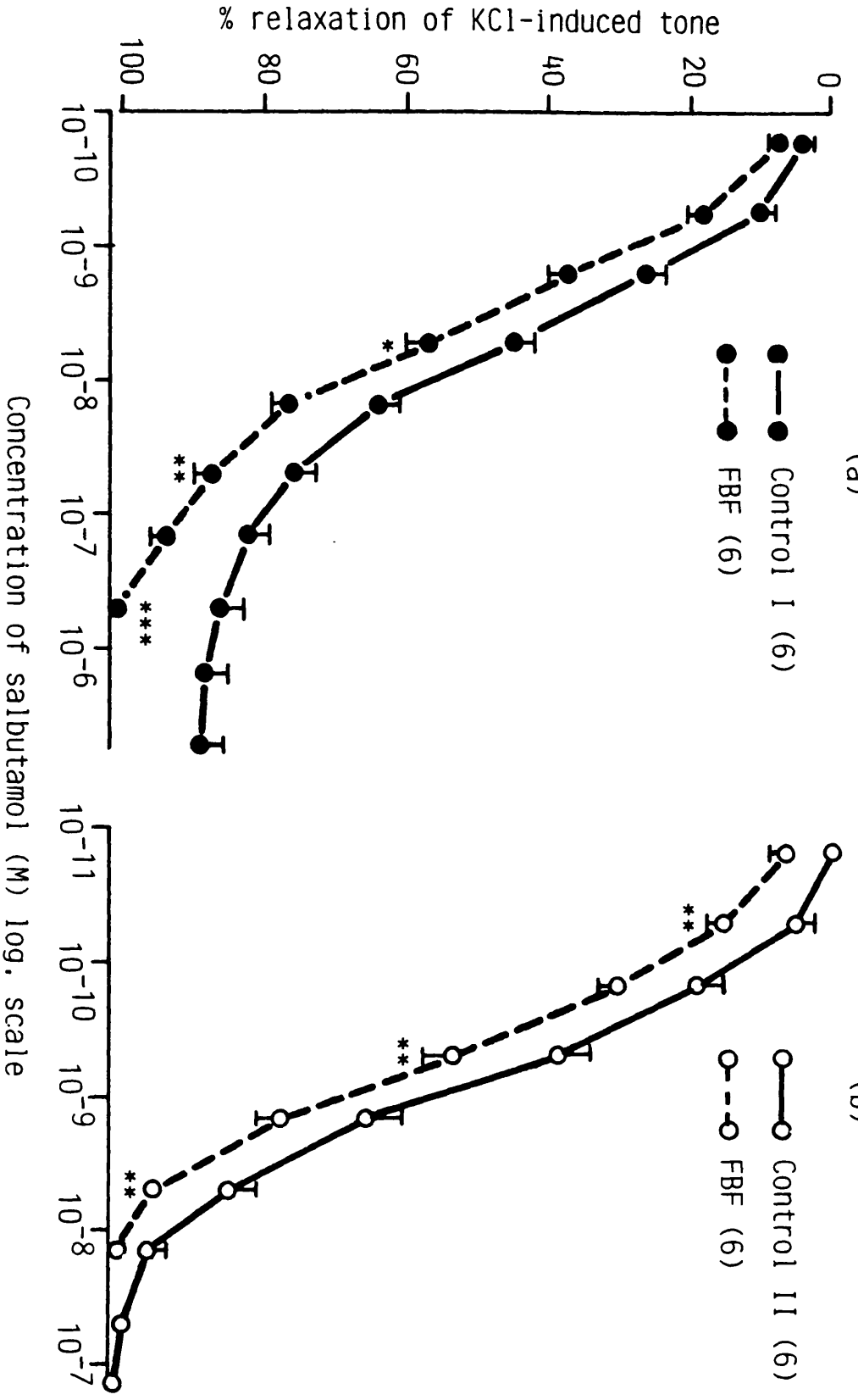

% relaxation of KCl-induced tone
100
80
60
40
20
0
(a)
Control I (6)
FBF (6)
***
Proestrus phase
(b)
Control II (6)
FBF (6)
**
**
***
10^{-10}
10^{-9}
10^{-8}
10^{-7}
10^{-6}
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
Concentration of salbutamol (M) log. scale

Table 19: Effect of flurbiprofen (FBF, 10^{-6}M) on salbutamol (SAL) potency (pD_2 values) in depolarized rat uterus.

Phase	Control I	After FBF	Control II	After FBF
Proestrus	9.17 ± 0.31	9.85 ± 0.04	9.77 ± 0.30	10.66 ± 0.09**
	(6)	(6)	(6)	(6)
Oestrus	9.12 ± 0.33	9.54 ± 0.07	9.88 ± 0.30	10.79 ± 0.03**
	(6)	(6)	(6)	(6)
Metoestrus	9.38 ± 0.10	9.99 ± 0.36	10.65 ± 0.05	10.88 ± 0.08**
	(5)	(5)	(5)	(5)
Dioestrus	9.33 ± 0.06	9.61 ± 0.21	10.25 ± 0.03	10.52 ± 0.08**
	(6)	(7)	(6)	(7)

Values are mean ± S.E.M.; Control I = agonists alone; Control II = agonists in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M); number of observations in brackets.

Fig. 31: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in oestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.


% relaxation of KCl-induced tone
100
80
60
40
20
0
(a)
Control I (6)
FBF (6)
Oestrus phase
(b)
Control II (6)
FBF (6)
10^{-10} 10^{-9} 10^{-8} 10^{-7} 10^{-6}
10^{-11} 10^{-10} 10^{-9} 10^{-8} 10^{-7}
Concentration of salbutamol (M) log. scale

experiments, there was a significant increase ($p < 0.01$) in the pD_2 value for SAL, in the presence of FBF. However, the increase in the Control I pD_2 value for SAL was not statistically significant (Table 19).

(c) Metoestrus (Figure 32)

FBF shifted significantly both Control I ($p < 0.05$) and Control II ($p < 0.01$) dose-response curves to the left. In Control II experiments, there was a significant increase ($p < 0.01$) in the pD_2 value for SAL, in the presence of FBF. However, the increase in the Control I pD_2 value for SAL was not statistically significant (Table 19).

(d) Dioestrus (Figure 33)

FBF shifted significantly ($p < 0.001$) the Control II dose-response curves to the left. In Control I experiments only the responses to the lower concentrations of SAL were enhanced significantly ($p < 0.01$). In Control II experiments there was a significant increase ($p < 0.01$) in the pD_2 value for SAL, in the presence of FBF. However, the increase in the Control I pD_2 value for SAL was not statistically significant (Table 19).

The inhibitory effect and pD_2 values for SAL in KCl-depolarized uteri were similar to those in which tone was induced with ACh. In addition, inhibition of cyclo-oxygenas with FBF produced corresponding changes in the dose-response curves and pD_2 values in the four phases of the oestrous

Fig. 32: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in metoestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.

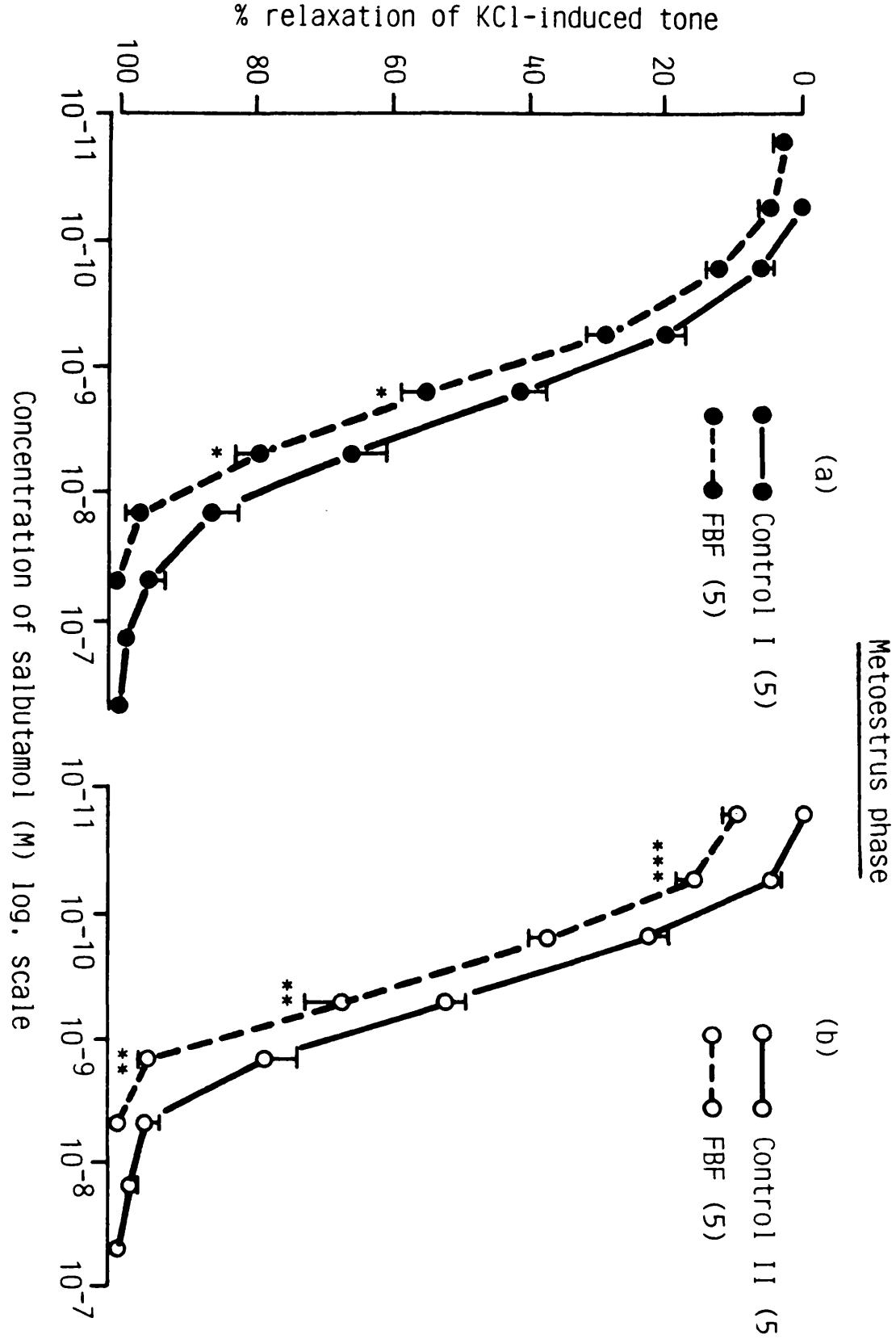
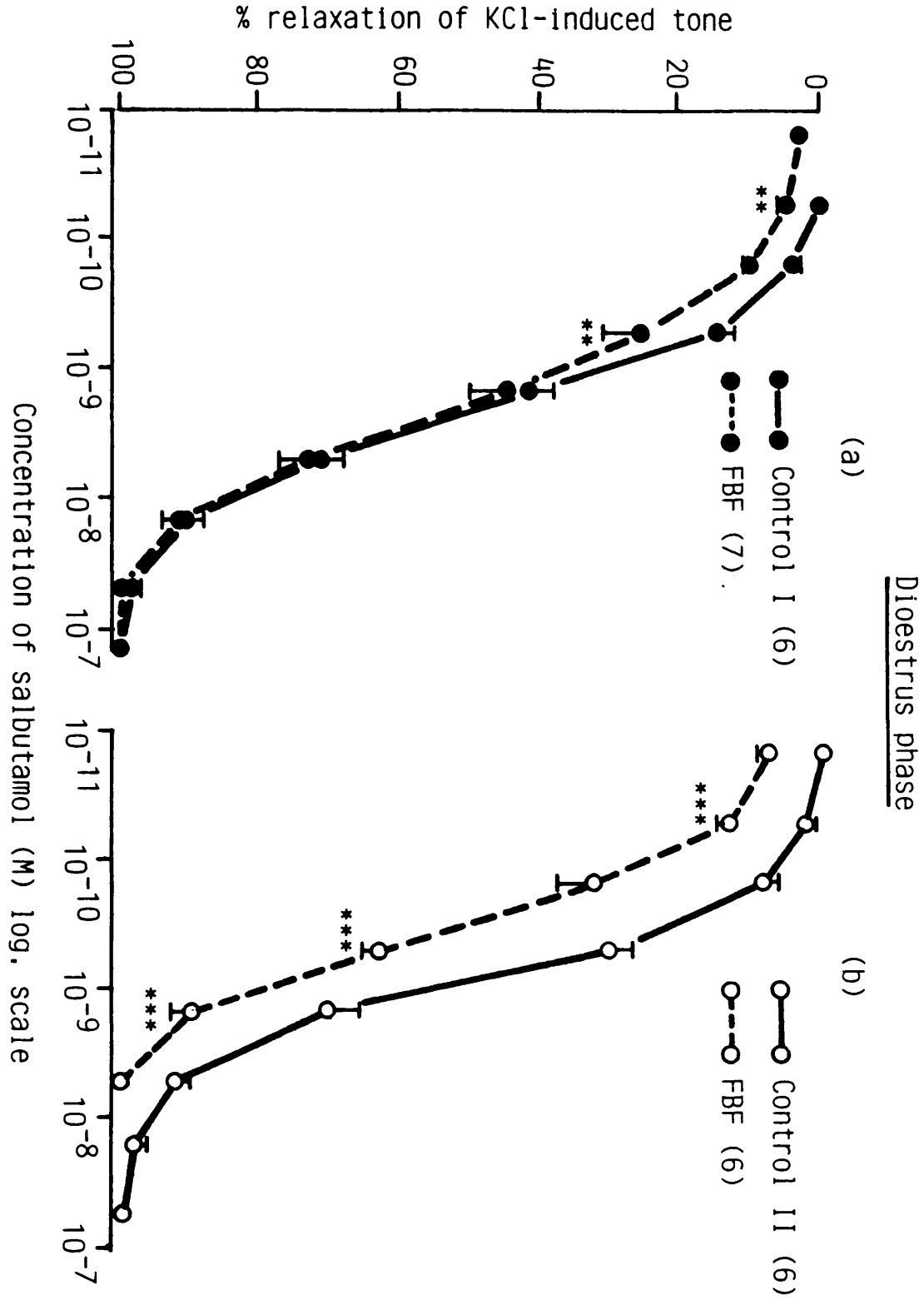

% relaxation of KCl-induced tone
100
80
60
40
20
0
Metoestrus phase
(a)
Control I (5)
FBF (5)
(b)
Control II (5)
FBF (5)
*
**
***
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
Concentration of salbutamol (M) log. scale

Fig. 33: Log. dose-response curves to salbutamol (SAL), in the rat isolated uterus in dioestrus: controls, and in presence of flurbiprofen (FBF, 10^{-6}M). Control I = SAL alone; Control II = SAL in presence of AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M). Number of observations in brackets.


% relaxation of KCl-induced tone
100
80
60
40
20
0
(a)
Control I (6)
FBF (7)
(b)
Control II (6)
FBF (6)
Dioestrus phase
**
**
***
***
***
10^{-11}
10^{-10}
10^{-9}
10^{-8}
10^{-7}
Concentration of salbutamol (M) log. scale

cycle. Thus, it would appear that both the observed inhibitory effect to adrenoceptor agonists, and the enhancement produced by cyclo-oxygenase inhibition, are independent of the method of inducing tone in the uterus.

K. Responses to SAL in the presence of an inhibitor of both cyclo-oxygenase and lipoxygenase in the four phases of the oestrous cycle

The prostaglandin precursor, arachidonic acid is a common substrate for both cyclo-oxygenase and lipoxygenase, and products of the latter pathway (i.e. the leukotrienes) may also be involved in uterine motility (Fig. 34). Indeed, by blocking the cyclo-oxygenase pathway, the substrate will be available solely for conversion to the leukotrienes. The possibility that the leukotrienes could be affecting the adrenoceptor agonists responses was, therefore investigated in a series of experiments. 3-amino-1-(m-trifluoromethyl)-phenyl-2-pyrazolone (BW 755C, 10^{-5}M), a dual inhibitor of both cyclo-oxygenase and lipoxygenase activities was used. Uterine tone was induced with KCl (5×10^{-2}M), and all three antagonists were present i.e. these experiments were of the Control II type.

Dose-response curves and pD_2 values for SAL in the absence, and presence of BW 755C in the four phases are shown in Figures 35 to 38, and Table 20, respectively, in which FBF values are included for comparison. BW 755C

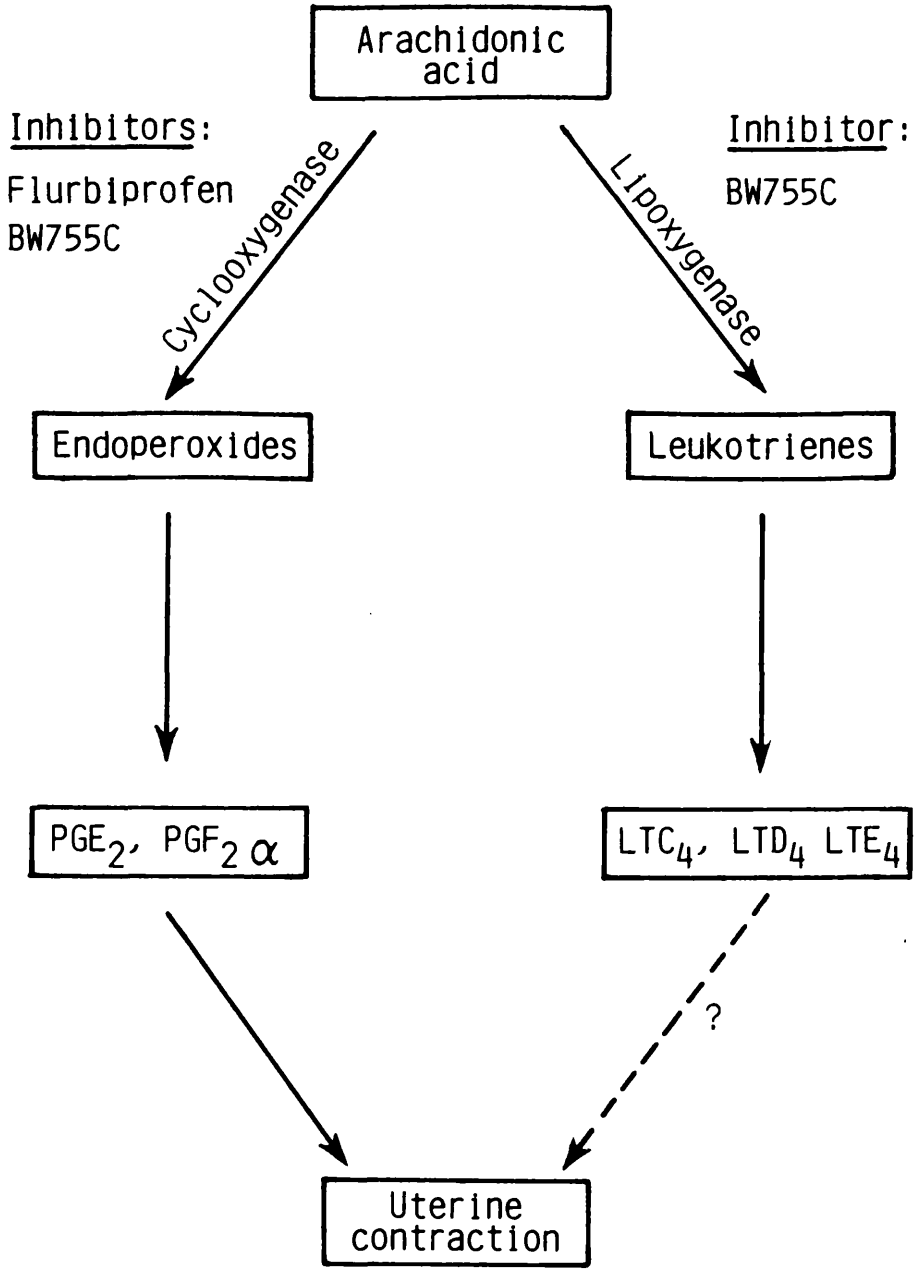


Fig. 34: Generation of prostaglandins and leukotrienes from arachidonic acid by cyclo-oxygenase and lipoxygenase, respectively. Flurbiprofen inhibits cyclo-oxygenase, while BW 755C inhibits both cyclo-oxygenase and lipoxygenase activities.

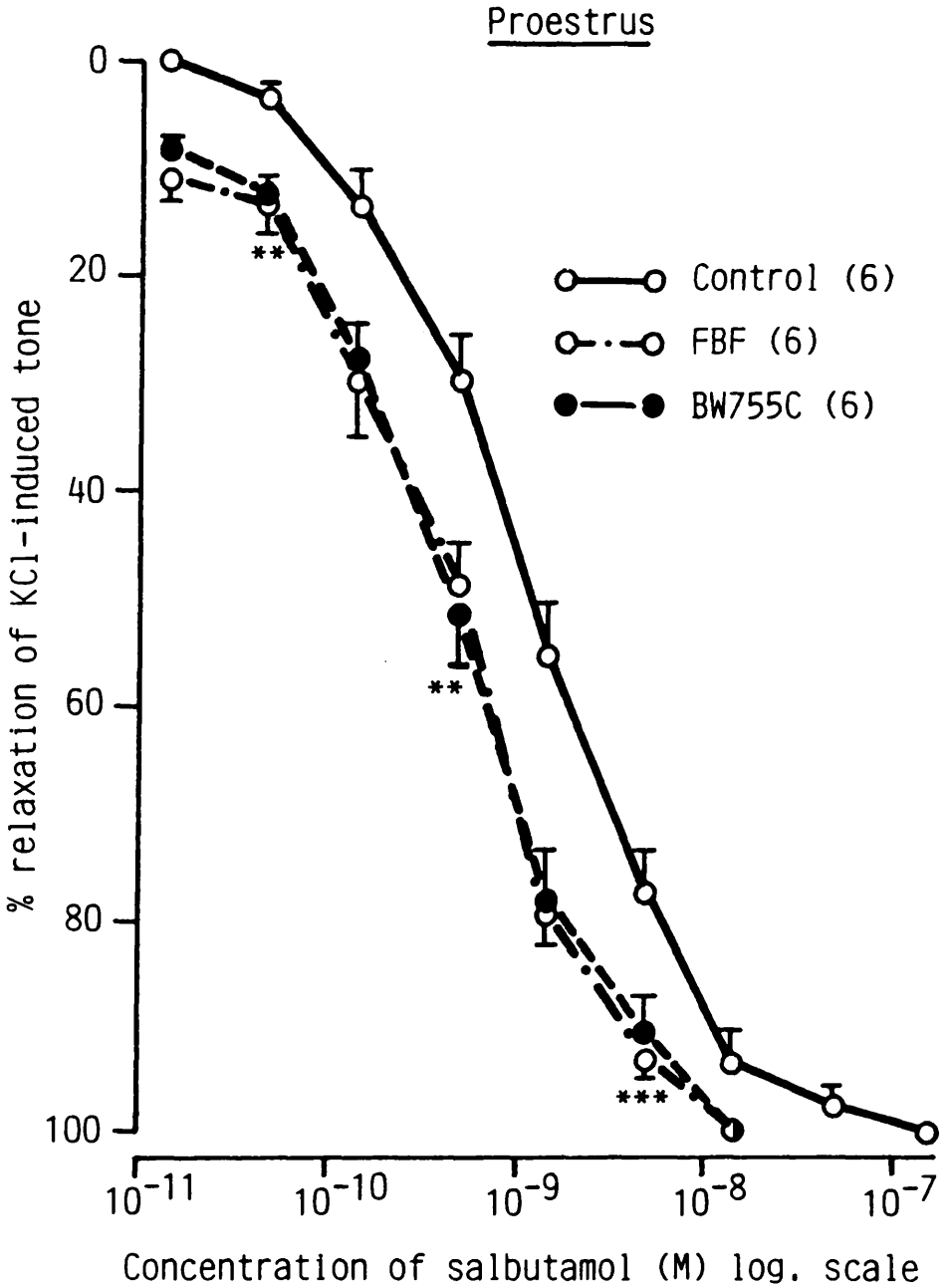


Fig. 35: Log. dose-response curves to salbutamol in the rat isolated uterus in proestrus: control, and in presence of FBF (10^{-6}M) and BW 755C (10^{-5}M). AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M) present in the Tyrode solution throughout the experiment. Number of observations in brackets. Note reversal of the ordinate scale.

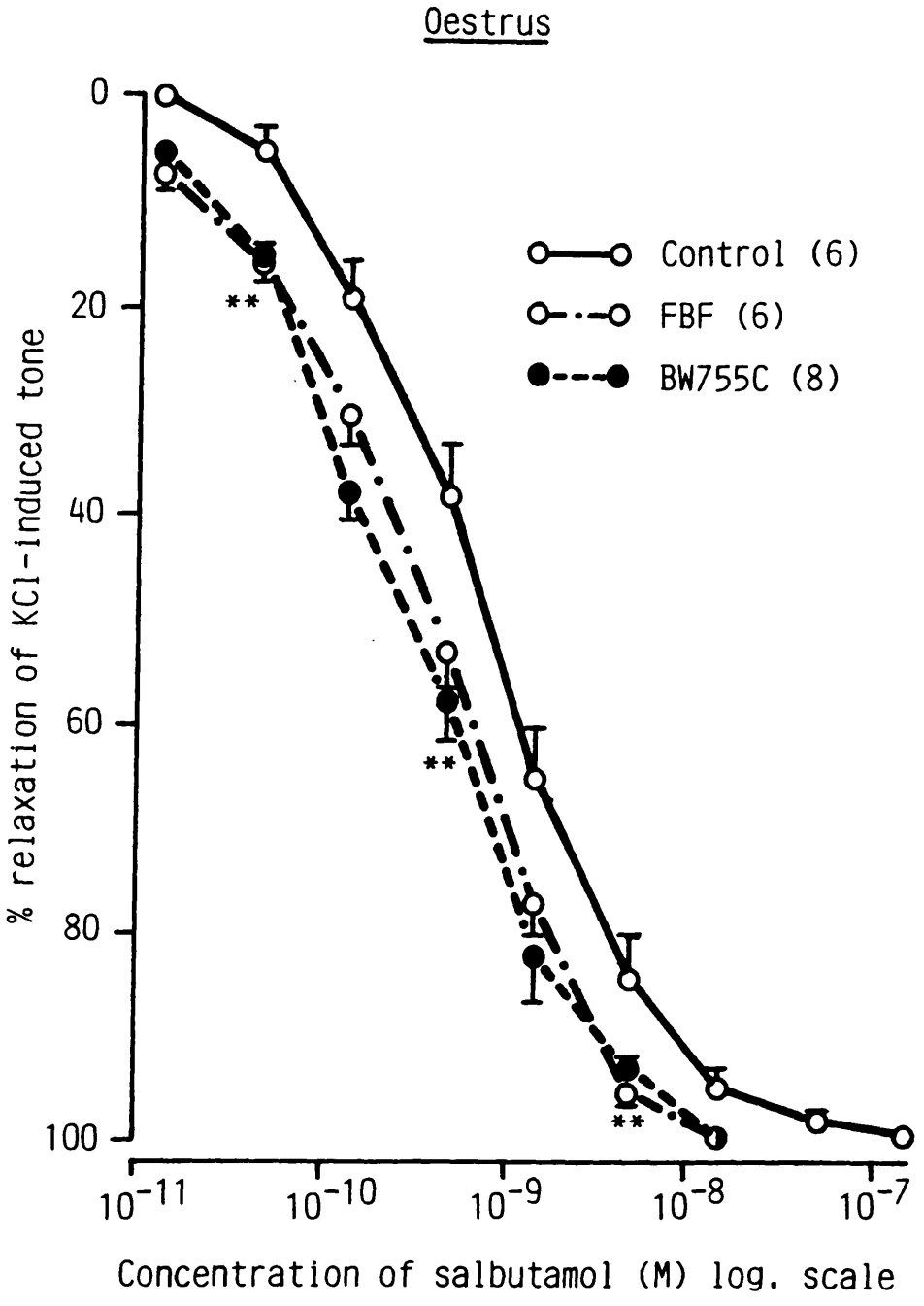


Fig. 36: Log. dose-response curves to salbutamol in the rat isolated uterus in oestrus: control, and in presence of FBF (10^{-6}M) and BW 755C (10^{-5}M). AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M) present in the Tyrode solution throughout the experiment. Number of observations in brackets.

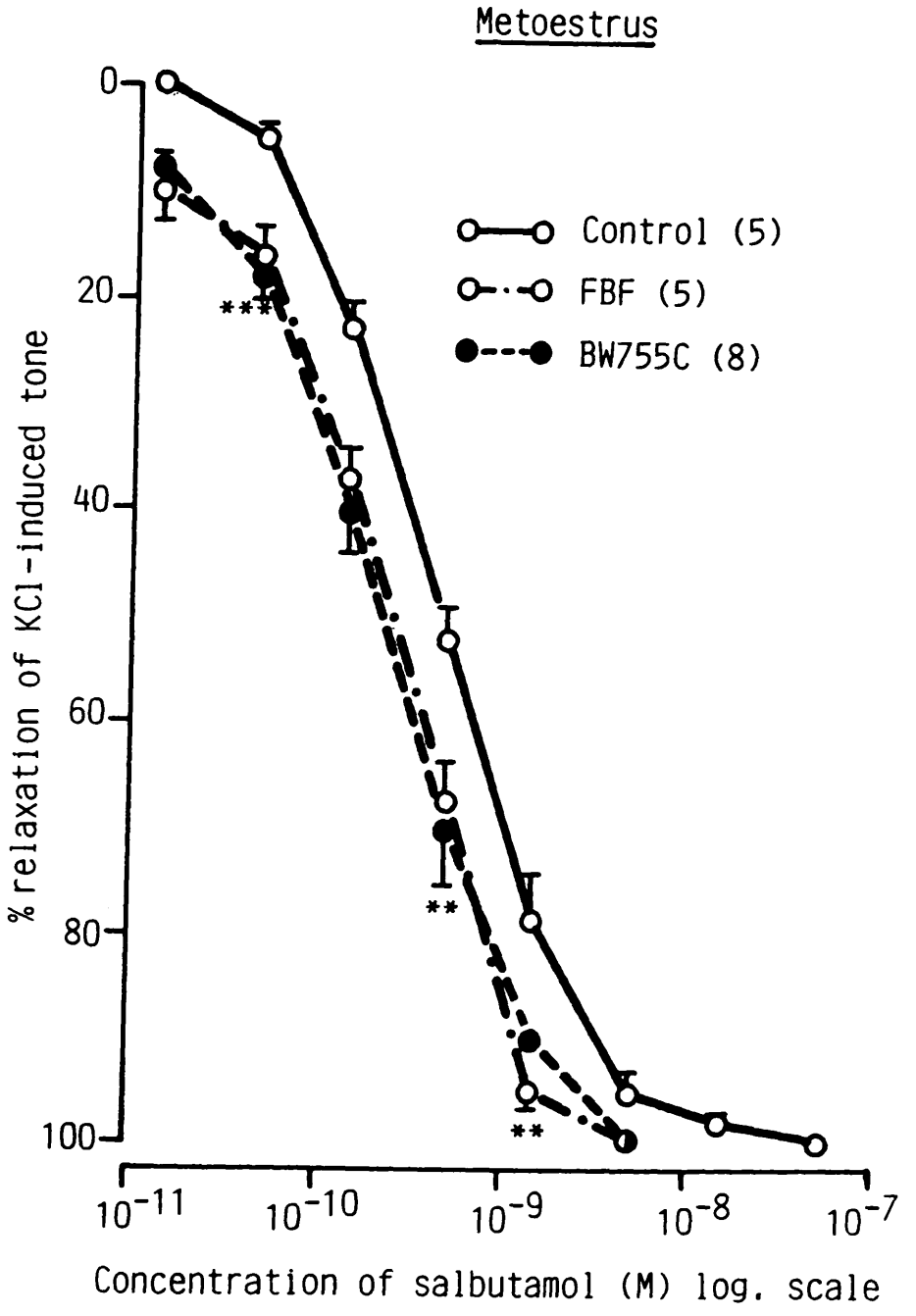


Fig. 37: Log. dose-response curves to salbutamol in the rat isolated uterus in metoestrus: control, and in presence of FBF (10^{-6}M) and BW 755C (10^{-5}M). AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M) present in the Tyrode solution throughout the experiment. Number of observations in brackets.

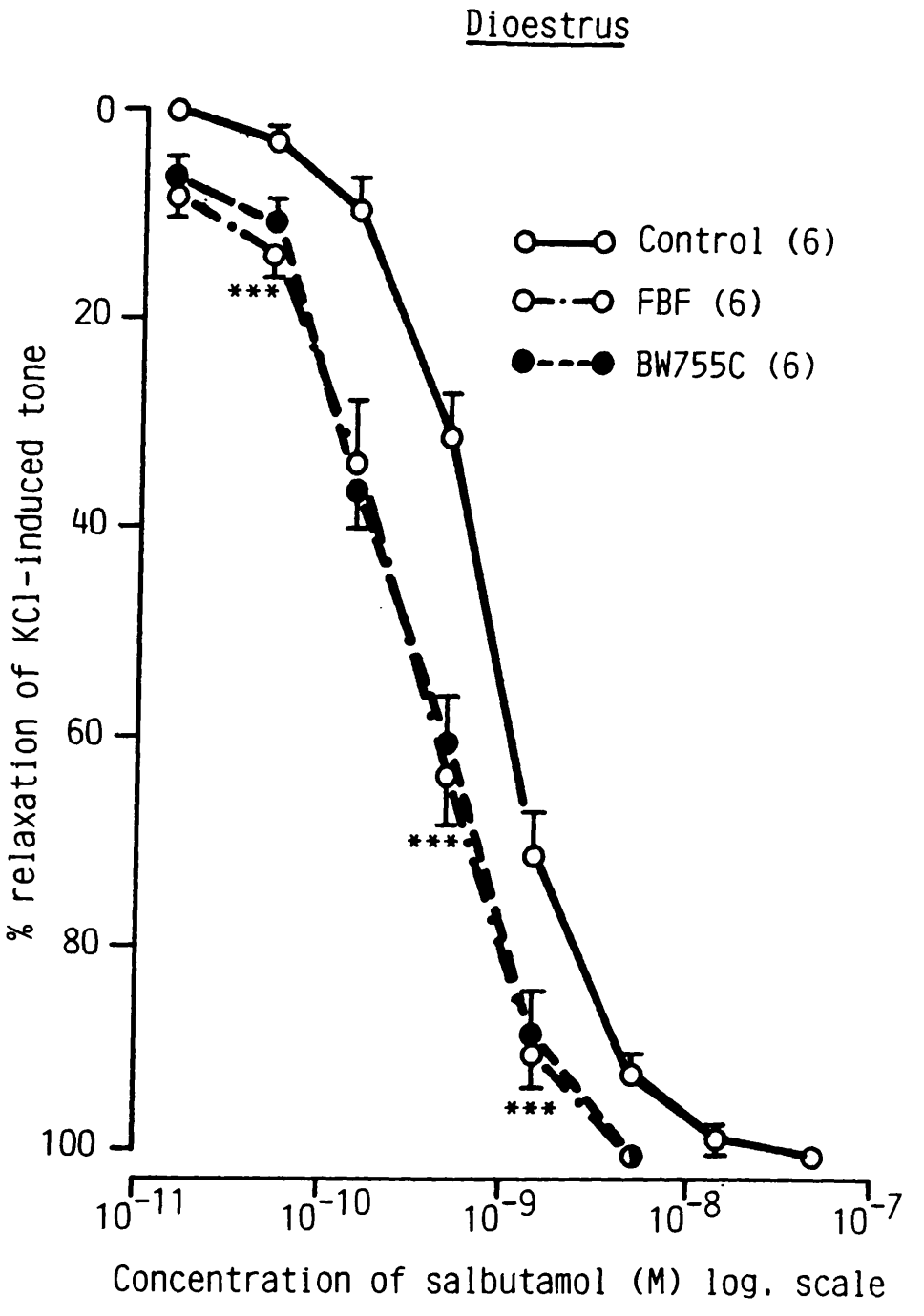


Fig. 38: Log. dose-response curves to salbutamol in the rat isolated uterus in dioestrus: control, and in presence of FBF (10^{-6}M) and BW 755C (10^{-5}M). AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M) present in the Tyrode solution throughout the experiment. Number of observations in brackets.

Table 20: Effect of flurbiprofen (FBF, 10^{-6}M) and BW 755C (10^{-5}M) on salbutamol (SAL) potency (pD_2 values) in depolarized rat uterus.

Phase	Control	After FBF	After BW 755C
Proestrus	9.77 ± 0.30	10.66 ± 0.09**	10.63 ± 0.11**
	(6)	(6)	(6)
Oestrus	9.88 ± 0.30	10.79 ± 0.03**	10.84 ± 0.02**
	(6)	(6)	(8)
Metoestrus	10.65 ± 0.05	10.88 ± 0.08**	10.86 ± 0.01**
	(5)	(5)	(8)
Dioestrus	10.25 ± 0.05	10.52 ± 0.08**	10.63 ± 0.10**
	(6)	(7)	(6)

Values are mean ± S.E.M.; number of observations in brackets.

shifted the SAL dose-response curves to the left, and increased the pD_2 values in the four phases of the oestrous cycle. All changes were significant at $p < 0.01$ or greater.

As can be seen from the figures and Table 20, BW 755C produced the same effect as did FBF, which would suggest that products of the lipoxygenase pathway are not involved in adrenoceptor agonists inhibitory responses in the uterus.

II. ADENOSINE 3'5' CYCLIC MONOPHOSPHATE ASSAY EXPERIMENTS IN THE FOUR PHASES OF THE OESTROUS CYCLE

Inhibition of intramural prostaglandin production reduced the variation between the adrenoceptor agonists in the different phases of the oestrous cycle and in ovariectomized animals but did not abolish it completely. Moreover, agonist effects on prostaglandin production appeared independent of hormonal influences. Thus the remaining variation may be due to the ovarian hormones. Since these have been reported to alter adenosine 3'5' cyclic monophosphate (cAMP) levels in the rat uterus (Kishikawa, 1981), and β-adrenoceptor agonists act via increased levels of cAMP, uterine levels of cAMP were examined in the four phases.

Basal cAMP levels were first measured in uteri from the four phases, and the effects of the combined antagonists (i.e. the Control II situation described previously) and SAL examined. A concentration of 5×10^{-6}M SAL was used because it produced the maximum inhibitory effect in isolated uterine horn preparations.

1. Basal cAMP levels

The basal cAMP levels in uteri from proestrus, oestrus, metoestrus and dioestrus are shown in Figure 39. There were no statistically significant differences in basal cAMP content in the four phases. Addition of the combined

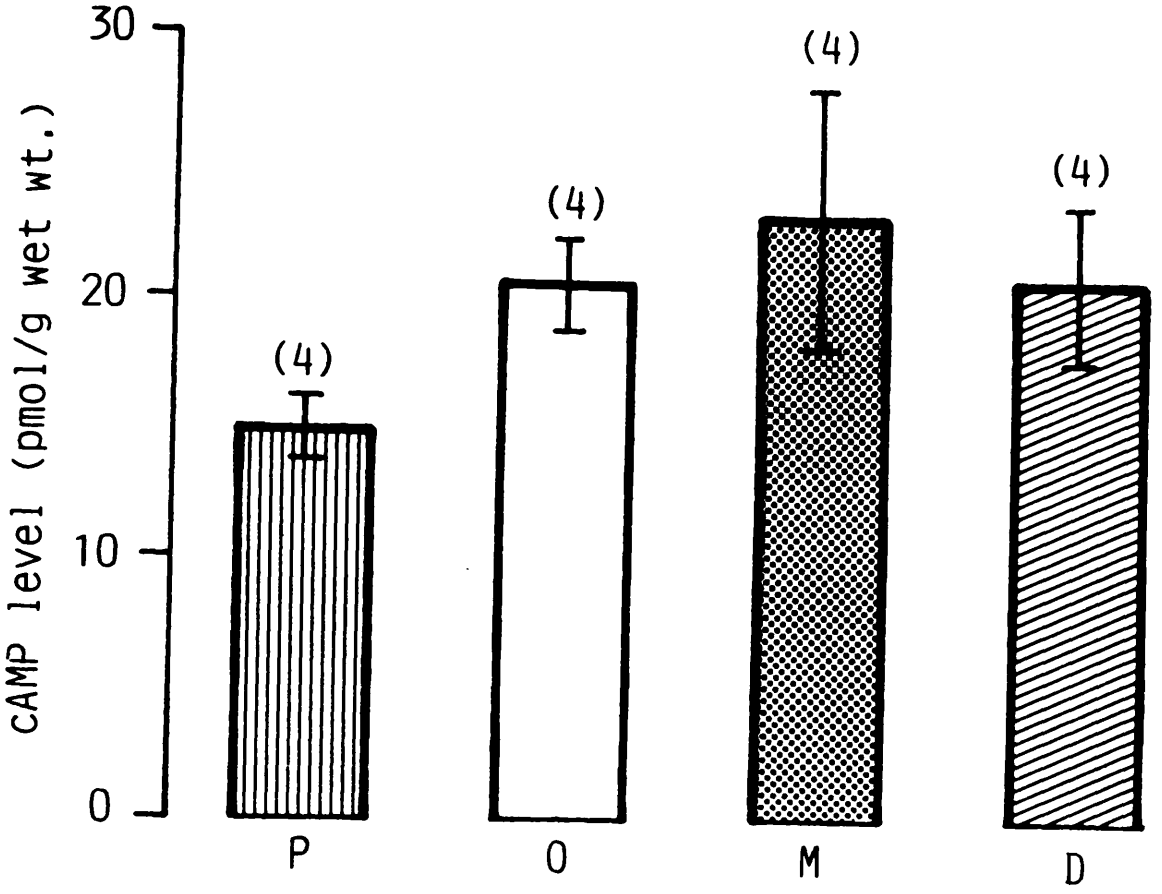


Fig. 39: Basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus, in the four phases of the oestrous cycle. P = proestrus; O = oestrus; M = metoestrus; D = dioestrus. Number of observations in brackets.

antagonists - AZA (10^{-6}M), DMI (10^{-6}M) and NMN (10^{-6}M), produced no significant differences in basal cAMP levels, and did not alter the effects of SAL on these levels.

2. Effect of SAL on cAMP levels

SAL (5×10^{-6}M) produced significant increases ($p < 0.001$) in cAMP content which were similar in all four phases (Fig. 40).

3. Effect of a cyclo-oxygenase inhibitor on cAMP levels

Prostaglandins have been shown to alter cAMP metabolism in the rat uterus (Krall, Barrett, Jamgotchian & Korenman, 1984). Therefore, the effect of cyclo-oxygenase inhibition on basal cAMP levels was investigated and the effects of SAL re-examined in preparations in which prostaglandin production had been inhibited. Addition of the combined antagonists (i.e. Control II situation) did not alter the effects of FBF (10^{-6}M) alone, or in combination with SAL.

The effects of FBF on basal cAMP levels are shown in Figures 41 and 42. There were no statistically significant differences in cAMP content in the presence of FBF, in any of the phases.

4. Effect of SAL on cAMP levels in uteri pretreated with a cyclo-oxygenase inhibitor

In a series of experiments, the effect of SAL (5×10^{-6}M)

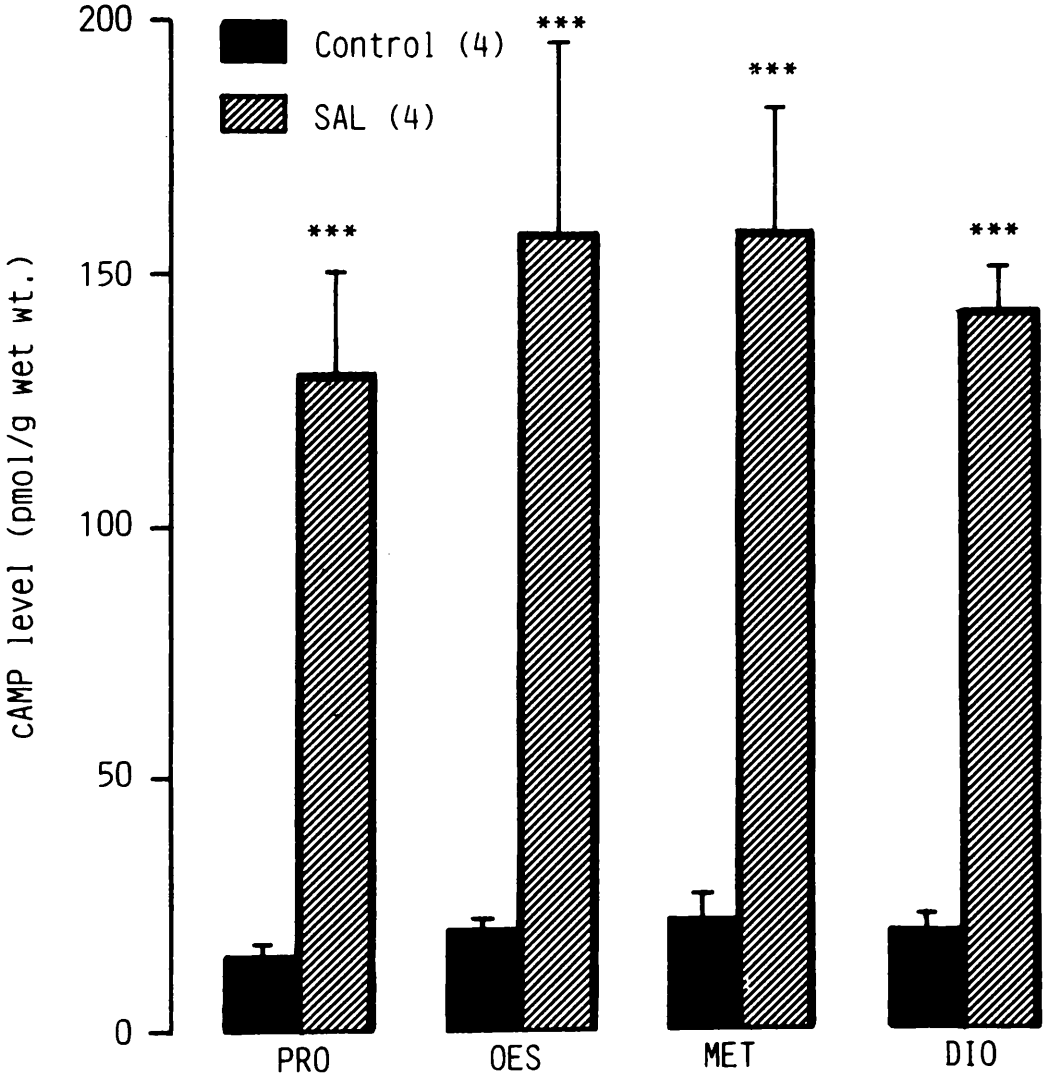


Fig. 40: Effect of salbutamol (SAL) on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the four phases of the oestrous cycle. PRO = proestrus; OES = oestrus; MET = metoestrus; DIO = dioestrus. Filled columns = controls; hatched columns = in presence of SAL (5×10^{-6}M). Number of observations in brackets.

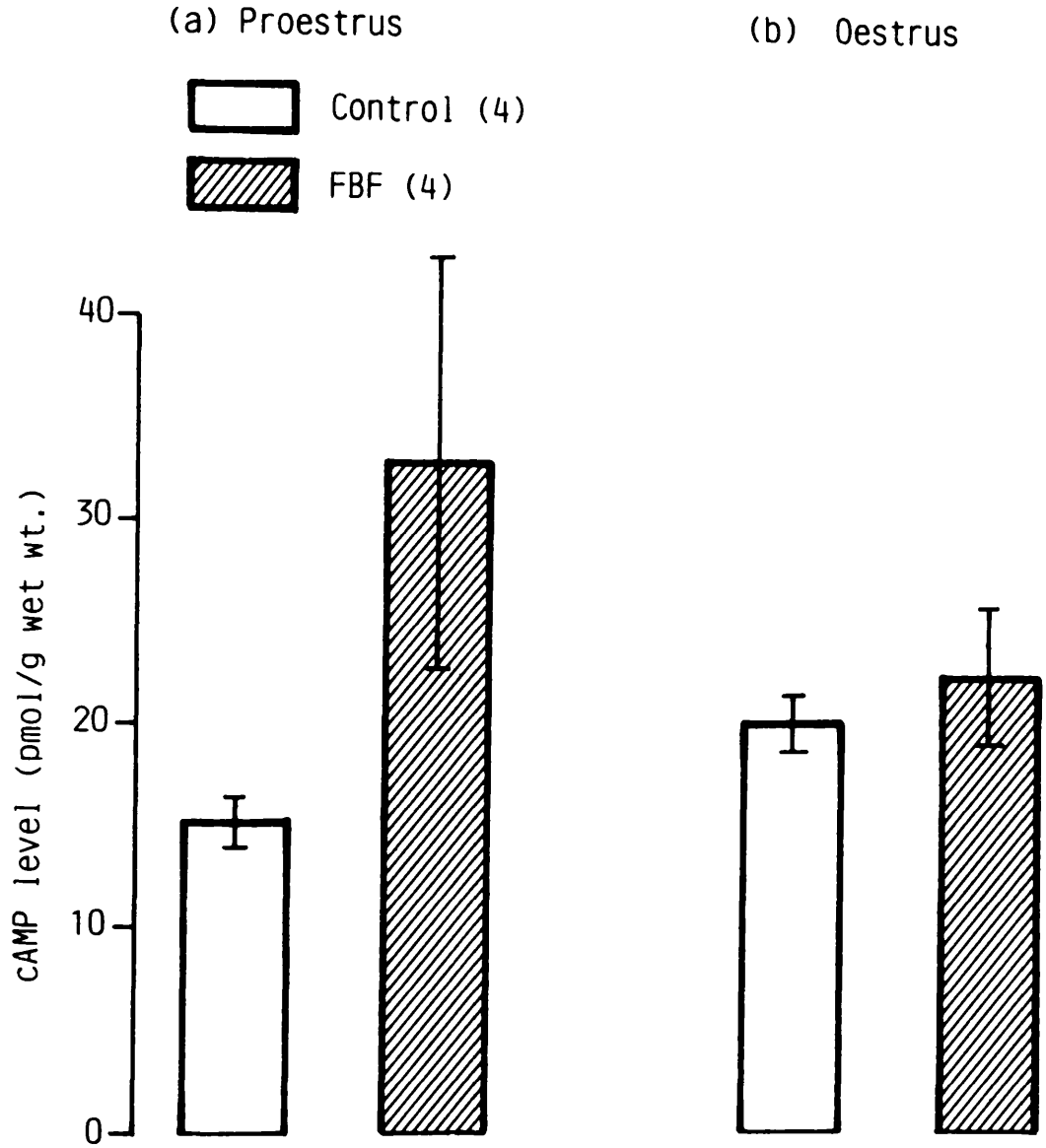
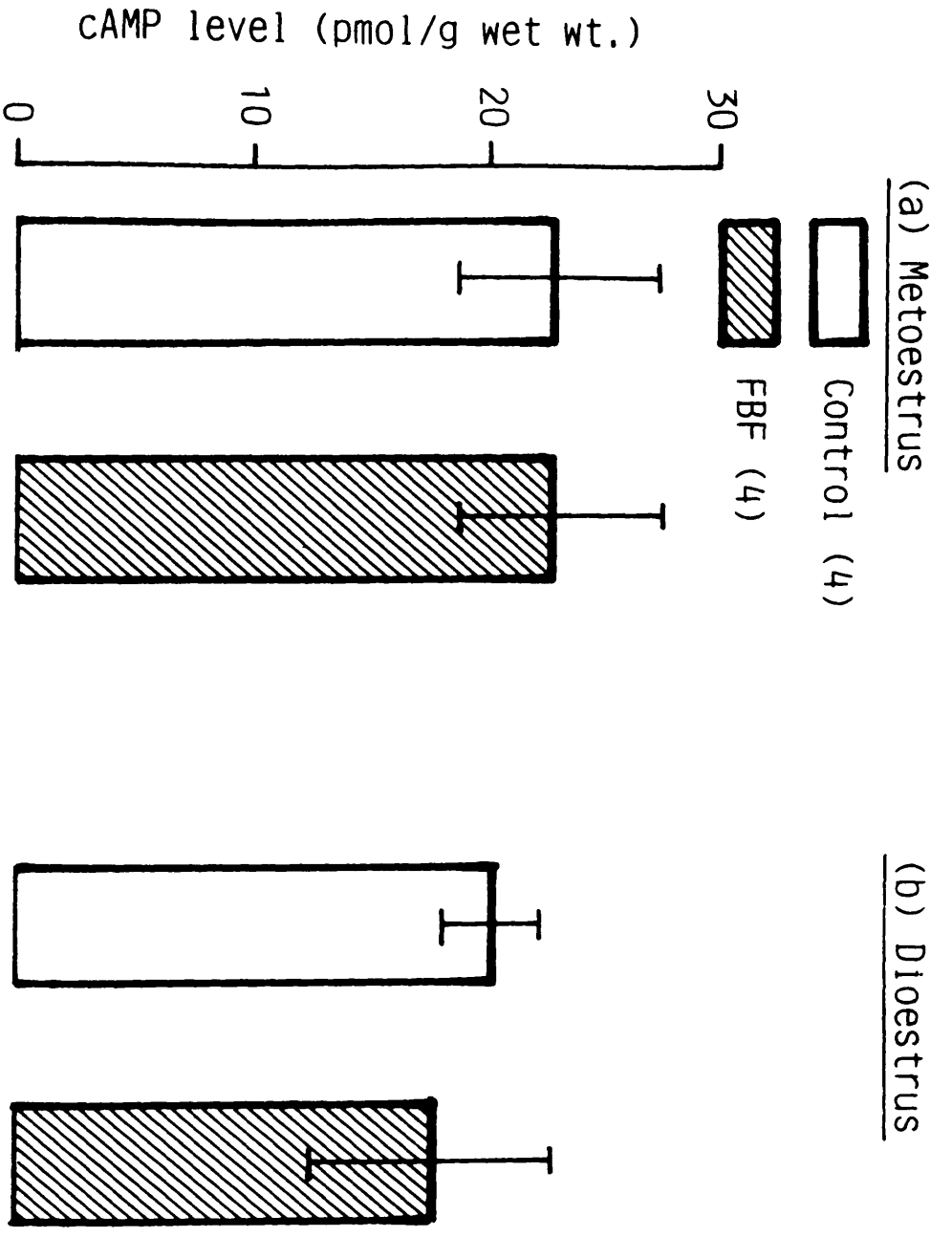


Fig. 41: Effect of flurbiprofen (FBF) on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in proestrus (a), and oestrus (b). Open columns = controls; hatched columns = in presence of FBF (10^{-6}M). Number of observations in brackets.

Fig. 42: Effect of flurbiprofen (FBF) on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in metoestrus (a), and dioestrus (b). Open columns = controls; hatched columns = in presence of FBF (10^{-6}M). Number of observations in brackets.


cAMP level (pmol/g wet wt.)
0
10
20
30
(a) Metoestrus
Control (4)
FBF (4)
(b) Dioestrus

was examined in preparations pretreated with FBF (10^{-6}M). The results obtained are shown in Figures 43 to 46. In the presence of FBF, SAL produced significant increases ($p < 0.001$) in cAMP content in uteri from all four phases, but the increases were not different from those in which prostaglandin production had not been inhibited.

Basal cAMP levels and the increases induced by SAL were similar in all four phases of the cycle. Cyclo-oxygenase inhibition had no effect on basal cAMP levels, nor on the ability of SAL to increase tissue cAMP content. This would suggest a limited role for cAMP in the observed variation in adrenoceptor agonists inhibitory effects during the oestrous cycle.

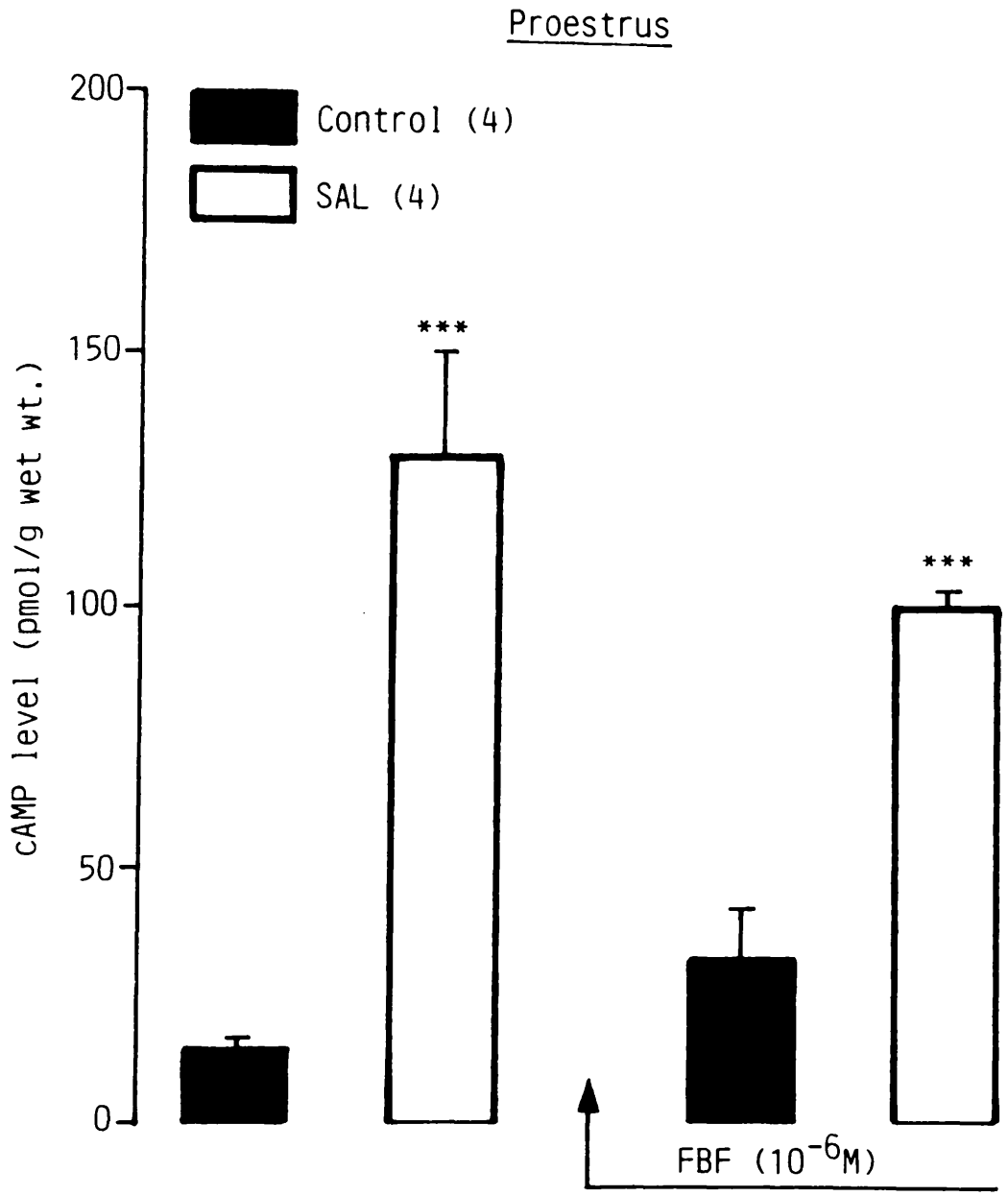


Fig. 43: Effect of salbutamol (SAL) on cAMP levels (pmol/g wet weight of tissue) in the rat isolated uterus in proestrus: before, and after treatment with FBF (10^{-6}M). Filled columns = controls; open columns = in presence of SAL (5 x 10^{-6}M). Number of observations in brackets.

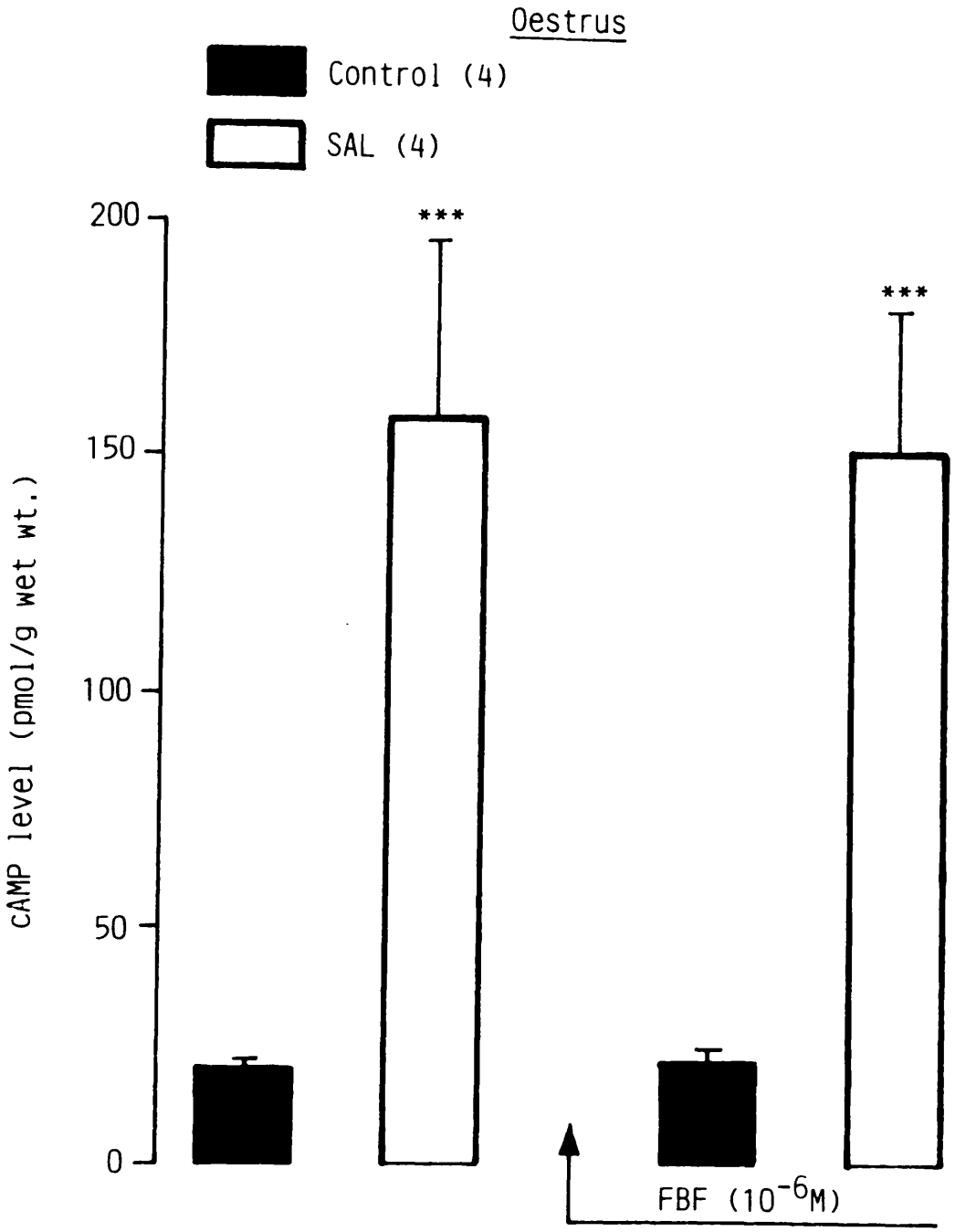


Fig. 44: Effect of salbutamol (SAL) on cAMP levels (pmol/g wet weight of tissue) in the rat isolated uterus in oestrus: before, and after treatment with FBF (10^{-6}M). Filled columns = controls; open columns = in presence of SAL (5 x 10^{-6}M). Number of observation in brackets.

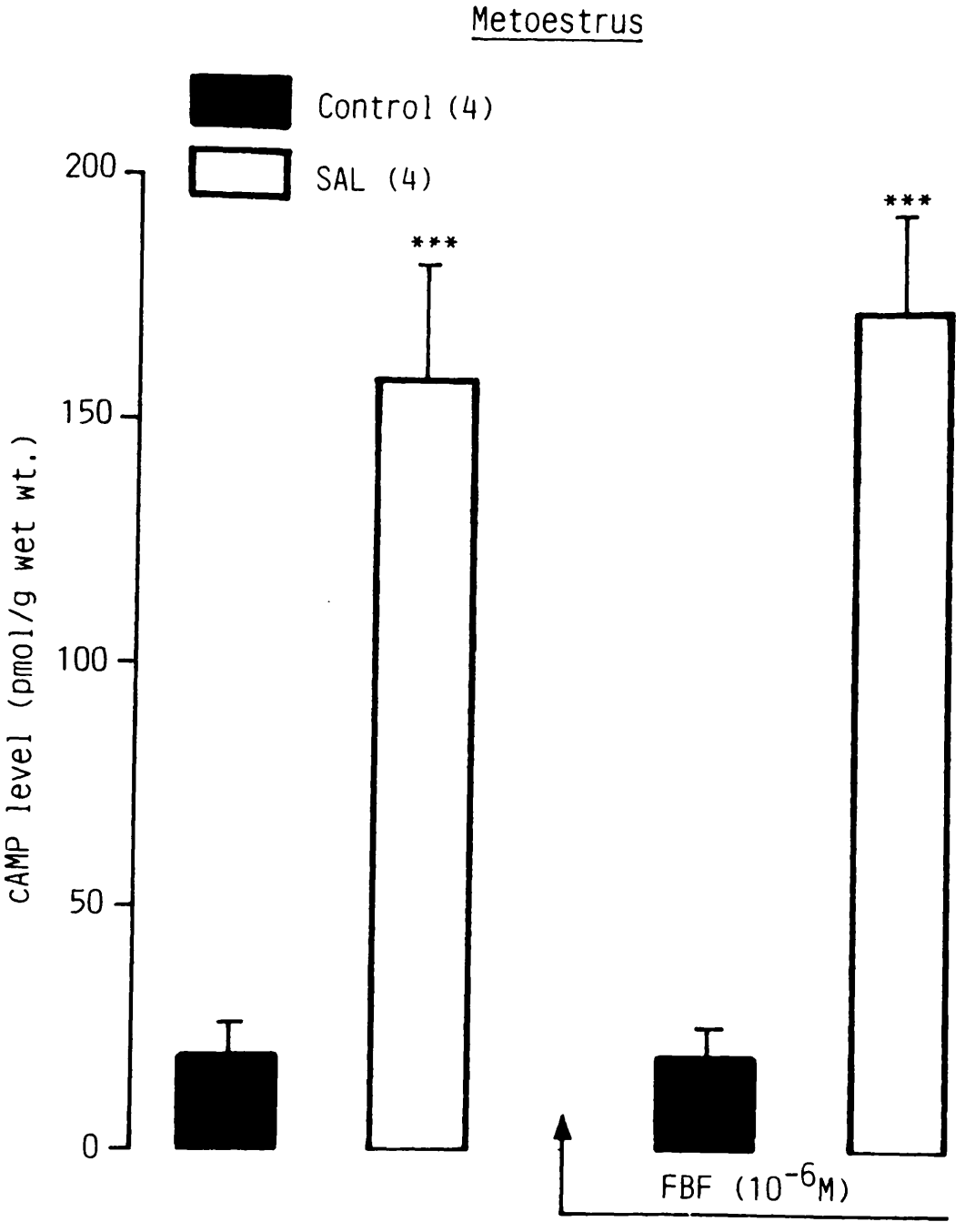


Fig. 45: Effect of salbutamol (SAL) on cAMP levels (pmol/g wet weight of tissue) in the rat isolated uterus in metoestrus: before and after treatment with FBF (10^{-6}M). Filled columns = controls; open columns = in presence of SAL (5 x 10^{-6}M). Number of observations in brackets.

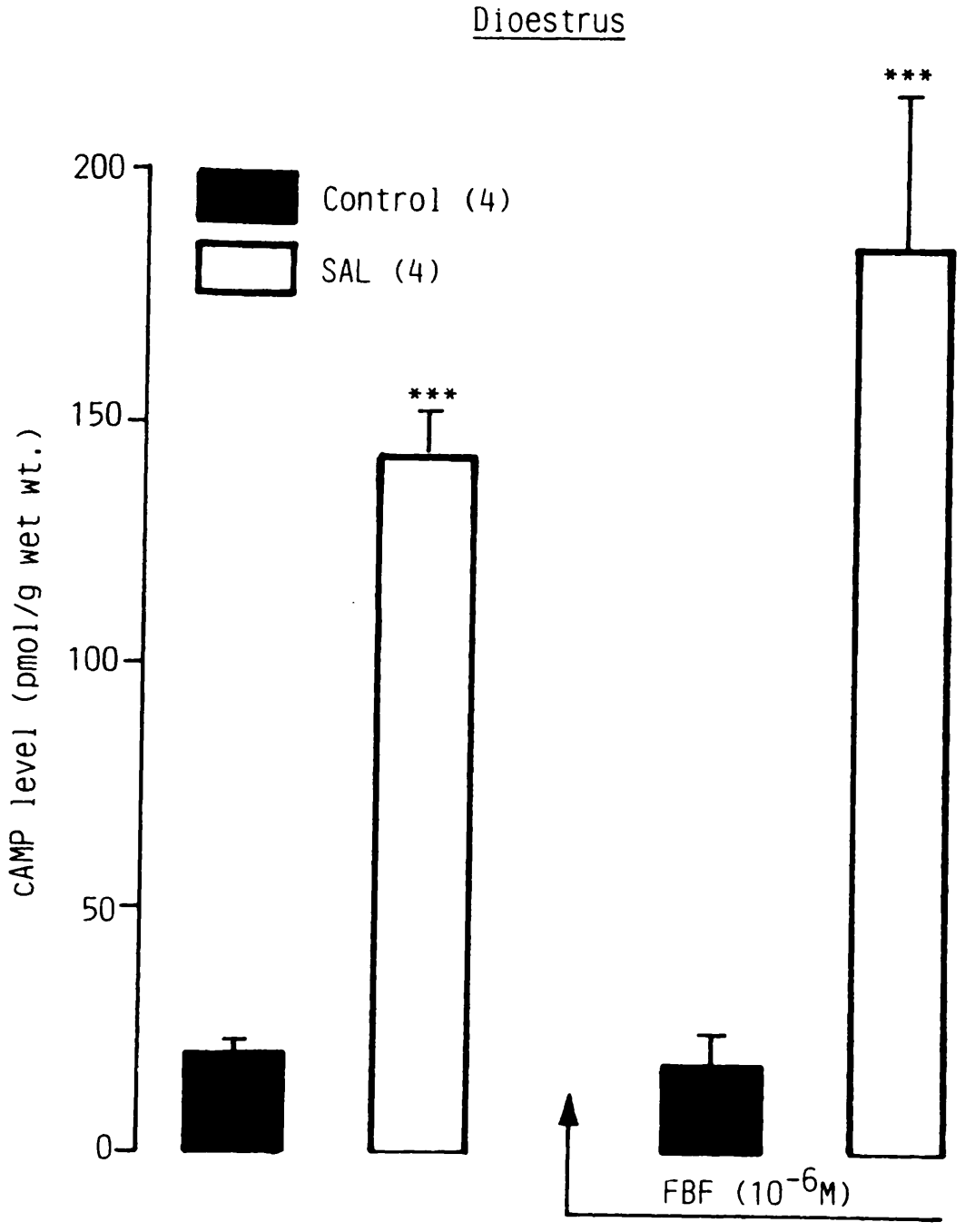


Fig. 46: Effect of salbutamol (SAL) on cAMP levels (pmol/g wet weight of tissue) in the rat isolated uterus in dioestrus: before, and after treatment with FBF (10^{-6}M). Filled columns = controls; open columns = in presence of SAL (5 x 10^{-6}M). Number of observations in brackets.

III. RADIOACTIVE CALCIUM ($^{45}Ca^{2+}$) EXPERIMENTS

Since adrenoceptor agonist effects on prostaglandin production and cAMP metabolism did not account wholly for the observed variation in their inhibition, a third possibility was then considered. Hormonally induced changes in calcium (Ca^{2+}) movements may play an indirect role in the altered responses because the final determinant of smooth muscle motility is the availability of intracellular free Ca^{2+}. Ca^{2+} movements were studied in uteri from four phases of the oestrous cycle, and in ovariectomized animals.

1. Determination of $^{45}Ca^{2+}$ concentration for efflux and influx experiments

The optimum tracer concentration to be used for efflux and influx studies was determined in preliminary experiments. Tissue $^{45}Ca^{2+}$ content was measured at different tracer concentrations in oestrus and metoestrus, which represented the extremes of the oestrous cycle, i.e. an oestrogen dominated and a progesterone dominated phase, respectively (Fig. 47). In both phases, tissue uptake of $^{45}Ca^{2+}$ was concentration dependent and exhibited a similar pattern. Tissue $^{45}Ca^{2+}$ increased gradually reaching a plateau at 0.05 μCi/ml. Above 0.05 μCi/ml, there was a very sharp rise in tissue $^{45}Ca^{2+}$ up to 5 μCi/ml.

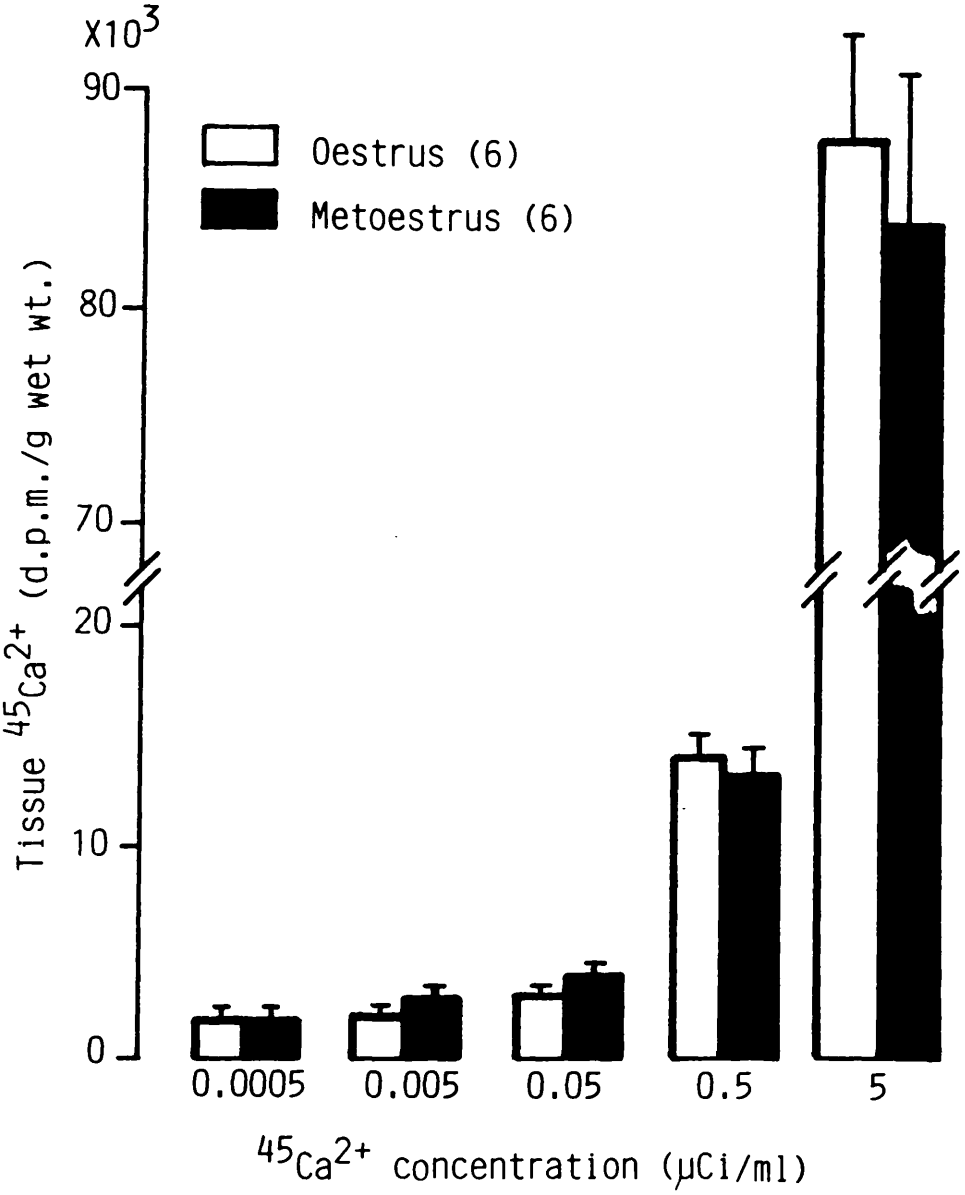


Fig. 47: Concentration-dependent uptake of $^{45}Ca^{2+}$ (expressed as d.p.m./g wet weight of tissue) in the rat isolated uterus in oestrus (open columns) and metoestrus (filled columns). Number of observations in brackets.

The plateau was attributed to attainment of equilibrium with the bathing medium $^{45}Ca^{2+}$ at the low tracer concentrations. The marked increase in tissue $^{45}Ca^{2+}$ at the higher concentrations reflected Ca^{2+} binding within the extracellular space. In order to minimize this non-specific binding of tracer in the extracellular space, a concentration of 0.25 μCi/ml was, therefore, chosen for influx experiments. Moreover, it would be difficult to measure small changes in tracer influx against a high background count. For efflux experiments, a concentration of 2 μCi/ml was chosen in order to ensure adequate loading of cells. In this case, some tracer will also be bound in the extracellular space.

2. $^{45}Ca^{2+}$ efflux in the four phases of the oestrous cycle

$^{45}Ca^{2+}$ efflux in uteri from animals in proestrus, oestrus, metoestrus and dioestrus was biphasic (Fig. 48). There was an initial rapid decline in tissue $^{45}Ca^{2+}$ in the first 30 minutes of efflux, followed by a slower decline until equilibrium was achieved after about 50 minutes. Uteri from all four phases showed a similar pattern of decline of tissue $^{45}Ca^{2+}$, but efflux in metoestrus was significantly lower ($p < 0.001$) than in the other three phases.

The rate constant of $^{45}Ca^{2+}$ loss (i.e. $^{45}Ca^{2+}$ leaving tissue during collection interval divided by $^{45}Ca^{2+}$ in the tissue at the beginning of the interval), was determined for each phase (Fig. 49). As with the $^{45}Ca^{2+}$ efflux curves, a biphasic decline was obtained. At equilibrium, the rate

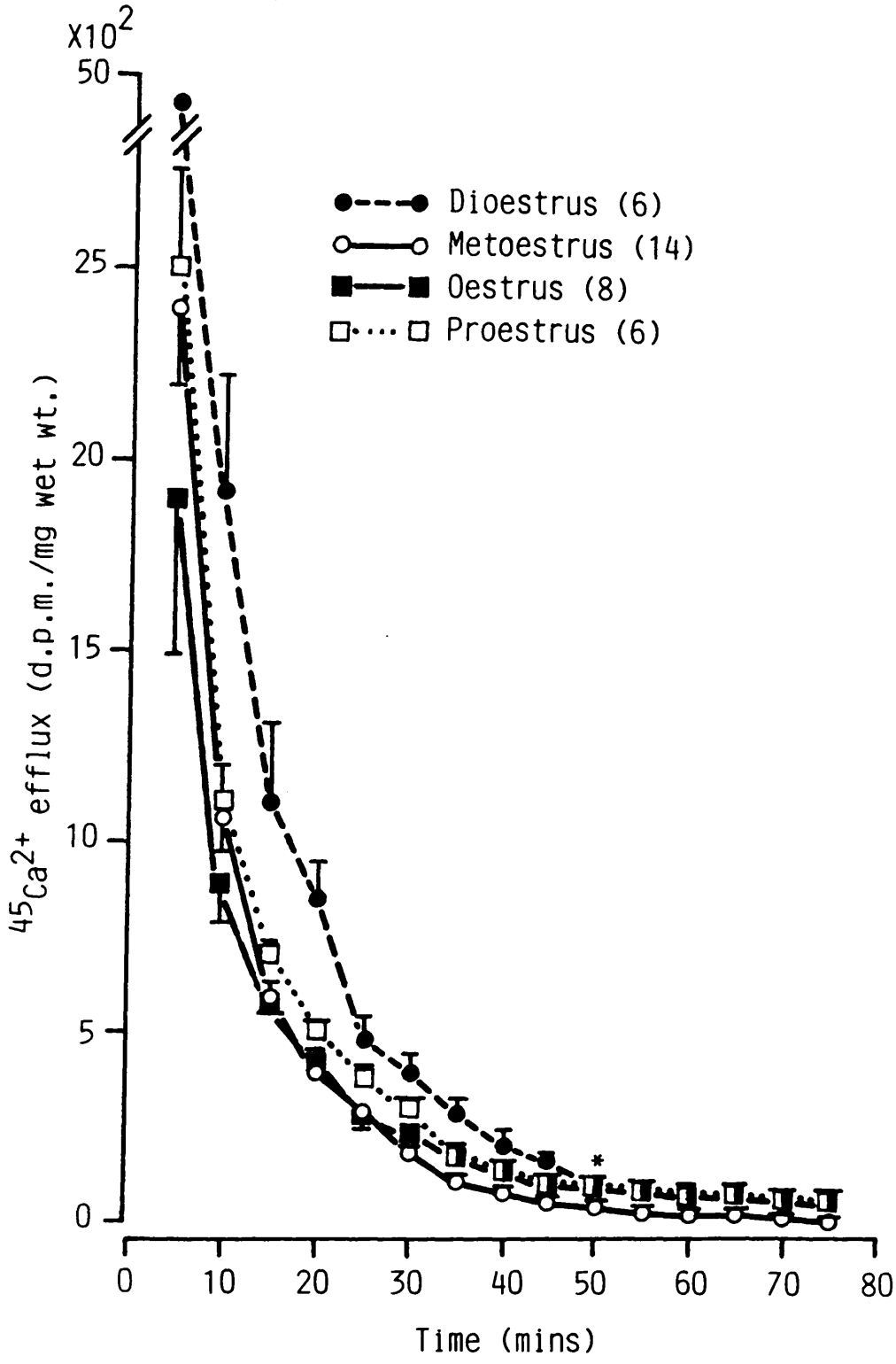


Fig. 48: $^{45}Ca^{2+}$ efflux curves (in d.p.m./mg wet weight of tissue against time) in the rat isolated uterus, in the four phases of the oestrous cycle. Number of observations in brackets.

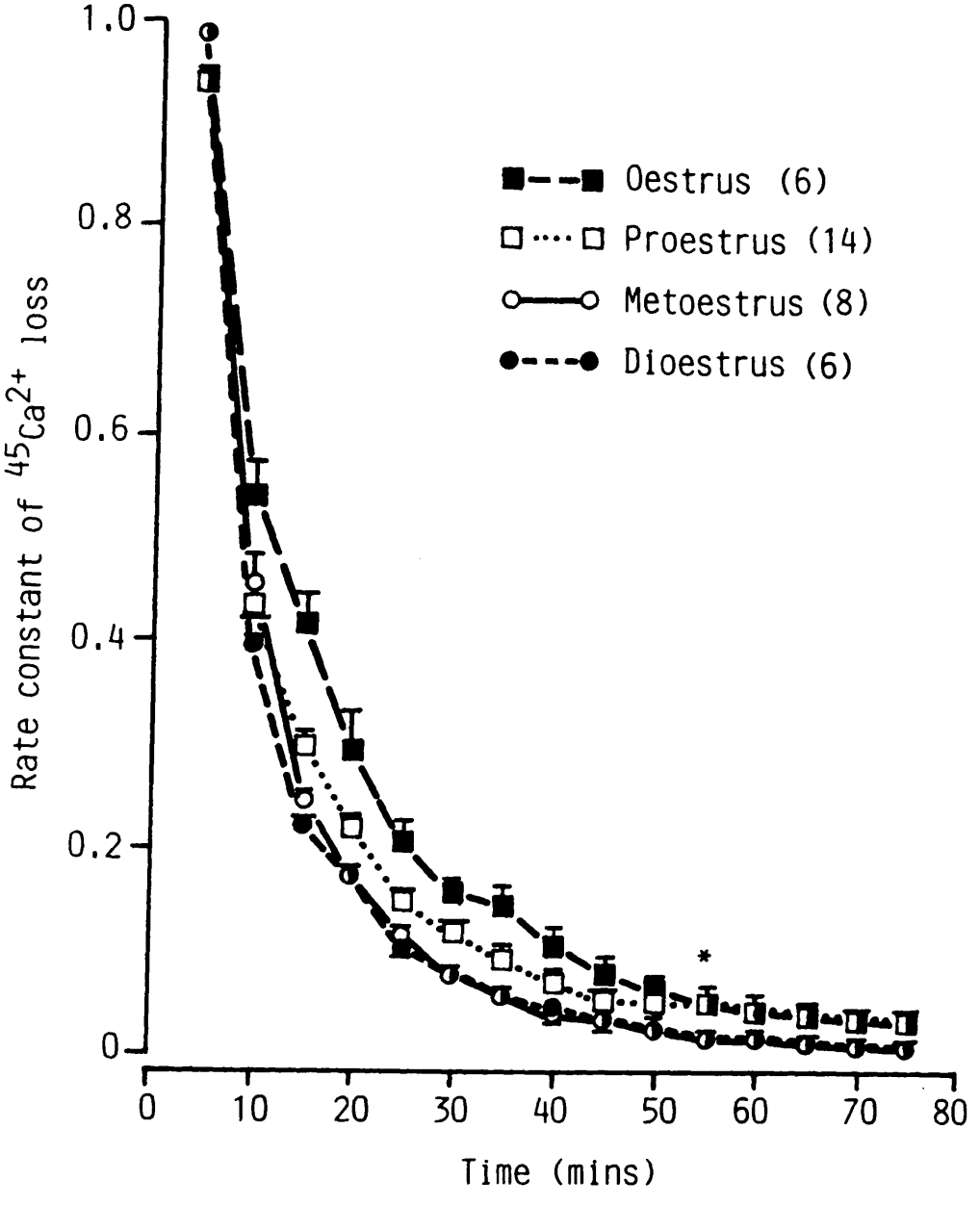


Fig. 49: Curves showing the rate constant of $^{45}Ca^{2+}$ loss (i.e. $^{45}Ca^{2+}$ leaving tissue during collection interval divided by $^{45}Ca^{2+}$ in the tissue before collection interval) with time, in the rat isolated uterus during the four phases of the oestrous cycle. Number of observations in brackets.

constants in metoestrus and dioestrus were similar, and were significantly lower ($p < 0.001$) than those in proestrus and oestrus.

3. $^{45}Ca^{2+}$ efflux after removal of ovarian hormonal influence

Since differences in hormonal status clearly influenced $^{45}Ca^{2+}$ efflux, it was of interest to examine efflux in uteri from ovariectomized animals. As in uteri from intact rats, $^{45}Ca^{2+}$ efflux showed a biphasic decline which was similar to efflux seen in metoestrus (Fig. 50). The rate constant of $^{45}Ca^{2+}$ loss was also close to that achieved in metoestrus.

After 80 minutes of efflux, the residual tissue $^{45}Ca^{2+}$ was measured in all uteri and related to their corresponding tissue weight (Fig. 51). The amount of $^{45}Ca^{2+}$ retained after efflux did not correlate in any way with tissue weight.

4. Effect of SAL on $^{45}Ca^{2+}$ efflux in normal and potassium chloride depolarized uteri

In order to produce inhibition, adrenoceptor agonists could lower the intracellular free Ca^{2+} concentration by increasing Ca^{2+} efflux from the cell. This possibility was investigated by studying the effect of SAL on $^{45}Ca^{2+}$ efflux in uteri in oestrus (oestrogen dominated) and metoestrus (progesterone dominated). A concentration of 5×10^{-6}M SAL was used because it produced the maximum inhibitory effect in isolated uterine horn preparations. SAL, added during the 65-70 minutes period of efflux (Fig. 52), did not alter

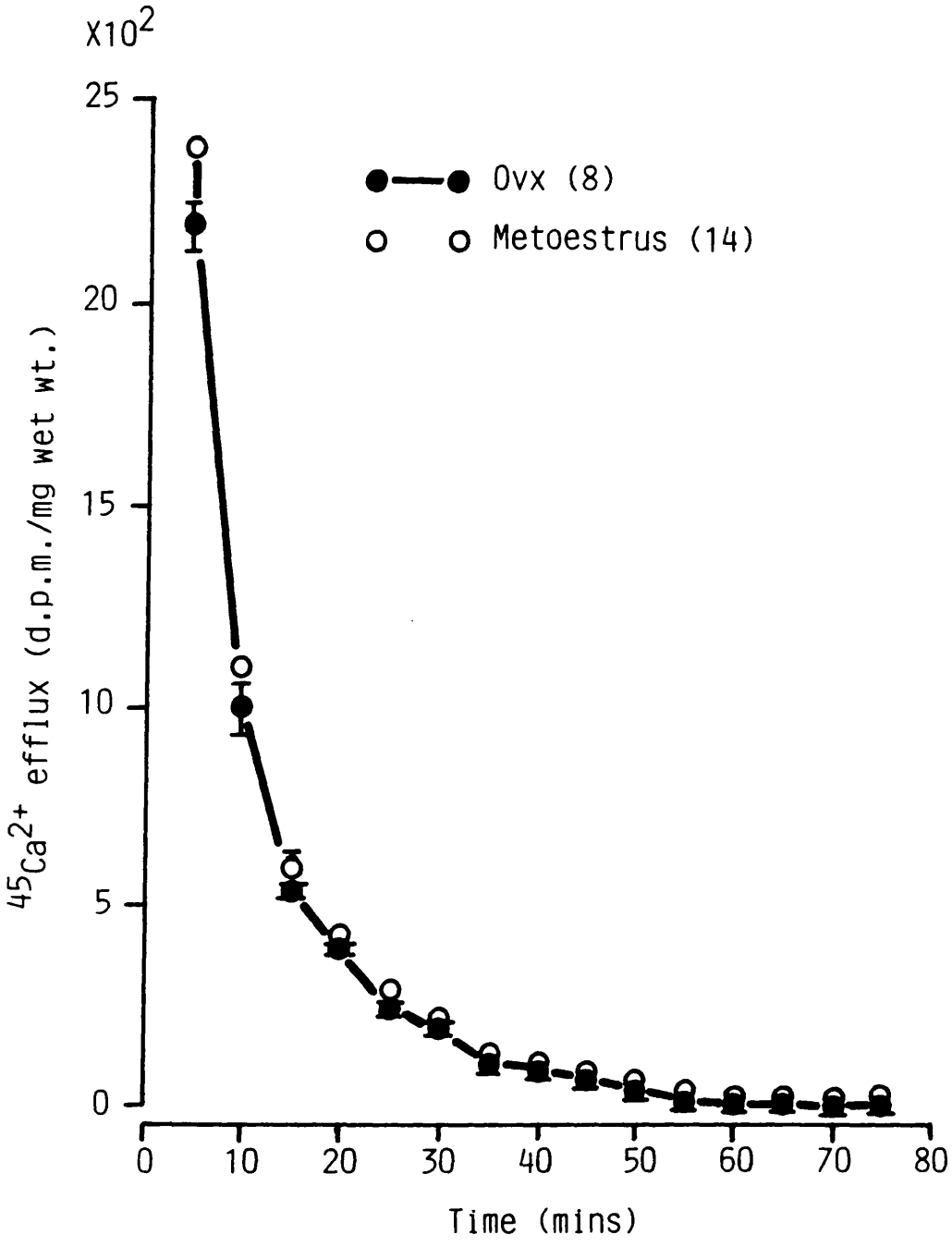
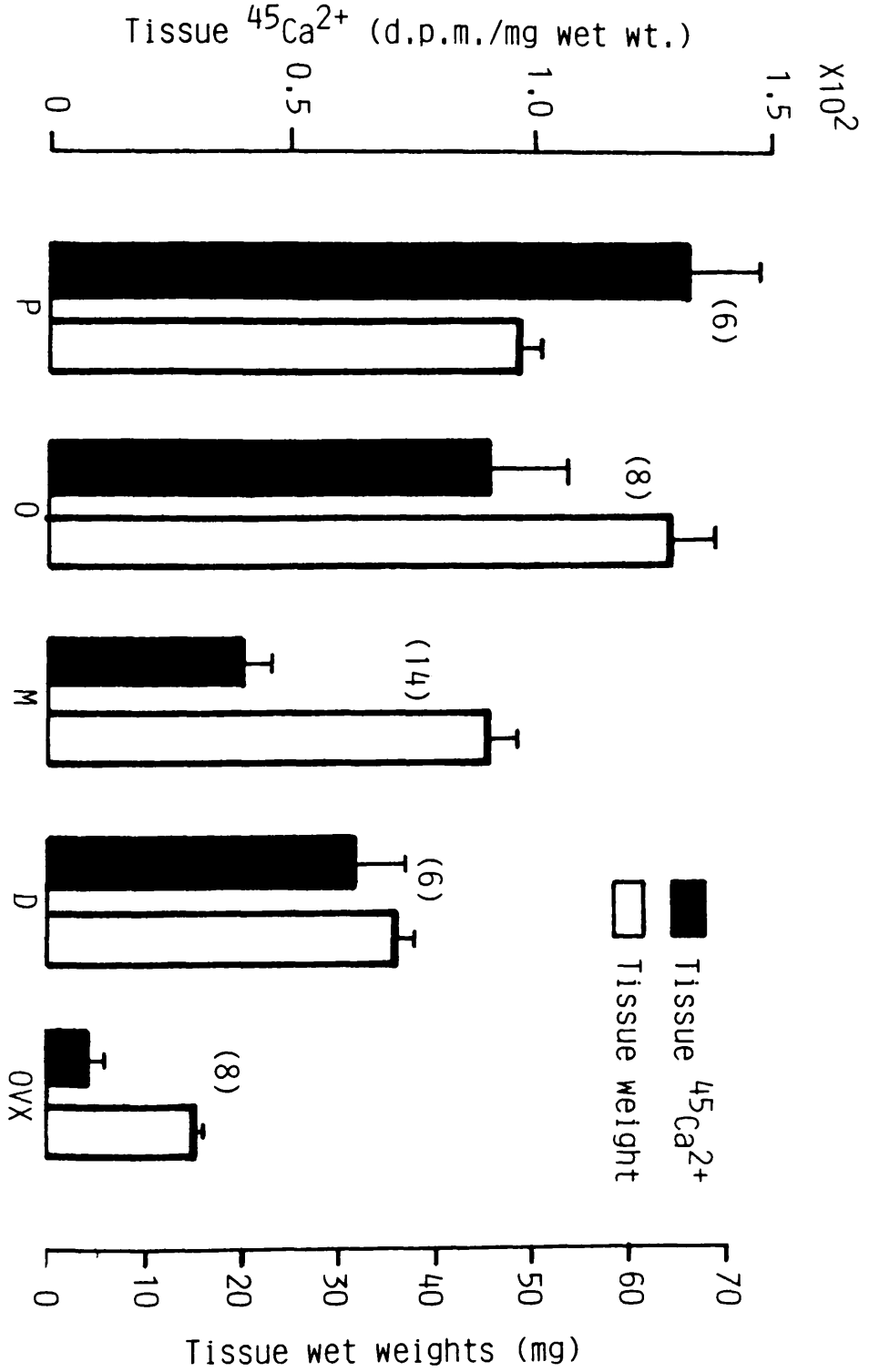


Fig. 50: $^{45}Ca^{2+}$ efflux curve (in d.p.m./mg wet weight of tissue against time) in uteri from ovariectomized rats. Values from uteri in metoestrus (open circles) are also shown. Number of observations in brackets.

Fig. 51: Residual $^{45}Ca^{2+}$ (expressed as d.p.m./mg wet weight of tissue) after efflux (filled columns), and their corresponding weights (open columns), in uteri from the four phases of the oestrous cycle, and after ovariectomy. P = proestrus; O = oestrus; M = metoestrus; D = dioestrus; OVX = after ovariectomy. Number of observations in brackets.


Tissue $^{45}Ca^{2+}$ (d.p.m./mg wet wt.)
0
0.5
1.0
1.5
X10^2
P
(6)
O
(8)
M
(14)
D
(6)
OVX
(8)
Tissue $^{45}Ca^{2+}$
Tissue weight
0
10
20
30
40
50
60
70
Tissue wet weights (mg)

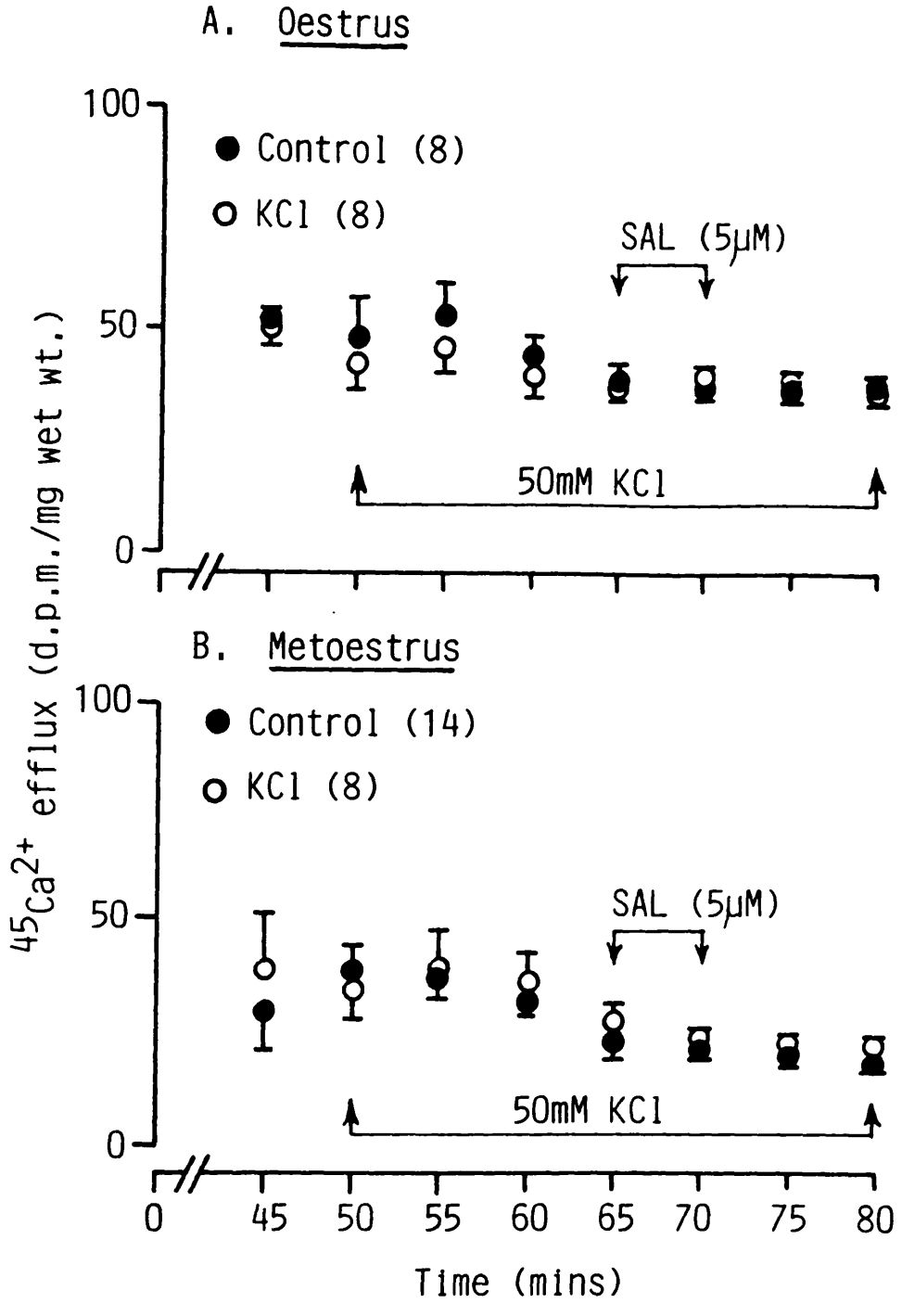


Fig. 52: Effect of salbutamol (SAL) on $^{45}Ca^{2+}$ efflux (in d.p.m./mg wet weight of tissue against time), in the rat isolated uterus in oestrus (A) and metoestrus (B): controls, and in uteri treated with potassium chloride (KCl). SAL (5×10^{-6}M) and KCl (5×10^{-2}M) added between arrows. Numbers of observations in brackets.

$^{45}Ca^{2+}$ efflux in oestrus and in metoestrus.

Since the inhibitory effects of SAL were shown in uteri which were contracted with ACh or KCl, a series of experiments was performed in which preparations from oestrus and metoestrus were depolarized with KCl (5×10^{-2}M) which was present during the 50-80 minutes period of efflux. Neither KCl alone nor SAL in the presence of KCl altered $^{45}Ca^{2+}$ efflux (Fig. 52).

5. $^{45}Ca^{2+}$ influx in uteri from the four phases of the oestrous cycle and after ovariectomy

Since the ovarian hormones altered $^{45}Ca^{2+}$ efflux during the oestrus cycle, their possible effects on $^{45}Ca^{2+}$ influx were also investigated. $^{45}Ca^{2+}$ influx curves for uteri in proestrus, oestrus, metoestrus and dioestrus and in those from ovariectomized animals are shown in Figure 53. Saturable curves were obtained in the four phases and after ovariectomy. At equilibrium, levels of $^{45}Ca^{2+}$ uptake in proestrus and oestrus were significantly higher ($p < 0.001$) when compared to uptake in metoestrus and dioestrus. $^{45}Ca^{2+}$ uptake in uteri from ovariectomized animals was significantly lower ($p < 0.001$) than in any of the phases of the oestrous cycle at equilibrium.

6. Effect of SAL on $^{45}Ca^{2+}$ influx

As an alternative to efflux, adrenoceptor agonists could also lower intracellular free Ca^{2+} concentration by decreasing

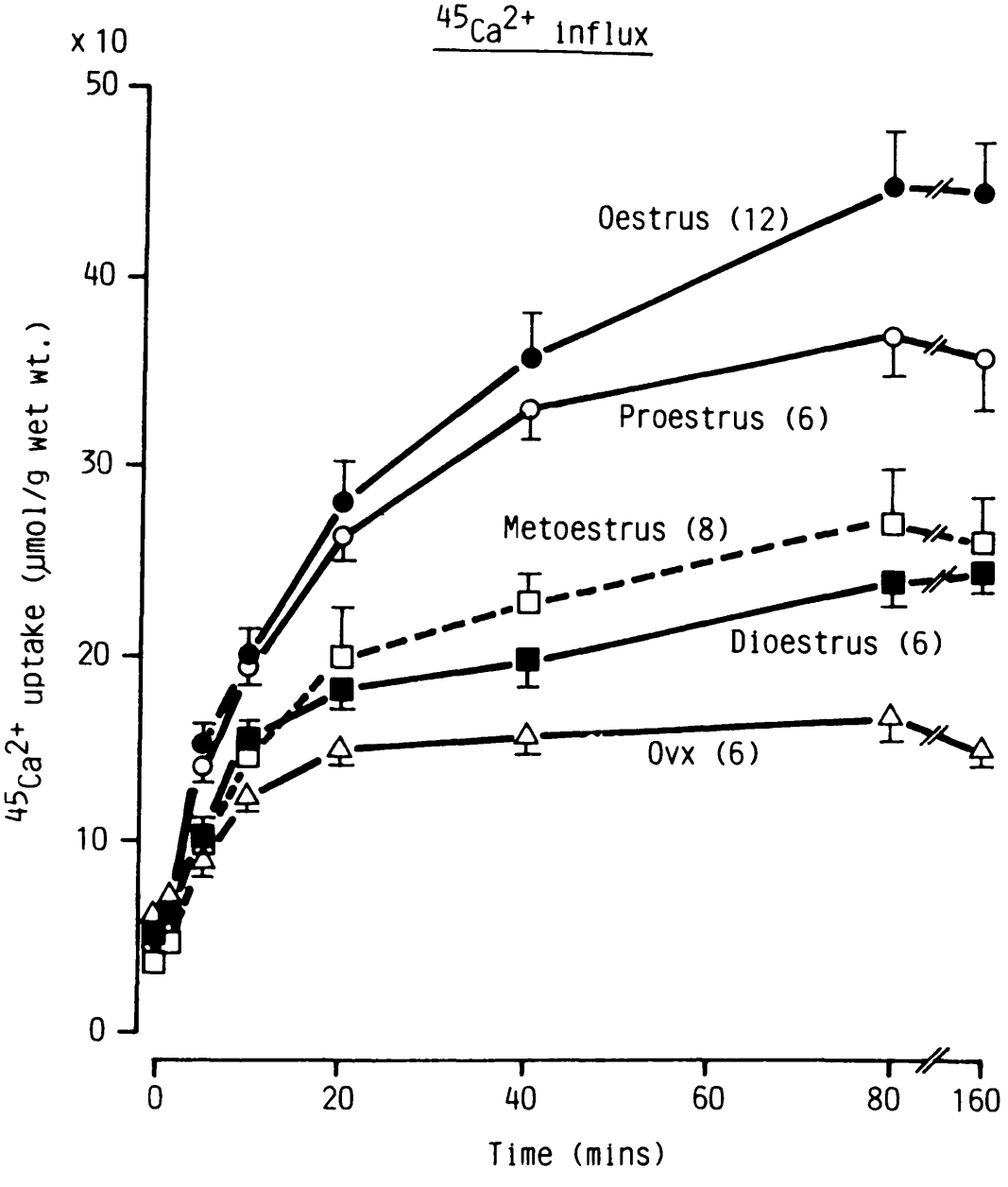


Fig. 53: $^{45}Ca^{2+}$ influx curves (in µmol/g wet weight of tissue against time) in the rat isolated uterus, in the four phases of the oestrous cycle, and after ovariectomy (OVX). Number of observations in brackets.

influx of Ca^{2+} into the cell. Since SAL did not affect efflux, its effects on $^{45}Ca^{2+}$ influx were examined in uteri from metoestrus and dioestrus. These phases were chosen because $^{45}Ca^{2+}$ influx was low, and thus it would be easier to demonstrate changes induced by SAL. SAL (5×10^{-6}M) was present in the Tyrode solution throughout the experiment. SAL had no statistically significant effect on $^{45}Ca^{2+}$ influx in metoestrus (Fig. 54) and dioestrus (Fig. 55).

The presence of the ovarian hormones influenced both influx and efflux of $^{45}Ca^{2+}$ during the oestrous cycle suggesting that the hormones could indirectly affect uterine response to the adrenoceptor agonists in this way. However, SAL had no effect on either $^{45}Ca^{2+}$ influx or efflux in both normal and KCl-depolarized uteri. This would suggest that adrenoceptor agonists may be lowering intracellular free Ca^{2+} concentration by promoting an increase in intracellular Ca^{2+} binding within its stores.

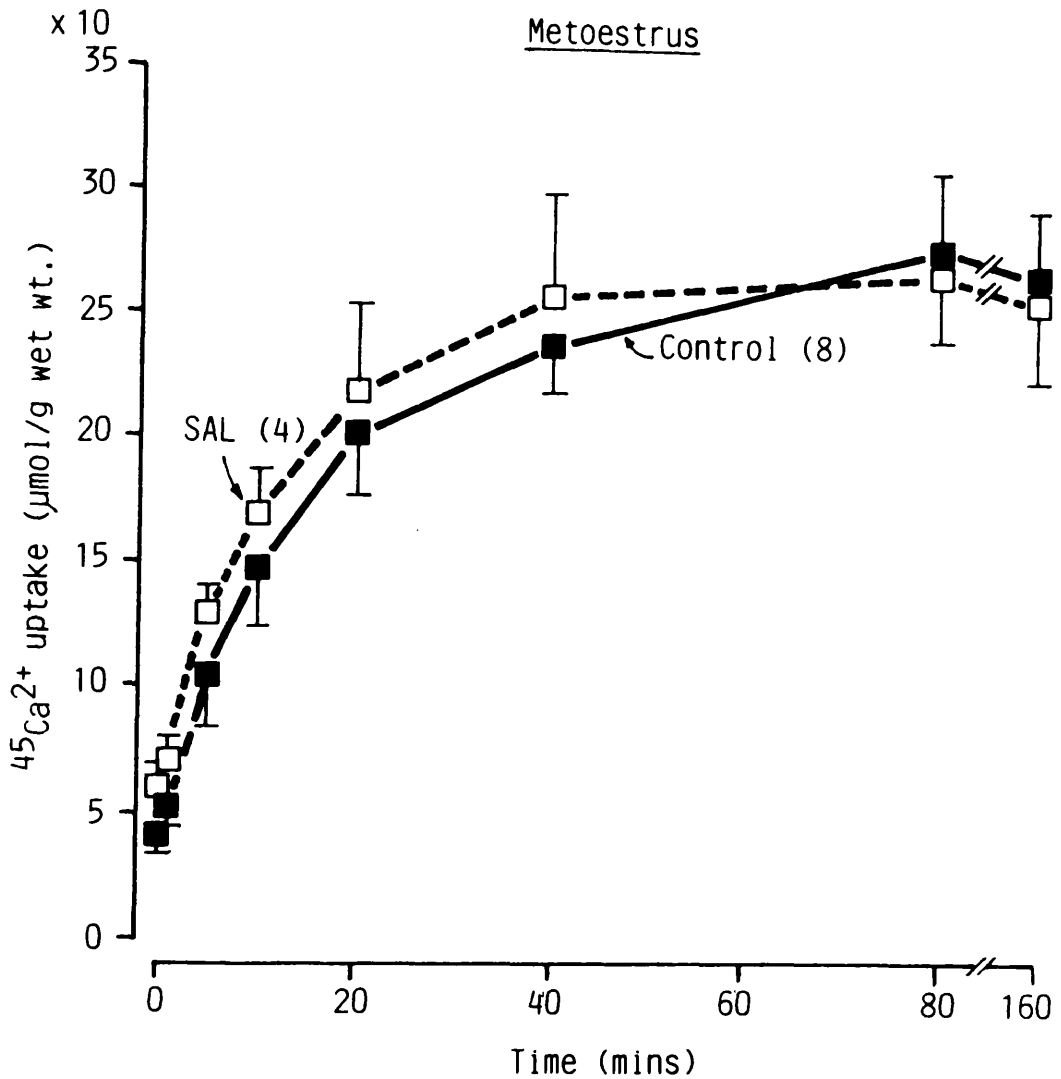


Fig. 54: Effect of salbutamol (SAL) on $^{45}Ca^{2+}$ influx (in µmol/g wet weight of tissue against time), in the rat isolated uterus in metoestrus. Solid line = control; broken line = in presence of SAL (5×10^{-6}M). Number of observations in brackets.

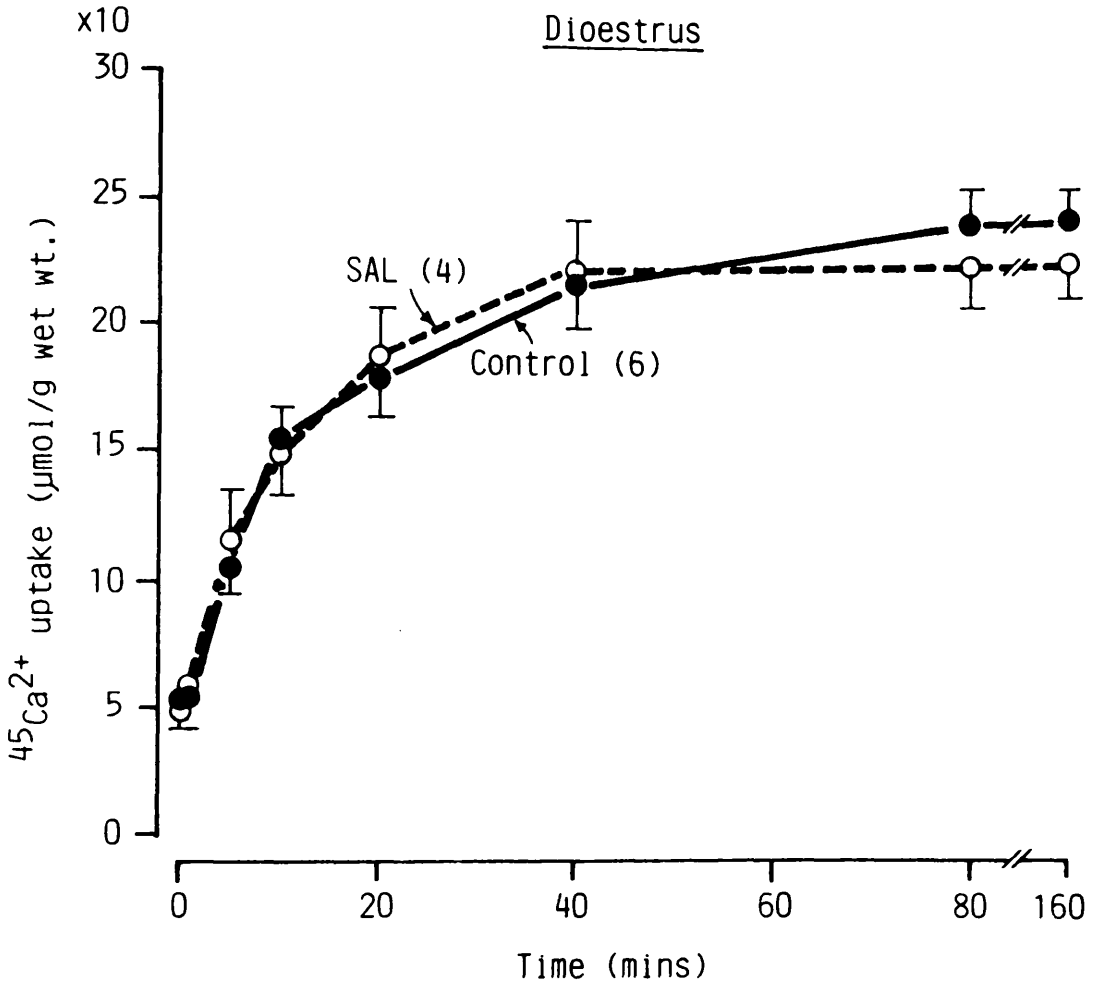


Fig. 55: Effect of salbutamol (SAL) on $^{45}Ca^{2+}$ influx (in µmol/g wet weight of tissue against time), in the rat isolated uterus in dioestrus. Solid line = control; broken line = in presence of SAL 5×10^{-6}M). Number of observations in brackets.

DISCUSSION

A. RESPONSES TO MOTOR AGENTS

ACh produced motor responses in all uteri, i.e., in the four phases of the oestrous cycle and in ovariectomized animals, and the ACh potency did not vary under the different conditions. Thus, it would appear that the receptor population for ACh is constant, and is not influenced by the ovarian hormones. However, the maximum tension developed in response to ACh varied being highest in oestrus, and lowest in ovariectomized animals. As with ACh, uteri from oestrus also developed the highest tension to KCl. The variations in tension to both ACh and KCl were similar and may reflect the observed differences in the thickness of the uterine smooth muscle during the oestrous cycle (Digges, 1980; Boyle & Digges, 1982a).

B. RESPONSES TO ADRENOCEPTOR AGONISTS IN THE OESTROUS CYCLE

All three adrenoceptor agonists - NA, ADR and SAL produced inhibitory responses in the four phases of the oestrous cycle. Antagonism of the ACh motor response by the adrenoceptor agonists is physiological, and is mediated through activation of β-adrenoceptors (Krall *et al.*, 1978; Boyle & Digges, 1982a; Acritopoulou-Fourcroy, Clabaut & Schrub, 1985). The rank order of potency of the agonists was: ADR > SAL > NA for uteri in proestrus, metoestrus and dioestrus as reported by other workers, which would suggest

that the β-adrenoceptor population is of the β_2-subtype (Levy & Apperley, 1978; Johansson, Andersson & Wikberg, 1980; Nahorski, 1981). However, in oestrus, the rank order of potency of the agonists was: ADR > NA > SAL, suggesting that β_1-adrenoceptors may be present in this phase. Oestrus is predominantly under the influence of oestrogen (Brenner & West, 1975; Spaziani, 1975) and the finding that both β_1- and β_2-adrenoceptors co-existed in an oestrogen dominated rat uterus (Johansson *et al.*, 1980; Nahorski, 1981) may explain the above observation.

For each adrenoceptor agonist, both the degree of inhibition and the potency varied throughout the oestrous cycle. The variation could have been partially due to activation of α-excitatory receptors by NA and ADR, thereby causing a reduction in their β-adrenoceptor mediated inhibition. At high concentrations, NA and ADR produced small motor responses in proestrus and oestrus, which were abolished by the α-adrenoceptor antagonist AZA. Similar AZA-sensitive motor responses observed in the presence of propanolol (Boyle & Digges, 1982a) support the existence of α-adrenoceptors in these phases. α-adrenoceptors have been demonstrated in the rat uterus during proestrus and oestrus by Krall *et al.* (1978) using the radioligand binding technique, and by Acritopoulou-Fourcroy *et al.* (1985) in *in vivo* spontaneously contracting uteri in proestrus. It thus appears that α-adrenoceptor activity in the uterus is

associated with conditions of oestrogen dominance, since oestrogen levels are high during proestrus and oestrus (Brenner & West, 1975; Spaziani, 1975). In the presence of AZA, the maximum degrees of inhibition produced by NA (proestrus and oestrus) and ADR (proestrus, oestrus and metoestrus) were enhanced. Brooks et al. (1965) and Acritopoulou-Fourcroy et al. (1985) reported similar enhancement of NA and ADR inhibitory effects in the rat uterus by α-adrenoceptor antagonists.

At low concentrations, AZA also caused paradoxical shifts in ADR dose-response curves to the right. This shift may be due to a non-specific excitatory effect of AZA independent of α-adrenoceptor antagonism as reported by Qayum and Yusuf (1977). Other α-adrenoceptor antagonists including phentolamine and tolazoline have also been shown to produce similar excitatory effects in the rat uterus (Tothill, 1967; Paton, 1968). Thus, the rightward shifts caused by AZA may be explained on the basis of a non-specific stimulant effect.

Notwithstanding the stimulant effect produced by AZA, the conclusions can be drawn that there are α-adrenoceptors in the rat uterus which subserve an excitatory function. However, SAL never produced motor responses and its inhibition was unaffected by AZA. Thus, while α-adrenoceptor involvement contributed to the variations seen with NA and ADR it could not explain the SAL variation.

The presence of an avid agonist removal mechanism into neuronal and extraneuronal tissue (Gillespie, 1973; Iversen, 1973; La Bella, 1985) would tend to reduce the concentration of adrenoceptor agonists at the receptor sites. The possibility that these uptake processes may be responsible for the variation in uterine response to these agonists was investigated in the presence of a neuronal uptake inhibitor (DMI), and an extraneuronal uptake inhibitor (NMN). DMI and NMN shifted NA (metoestrus and dioestrus) ADR and SAL dose-response curves to the left, and enhanced the maximum degrees of inhibition. Fleming (1975) and Guimaraes and Trendelenburg (1985) described the increased sensitivity induced by inhibition of agonists sites of loss as deviation supersensitivity. For an agonist that is subject to the removal processes, its average concentration in the receptor biophase is lower than that in the incubation medium. Thus supersensitivity follows when inhibition of the sites of loss raises the agonist concentration in the biophase (Guimaraes & Trendelenburg, 1985).

Despite the fact that DMI and NMN shifted agonists dose-response curves to the left, corresponding increases in their pD_2 values were not achieved in all cases. This may be due to the fact that in addition to the observed leftward shifts, there was an increase in the maximum degrees of inhibition produced by the agonists. Thus, changes in potency may be occurring, but are concealed by the increased intensity of the effect.

The agonists increased maximum response observed in presence of the uptake inhibitors, has also been demonstrated in the rat vasa deferentia (Kenakin, 1980, 1984). Kenakin, (1984) interpreted the increased maximum response to NA in the rat vas deferens in terms of an altered agonist concentration gradient within the muscle. Since the rat vasa deferentia is a densely innervated tissue, Kenakin's explanation may not be applicable to the rat uterus. The agonists enhanced maximum degrees of inhibition observed in the present study cannot, therefore, be explained solely on the basis of an increased agonist concentration in the receptor biophase. Gillespie (1973) proposed that extraneuronally accumulated catecholamines may act intracellularly to regulate other processes including cell division and smooth muscle motility. Because the rat has a sparse adrenergic innervation (Silva 1966), extraneuronal uptake may be more important than neuronal uptake in the disposal of the catecholamines. Indeed, Boyle and Digges (1982a) found that inhibition of the extraneuronal process alone produced effects similar to blocking both neuronal and extraneuronal uptake processes.

In the present study, inhibition of the uptake processes produced greater effects, i.e., shifts in agonists dose-response curves and increases in the maximum degree of inhibition, than blockade of the α-excitatory adrenoceptors. This would suggest that the removal mechanisms play a more important role than α-adrenoceptor activity in the observed variation in uterine response during the oestrous cycle.

In order to ensure that an effect on β-adrenoceptors uncomplicated by α-adrenoceptor activity and agonist removal mechanisms could be observed, a series of experiments was performed in the presence of AZA , DMI and NMN. The combined antagonists shifted NA, ADR (proestrus and oestrus) and SAL dose-response curves to the left, and enhanced their maximum degrees of inhibition. However, in dioestrus, ADR dose-response curve was shifted to the right at the lower concentrations. This shift may be attributed to the AZA-induced non-specific stimulant action as described previously, since it was similar to that observed in the presence of AZA alone.

The effects produced by the combined antagonists were similar to those achieved in the presence of the uptake inhibitors alone again supporting the view that agonist removal mechanisms are more important than α-adrenoceptor activity. However, the variation in uterine response to the adrenoceptor agonists persisted even in the presence of AZA, DMI and NMN , indicating that other factors may be involved.

C. RESPONSES TO ADRENOCEPTOR AGONISTS IN OVARIECTOMIZED ANIMALS

The effects of NA , ADR and SAL were re-examined in uteri from animals with bilateral ovariectomy in order to assess the influence of the ovarian hormones on their inhibitory activity. In contrast to the intact animals

all three agonists produced complete inhibition of the ACh-induced contraction. The rank order of potency of the agonists was: SAL > ADR > NA. SAL was more potent in uteri from ovariectomized animals, than from any phase of the oestrous cycle in which ADR was the most potent. It may well be that the β-adrenoceptor population following ovariectomy is predominantly of the β_2-subtype.

The possibility that α-adrenoceptors may also be present in uteri from ovariectomized animals was investigated. NA and ADR did not produce any motor responses suggesting that α-excitatory receptors are absent in this condition. In the presence of AZA, both ADR and SAL dose-response curves were shifted to the right, while the NA curve was unaffected. Thus, AZA produced its non-specific excitatory effects as in uteri from intact animals.

Since both neuronal and extraneuronal uptake processes appeared to play a role in adrenoceptor agonists responses during the oestrous cycle, it was of interest to examine their effects in uteri from ovariectomized animals. DMI and NMN produced classical leftward shifts in NA and ADR dose-response curves, while for SAL, only responses to the lower concentrations were enhanced. The leftward shifts may be ascribed to increased agonist concentrations at the receptor sites (Fleming, 1975; Guimaraes & Trendelenburg, 1985). It thus appears that the removal mechanisms could also play a role in adrenoceptor agonists responses in uteri from ovariectomized animals.

To allow comparison with experiments in intact animals, the effect of controlling both α-adrenoceptor activity and the uptake processes, was also investigated in uteri from ovariectomized rats. In the presence of the combined antagonists, the SAL dose-response curve lay to the right of its control, while those for NA and ADR coincided with the antagonist-free controls. The observed rightward shift in the SAL dose-response curve was similar to that achieved in the presence of AZA alone. Since both SAL and ADR were the most potent in uteri from ovariectomized and intact animals respectively, these agonists appeared to be more sensitive to the non-specific stimulant effect of AZA.

The result of the present study shows that in uteri from ovariectomized rats, NA, ADR and SAL produced complete physiological antagonism of the ACh-induced contraction. Thus, the presence of the ovarian hormones during the oestrous cycle may underlie the observed variations in uterine responses to the adrenoceptor agonists.

One possible explanation as to the mechanism of ovarian hormone-induced alteration in uterine response to the adrenoceptor agonists may be that the hormones were affecting adrenoceptor activity by a direct action on the receptor. When the maximum degrees of inhibition achieved by NA, ADR and SAL, in uteri from both intact and ovariectomized animals were compared with their potencies at the receptor

site, no correlation was found. Thus, events responsible for the variation in adrenoceptor agonists responses during the oestrous cycle lay beyond the receptor.

Another explanation may be that the ovarian hormones are affecting an intracellular process which in turn would account for the variation in uterine response to the adrenoceptor agonists. The ovarian hormones (especially oestrogen) have been reported to increase uterine prostaglandin production in the rat uterus (Ham et al., 1975; Sterin-Speziale et al., 1980; Wilson, 1983; Gimeno & Gimeno, 1984). In consequence, higher levels of endogenous prostaglandins would be expected in uteri from rats during the oestrous cycle, than from ovariectomized animals. It thus seems that the inability of the adrenoceptor agonists to produce complete physiological antagonism of the ACh contraction could be partially due to the hormone-dependent high levels of prostaglandins during the oestrous cycle.

D. CELLULAR MECHANISMS INVOLVED IN ADRENOCEPTOR AGONISTS INHIBITORY RESPONSES

1. Effect of adrenoceptor agonists on intramural prostaglandin production

Catecholamines have been shown to stimulate cyclo-oxygenase in tissue homogenates and isolated cells (Egan, Humes & Kuehl, 1978; Bauman, Von Bruchhausen & Wurm, 1979).

Thus, the present study investigated the possibility that by stimulating cyclo-oxygenase, and hence prostaglandin biosynthesis in the uterus the adrenoceptor agonists could oppose or reduce their own inhibition. The effects of the cyclo-oxygenase inhibitor, FBF, were examined under two conditions - the first in which only the agonists were present (referred to as Control I experiments), and the second in which both the agonists and the antagonists AZA, DMI and NMN were present (referred to as Control II experiments). The Control II experiments were performed to ensure that an effect on β-adrenoceptors only, was being examined since antagonism of α-adrenoceptor activity, neuronal and extraneuronal uptake processes had been shown to modify the agonists inhibitory responses.

With some exceptions, FBF shifted NA, ADR and SAL Control I and Control II dose-response curves to the left and enhanced their maximum degrees of inhibition, such that all three agonists produced complete inhibition of the ACh contraction. There were corresponding increases in agonist potencies, but these did not reach significance in all experiments. This failure may be due to the fact that in addition to the observed leftward shifts in agonists dose-response curves, there was an increase in their maximum degrees of inhibition. As observed earlier with the uptake inhibitors, changes in potency may be occurring but are concealed by the increased intensity of the agonist response in the presence of FBF.

Cyclo-oxygenase inhibition potentiated the inhibitory effects produced by all three agonists in the four phases of the oestrous cycle. A similar enhancement by indomethacin of β-adrenoceptor mediated relaxation in canine coronary arteries has been reported by Rubanyi and Vanhoutte (1985). It thus appears that in the rat uterus, NA, ADR and SAL, may stimulate prostaglandin biosynthesis , and the prostaglandins thus formed, would then oppose the β-adrenoceptor mediated relaxation.

The mechanism by which cyclo-oxygenase activity could be stimulated by adrenoceptor agonists is not well understood. Egan et al. (1978) and Bauman et al. (1979) have suggested that the agonists may participate as phenolic cofactors for the cyclo-oxygenation of arachidonic acid. It may also be possible that stimulation of cyclo-oxygenase could be an indirect event evoked by activation of the adrenoceptors. Stimulation of α-adrenoceptors has been shown to increase prostaglandin generation in the pregnant human myometrium (Quaas & Zahradnik, 1985; Wikland, Lindblom & Wiqvist, 1985). Thus while α-adrenoceptors may contribute to the prostaglandins produced in the rat uterus, the β-adrenoceptors are also important since agonists effects on intramural prostaglandin generation persists in the presence of AZA. However, the link between adrenoceptor activation, and increases in cyclo-oxygenase activity remains to be determined. Although both the myometrium and endometrium have been demonstrated to generate prostaglandins in the

rat, the endometrium appears to be the major source (Campos et al., 1980; Brown & Poyser, 1985).

Although NA responses in Control I experiments were enhanced by FBF, complete inhibition of the ACh contraction was not achieved. Thus, for NA, inhibition of both α-adrenoceptor activity and the uptake processes may be necessary in order to obtain complete physiological antagonism of the ACh-induced contraction in the presence of FBF. The exceptions to the leftward shift in agonists Control II dose-response curves were in metoestrus (NA, ADR and SAL) and proestrus (ADR and SAL). It appears that under these conditions blocking both α-adrenoceptors and the uptake processes may prevent agonists effects on cyclo-oxygenase.

In summary, the present results tend to suggest that an effect on intramural prostaglandin production could be partially responsible for the observed variation in uterine response to the adrenoceptor agonists during the oestrous cycle. Stimulation of cyclo-oxygenase by the adrenoceptor agonists may be achieved in two ways: (a) by a direct effect of the agonists on the enzyme, and/or (b) indirectly through an effect on adrenoceptors.

In uteri from ovariectomized animals, FBF shifted both Control I and Control II dose-response curves for ADR and SAL to the left. Just as FBF effects on the NA response in Control I experiments were less than in Control II in

uteri from intact rats, FBF was without effect in Control I experiments in uteri from ovariectomized animals, while shifting the Control II curve to the left. This would suggest that for NA, blockade of both α-adrenoceptor activity and the uptake processes may be necessary before an effect on cyclo-oxygenase could be demonstrated. The rightward shift in the ADR Control II curve at the lower concentrations may be attributed to the non-specific excitatory action of AZA as described earlier. In general, FBF produced less effect in uteri from ovariectomized rats, than in those from intact animals. One possible explanation may be that since ovarian hormonal influence on uterine prostaglandin production was absent in ovariectomized animals, a lower basal prostaglandin activity would lead to a reduced FBF-induced effect.

In order to ascertain whether cyclo-oxygenase inhibition could also modify the responses produced by other uterine relaxants, experiments were performed using HIS and PAP. HIS inhibitory responses in the rat uterus involve activation of H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972; Bertaccini, Molina, Vitali & Zappia, 1979; Ohia & Okpako, 1981). Since there is evidence that H_1-excitatory receptors may also be present in this preparation (Ohia & Okpako, 1981), HIS responses were examined in the presence of mepyramine. HIS, like the adrenoceptor agonists, produced different degrees of inhibition of the ACh-induced

contraction throughout the oestrous cycle but HIS was unaffected by FBF treatment. The non-specific smooth muscle relaxant, PAP produced complete inhibition of the ACh-induced contraction in proestrus, oestrus, metoestrus and dioestrus. This observation demonstrates that it is possible to achieve complete physiological antagonism of the ACh motor response. As with HIS, FBF treatment had no effect on PAP inhibitory responses in the four phases of the oestrous cycle. Since neither HIS nor PAP responses were modified by FBF, the effects produced by cyclo-oxygenase inhibition appeared to be specific for adrenoceptor agonists NA, ADR and SAL.

Daniel (1982) suggested that drug-induced inhibition in smooth muscles which possess no intrinsic tone may be influenced differentially by the mechanisms whereby tension was increased. Since the rat uterus has no intrinsic tone the inhibitory responses to the adrenoceptor agonists have been assessed either on spontaneously contracting preparations (Rudzik & Miller,1962; Levy & Tozzi, 1963; Pharriss & Russell, 1968; Downing & Porter, 1980), or under conditions in which tone was induced with 5-hydroxytryptamine (Jensen & Vennerod, 1961), KCl (Schild, 1966, 1967; Diamond & Marshall, 1969a,b; Marshall & Kroeger, 1973), ACh (Ash & Schild, 1966; Olsson & Persson, 1971; Wasserman & Levy, 1972; Boyle & Digges, 1982a,b) and by field stimulation (Paterson, 1965; Clegg, 1966). In order to investigate

the possibility that the effects produced by FBF was not dependent on tone induced with ACh, series of experiments were performed using KCl.

KCl produced a biphasic contraction in uteri from the four phases of the cycle, as has been reported by other workers (Osa & Kuriyama, 1975; Van Breemen, Aaronson & Loutzenhiser, 1979). The maintained tension in the secondary phase has been associated with an increased intracellular free calcium concentration (Bolton, 1979; Granger, Hollingworth & Weston, 1986). In previous studies, induction of uterine tone with KCl involved replacing sodium ions in the bathing medium with equimolar concentrations of potassium ions (Van Breemen & Daniel, 1966; Marshall & Kroeger, 1973; Bengtsson, 1978). Since sodium ions have been shown to be necessary for both catecholamine uptake processes (Gillespie, 1973; La Bella, 1985) and β-adrenoceptor mediated relaxation of uterine smooth muscle (Meisheri & McNeill, 1979b), KCl-induced tone in the present study was achieved by the addition of potassium ions to the bathing medium. SAL produced similar effects in preparations in which tone was induced with either KCl or ACh, suggesting that the receptors mediating inhibition under the two experimental conditions are alike (Schild, 1967; O'Donnell, Persson & Wanstall, 1978). The combined antagonists, i.e., AZA, DMI and NMN, also potentiated SAL inhibitory responses in uteri in which tone was induced with KCl.

As in experiments in which tone was induced with ACh, both Control I and Control II type studies were performed for SAL in KCl-depolarized preparations. FBF shifted both Control I and Control II dose-response curves to SAL to the left, and produced complete relaxation of the KCl-induced tone. The effects produced by FBF in these experiments were similar to those in which ACh was used to induce tone. This would suggest that the effects produced by cyclo-oxygenase inhibition were not dependent on the motor agent used.

Since arachidonic acid is a precursor of not only the prostaglandins, but also the leukotrienes through lipoxygenase activity, inhibition of cyclo-oxygenase may lead to an increased leukotriene formation. Carraher et al. (1983) suggested that leukotrienes may also be involved in the regulation of uterine motility. The possibility that leukotrienes may be affecting the adrenoceptor agonists responses was investigated, therefore, in the presence of BW 755C, an inhibitor of both cyclo-oxygenase and lipoxygenase (Rainsford, 1985). Control II type experiments were performed for SAL in the four phases of the oestrous cycle. BW 755C shifted SAL dose-response curves to the left and increased SAL potency throughout the oestrous cycle. The effects produced by BW 755C were similar to those achieved in the presence of FBF suggesting that leukotrienes are not involved in the adrenoceptor agonists responses.

In summary, the results of the present study show that adrenoceptor agonists could affect intramural prostaglandin production in the rat uterus. The exact mechanism responsible for these interactions are unclear but they may involve either: (a) a direct action of the agonists on cyclo-oxygenase, and/or (b) an indirect action on the enzyme via stimulation of adrenoceptors. The adrenoceptor agonists appeared to stimulate uterine prostaglandin production in the different hormonal conditions of the oestrous cycle, and in the absence of the hormones following ovariectomy. The effect on prostaglandin production was specific since cyclo-oxygenase inhibition had no effect on the inhibitory responses to HIS and PAP. The method of inducing tone appeared to be unimportant because cyclo-oxygenase inhibition produced similar effects in uteri in which tone was induced with ACh and KCl. The effect of cyclo-oxygenase inhibition may be of pharmacological importance since adrenoceptor agonists potencies were increased in uteri from both intact and ovariectomized animals.

Thus, ADR and SAL effects on prostaglandin production could be responsible for the observed variation in uterine response during the oestrous cycle. An effect on intramural prostaglandin generation may partially explain the observed variation to NA since, complete inhibition of the ACh contraction was achieved only in Control II experiments.

2. cAMP metabolism

The stimulation of rat uterine β-adrenoceptors by agonists has been shown to increase intracellular cAMP concentrations (Triner et al., 1971; Kroeger & Marshall, 1974; Johansson & Andersson, 1978; Meisheri, Diamond & McNeill, 1981; Marshall & Fain, 1985). The cAMP concentration has also been reported to be elevated in the uterus following injection of rats with oestrogen (Szego & Davies, 1967, 1969; Rosenfeld & O'Malley, 1970; Sandborn et al., 1973; Flandroy & Galand, 1978; Kishikawa, 1981), and after in vitro treatment of tissues with prostaglandin E_2 (Vesin & Harbon, 1974; Vesin, Do Khac & Harbon, 1978, 1979). It was of interest, therefore, to investigate the possibility that interactions of the adrenoceptor agonists, oestrogen and prostaglandins at the level of cAMP metabolism could partially account for the differences in uterine response to the adrenoceptor agonists during the oestrous cycle.

In the first series of experiments, the possibility that changes in cAMP concentrations induced by endogenous oestrogen may play a role in the variation in uterine response to the adrenoceptor agonists was investigated. Basal cAMP concentrations were measured in uteri from proestrus, oestrus, metoestrus and dioestrus. Uterine cAMP content in the four phases were similar suggesting that in physiological concentrations, oestrogen does not affect basal cAMP production. Since the basal levels

were similar, the variation in adrenoceptor agonists responses might have been due to the differential effect of the agonists on cAMP formation. The effect of the ρ_2-adrenoceptor agonist, SAL was examined on basal cAMP levels in the four phases of the oestrous cycle. The concentration of SAL used was that which had produced the maximum inhibitory effect in isolated uterine horn preparations. SAL caused an approximately seven-fold increase in cAMP concentrations over the basal levels, in all four phases. It thus appears that the ability of SAL to increase uterine cAMP formation via ρ-adrenoceptor activation is unaltered throughout the oestrous cycle. The variation in the degree of inhibition produced by the adrenoceptor agonists cannot, therefore, be attributed to: (a) effects of endogenous oestrogen on cAMP, and (b) the differential effects of the agonists on cAMP formation.

Since exogenous prostaglandin E_2 can affect uterine cAMP production, experiments were performed to determine the possible effects of intramurally generated prostaglandins on basal cAMP formation. FBF had no effect on basal cAMP levels in the four phases of the oestrous cycle suggesting that in physiological concentrations, prostaglandins may not affect uterine cAMP production.

Krall *et al.* (1984) suggested that exogenous prostaglandin E_2 may antagonize uterine inhibitory responses to ρ-adrenoceptor agonists by a desensitizing action on adenylate

cyclase. The possibility that prostaglandins generated intramurally may have an effect on the ability of SAL to increase cAMP levels was, therefore, investigated. SAL produced similar increases in uterine cAMP concentrations over the basal levels, in both the absence, and presence of FBF. Thus it would appear that the site of interaction between the adrenoceptor agonists and the endogenous prostaglandins produced by them, is not at the level of cAMP formation. The results of the present study support the view proposed by Harbon and her co-workers (Harbon & Clauser, 1971; Vesin & Harbon, 1974) that the excitatory effects produced by prostaglandins in the uterus do not involve changes in cAMP metabolism. Indeed, only prostaglandins of the E series have been shown to affect cAMP production in the rat uterus (Bhalla, Sandborn & Korenman, 1972; Vesin & Harbon, 1974; Krall *et al.*, 1984). In order to explain the differential effects produced by agents acting via cAMP metabolism, Vesin and Harbon (1974) and Marshall and Fain (1985), suggested that some form of compartmentalization may exist for cAMP in the rat uterus.

cAMP has been reported to induce prostaglandin biosynthesis in some tissues including the thyroid (Haye, Champion & Jacquemin, 1974), adipose tissue (Feher & Gidali, 1974) and the adrenal cortex (Laychock, Warner & Rubin, 1977). However, results from the present study do not support the idea that cAMP formed as a result of

β-adrenoceptor activation could have directly induced rat uterine prostaglandin production. The inhibitory responses produced by HIS and PAP were unaffected by cyclo-oxygenase inhibition, despite the fact that both agents have been shown to increase cAMP levels in this tissue (Mitznegg, Schubert & Fuchs, 1975; Bolton, 1979).

In summary, basal cAMP levels did not vary significantly in the four phases of the oestrous cycle. SAL increased uterine cAMP concentrations to the same extent in each phase, and the ability of SAL to increase cAMP levels was unaffected by cyclo-oxygenase inhibition. Thus the variation in adrenoceptor agonists responses throughout the oestrous cycle may not involve the differential effects of the agonists, oestrogen and intramurally generated prostaglandins on uterine cAMP formation.

3. Regulation of intracellular free calcium concentration

The cytoplasmic free calcium concentration which controls smooth muscle motility is regulated by three processes - influx , efflux and intracellular binding. Previous electrophysiological studies by Goto and Csapo (1959), and Marshall (1962) showed that oestrogen and progesterone can affect calcium movements in the rat uterus. In the present study, the possible effects of the hormones on calcium movements were examined using radioactive calcium ($^{45}Ca^{2+}$) to study influx and efflux of this ion in uteri from both intact and ovariectomized rats.

A preliminary series of experiments was performed to determine the optimum tracer concentrations to be used since the literature revealed a wide range in the $^{45}Ca^{2+}$ concentration used by previous workers. For instance, $^{45}Ca^{2+}$ concentrations of 4 μCi/ml (Feinstein, 1966) and 1 μCi/ml (Kroeger, Marshall & Bianchi, 1975) have been used for efflux, and 0.01 μCi/ml (Hodgson & Daniel, 1973) and 0.1 μCi/ml (Hodgson, 1976) for influx experiments in the rat uterus. Indeed, some workers did not indicate the concentration of $^{45}Ca^{2+}$ used in their studies (Van Breemen, Daniel & Van Breemen, 1966; Van Breemen & Daniel, 1966; Krejci & Daniel, 1970a,b; Batra & Bengtsson, 1978; Batra, 1982). Uteri from oestrus and metoestrus were used for the determination of the optimum tracer concentrations for the flux experiments since these phases are under oestrogen and progesterone influence respectively (Brenner & West, 1975; Spaziani, 1975). The phases demonstrated a similar pattern in their concentration-dependent uptake of $^{45}Ca^{2+}$. At low tracer concentrations (0.0005 - 0.05 μCi/ml), tissue uptake of $^{45}Ca^{2+}$ reached a plateau which may be attributed to the attainment of equilibrium with the bathing medium $^{45}Ca^{2+}$. A marked rise in tissue $^{45}Ca^{2+}$ was observed at higher tracer concentrations (0.05 - 5 μCi/ml) which may be due to appreciable binding in the extracellular space. A $^{45}Ca^{2+}$ concentration of 2 μCi/ml was chosen for the efflux experiments in order to ensure adequate loading of the cells and their extracellular compartment. For influx

experiments, a concentration of 0.25 μCi/ml was used so as to reduce the non-specific binding of tracer in the extracellular compartment.

$^{45}Ca^{2+}$ efflux from uteri in the four phases of the oestrous cycle and from ovariectomized animals, exhibited a biphasic pattern of decline. The initial rapid phase of decline represented $^{45}Ca^{2+}$ loss mainly from the extracellular space, while the secondary slower phase showed loss from more firmly bound intracellular sites. Similar biphasic curves have been demonstrated by other workers in uteri from rats pretreated with oestrogen and progesterone (Van Breemen et al., 1966; Krejci & Daniel, 1970 a,b) and those from late pregnancy (Kroeger et al., 1975). After 50 minutes, the efflux of $^{45}Ca^{2+}$ slowed down considerably in all tissues indicating that equilibrium was being approached. Uteri from proestrus, oestrus and dioestrus released more $^{45}Ca^{2+}$ into the bathing medium than did those from metoestrus. $^{45}Ca^{2+}$ efflux in uteri from ovariectomized animals was similar to that in metoestrus.

However, when the rate constants for $^{45}Ca^{2+}$ loss (i.e. $^{45}Ca^{2+}$ leaving tissue during collection interval divided by the $^{45}Ca^{2+}$ in the tissue before collection interval) were compared after 50 minutes, uteri from proestrus and oestrus had similar values which were higher than those from metoestrus, dioestrus and ovariectomized animals. The finding that the rate constants of $^{45}Ca^{2+}$ loss in uteri

from metoestrus and dioestrus were smaller would tend to support the hypothesis that calcium may be more firmly bound under progesterone influence (Goto & Csapo, 1959; Marshall, 1962). The rate constants of $^{45}Ca^{2+}$ loss in uteri devoid of the ovarian hormonal influence were found to be similar to those under progesterone dominance. Thus it would appear that oestrogen rather than progesterone may be responsible for the changes in $^{45}Ca^{2+}$ efflux in the rat uterus. Marshall (1962) suggested that myometrial cells under the influence of oestrogen may be freely permeable to calcium ions.

In order to determine whether the observed variation in $^{45}Ca^{2+}$ efflux might have been due to ovarian hormone-induced changes in the size of the uterus, the residual $^{45}Ca^{2+}$ in each tissue was measured at the end of efflux experiments. No correlation was found between the residual $^{45}Ca^{2+}$ in tissues and the corresponding tissue weights in uteri from both intact and ovariectomized animals. Thus the observed variation in $^{45}Ca^{2+}$ efflux is specific, and is not dependent on the morphology of the uterus. The finding that $^{45}Ca^{2+}$ was retained in the tissues after the efflux experiments would tend to suggest that there may be sites of $^{45}Ca^{2+}$ sequestration which are not readily available for exchange. These uterine $^{45}Ca^{2+}$ stores appeared to vary during the oestrous cycle and in ovariectomized animals.

One way in which β-adrenoceptor agonists could reduce cytoplasmic free calcium concentration would be via increased efflux of this ion from the cell. This possibility was investigated using SAL, at a concentration which had produced the maximum inhibitory effect in isolated uterine horn preparations. SAL had no effect on $^{45}Ca^{2+}$ efflux in uteri from oestrus and metoestrus. Feinstein (1966) also found that ADR did not alter $^{45}Ca^{2+}$ efflux in the rat uterus. Since SAL inhibitory responses in isolated uterine horn preparations were examined under conditions of induced tone, a series of experiments was performed in which KCl (50 mM) was present after 50 minutes of efflux. SAL also had no effect on $^{45}Ca^{2+}$ efflux in KCl-depolarized uteri from oestrus and metoestrus. In these experiments, KCl alone did not alter $^{45}Ca^{2+}$ efflux. Similar results were obtained by Krejci and Daniel (1970a) in uteri from oestrogen pretreated rats. Failure of KCl to affect $^{45}Ca^{2+}$ efflux was surprising since KCl depolarization has been shown to involve an influx of calcium into the cell (Batra, 1985; Granger et al., 1986). The influx of extracellular calcium might have been expected to displace $^{45}Ca^{2+}$ from the cell and hence increased $^{45}Ca^{2+}$ efflux. Van Breemen and Daniel (1966) demonstrated an increased $^{45}Ca^{2+}$ efflux in the presence of KCl, in uteri from oestrogen pretreated immature rats. However, these workers used a much higher concentration of KCl (191 mM), and did not observe any effect on $^{45}Ca^{2+}$

efflux before the 120 minutes period. In the present study and that reported by Krejci and Daniel (1970a) KCl was added before the 120 minutes period and this, could explain the inability to observe an effect on $^{45}Ca^{2+}$ efflux. The effect of KCl on $^{45}Ca^{2+}$ efflux in smooth muscles other than the uterus is also controversial (Bolton, 1979).

In summary, the ovarian hormone, oestrogen appeared to promote an increased efflux of $^{45}Ca^{2+}$ from the rat uterus during the oestrous cycle. SAL had no effect on $^{45}Ca^{2+}$ efflux in both normal and KCl-depolarized uteri, suggesting that the adrenoceptor agonists may not be reducing cytoplasmic free calcium concentration by increasing calcium efflux. Alternatively, the inability to demonstrate an effect on efflux by the adrenoceptor agonists may be due to the retention of $^{45}Ca^{2+}$ within the extracellular space which would tend to obscure the smaller cellular efflux of $^{45}Ca^{2+}$ (Spaziani, 1975).

Since the presence of the ovarian hormones did affect $^{45}Ca^{2+}$ efflux as seen from the differences in uteri from both intact and ovariectomized animals, it was of interest to investigate hormonal effects on $^{45}Ca^{2+}$ influx. In uteri from both intact and ovariectomized rats, $^{45}Ca^{2+}$ influx was biphasic with an initial rapid phase, followed by a slower phase which eventually reached a plateau. At equilibrium, influx of $^{45}Ca^{2+}$ was higher in uteri from

oestrus and proestrus, than in those from metoestrus and dioestrus. Similar observations have been made in uteri from rats pretreated with oestrogen and progesterone (Van Breemen et al., 1966; Batra & Sjogren, 1983). $^{45}Ca^{2+}$ influx in uteri from ovariectomized rats was less than in those from intact animals suggesting a role for the ovarian hormones in the regulation of calcium entry into the cell. Thus, oestrogen and progesterone may alter the ionic permeability of the membrane which could lead to an increased calcium influx into the cell. However, the precise mechanism responsible for the hormone-induced changes in calcium permeability is unclear.

β-adrenoceptor agonists could lower cytoplasmic free calcium concentration via a reduction of its influx into the cell. The effect of SAL on $^{45}Ca^{2+}$ influx was, therefore, investigated in uteri from animals in metoestrus and dioestrus. As described above, the concentration of SAL used in these experiments produced the maximum inhibitory effect in isolated uterine horn preparations. Metoestrus and dioestrus phases were chosen since the degree of $^{45}Ca^{2+}$ influx was lower than those in proestrus and oestrus, and thus SAL induced changes may be more easily observed. SAL did not affect $^{45}Ca^{2+}$ influx in these phases suggesting that it was unlikely that β-adrenoceptor mediated inhibition involved a reduced calcium influx into the cell. Hodgson

(1976) also found that isoprenaline had no effect on $^{45}Ca^{2+}$ influx in the rat uterus during late pregnancy.

In summary, $^{45}Ca^{2+}$ efflux and influx varied in uteri from proestrus, oestrus, metoestrus and dioestrus with fluxes generally higher in oestrogen than in progesterone dominated phases. $^{45}Ca^{2+}$ efflux in uteri from ovariectomized animals was similar to those in the metoestrus phase of the oestrous cycle. In contrast $^{45}Ca^{2+}$ influx in uteri from ovariectomized rats was lower than in those from intact animals. Thus, the mechanism responsible for the altered $^{45}Ca^{2+}$ fluxes may be complex, since efflux appeared to be controlled mainly by oestrogen, while influx could be regulated by both oestrogen and progesterone. SAL had no effect on either $^{45}Ca^{2+}$ efflux or influx.

The observation that ovarian hormones play a role in calcium movements in the rat uterus merits further investigation. Recently, Batra (1985) showed that KCl-stimulated $^{45}Ca^{2+}$ influx in myometrium from oestrogen pretreated rats was inhibited by calcium channel blockers, nitrendipine and D-600. However, there was no correlation between the action of D-600 on $^{45}Ca^{2+}$ influx and its inhibitory effect on $\left[^{3}H\right]$-nitrendipine binding, suggesting that calcium channels in the myometrial membranes may possess multiple sites. It is therefore possible that the ovarian hormones may act at different sites on membrane calcium channels to

regulate efflux and influx of calcium ions into the cell. The altered calcium fluxes in uteri during the oestrous cycle could result in differences in the cytoplasmic free calcium concentration in each phase. Thus, ovarian hormone-induced changes in calcium function may be partially responsible for the observed variation in uterine response to the adrenoceptor agonists. As a consequence of their effects on calcium movements, the ovarian hormones may also be involved in the maintenance of uterine tone.

However, interpretation of results from radiolabelled ion flux experiments requires caution, since factors responsible for their movements are unclear. The fluxes of ions into, or from smooth muscle cells may be determined both by their permeability (i.e., the existence of ion channels), and by the activity of carrier molecules in the membrane (Bolton, 1979). There are two factors which may complicate interpretation of data when using $^{45}Ca^{2+}$ to study calcium movements: (a) appreciable binding of the ion to extracellular sites, (b) existence of intracellularly bound calcium in the mitochondria, endoplasmic reticulum or other cell organelles. The non-specific binding of tracer calcium may be of such magnitude as to obscure subtle changes induced by agents (Van Breemen, Farinas, Casteels, Gerba, Wuytack & Deth, 1973; Bolton, 1979).

SAL had no effect on $^{45}Ca^{2+}$ fluxes in this study and this would support the hypothesis that β-adrenoceptor mediated inhibition could be the consequence of an increased intracellular calcium binding (Mueller & Van Breemen, 1979; Kamm & Stull, 1985). This increased binding would lead to a reduction in the concentration of free calcium ions in the cytoplasm. However, controversy exists regarding the main site of calcium sequestration in uteri from different species (Carsten, 1969; Batra, 1972, 1973). In the rat, both the plasma membrane (Rangachari, Pernollet & Worcel, 1976) and sarcoplasmic reticulum (Krall, Swensen & Korenman, 1976; Chaturvedi, Fox, Rama Sastry & Landon, 1979) have been shown to be major sites of calcium binding.

In conclusion, the observed variation in uterine response to the adrenoceptor agonists during the oestrous cycle is due to the effect of several factors on the response mechanisms. At the β-adrenoceptor site, agonist uptake processes, and to a lesser extent α-adrenoceptor activity (for combined α- and β-agonists) contributed to the variation in response. Blockade of the uptake processes increased both the potency and the maximum degree of inhibition produced by the agonists suggesting that effects on other cellular processes could also be involved in the observed variation in response. Ovariectomy enhanced the adrenoceptor agonists inhibitory responses suggesting a role for the

ovarian hormones mediated via an intracellular mechanism. Agonists effect on intramurally generated prostaglandins could also be involved in the observed variation since cyclo-oxygenase inhibition increased both agonist potency and the maximum degree of inhibition. cAMP metabolism did not appear to play a role because adrenoceptor agonist effects on cAMP formation was unaltered in the four phases. Ovarian hormones do affect calcium movements into, and out of uterine cells and thus may control the intracellular free calcium concentration. In this way, the ovarian hormones could alter the calcium environment in which cAMP operates.

ADDENDUM

The time courses for the inhibitory effects produced by the adrenoceptor agonists and PAP and HIS were determined in preliminary experiments using uteri from rats in oestrus and metoestrus. ACh was used to induce tone in each preparation as described previously (See Methods C.1(a)). After obtaining the standard submaximal response to ACh, the Tyrode solution was changed to one containing the inhibitory agonist. The standard ACh dose was then added after 30 seconds, and thereafter at 1 minute intervals until a constant response was achieved in the presence of the agonist. The times required for the maximal inhibitory effect of the agonists were: (a) adrenoceptor agonists, 30 seconds, (b) PAP, 1 minute and (c) HIS, 2 minutes.

Similar time courses were reported by Marshall & Kroeger (1973) for the effects of isoprenaline and PAP in the rat uterus. These workers also found that the time course for the increase in cAMP produced by both agents correlated with that for relaxation. On the basis of the preliminary experiments described above and the work of Marshall and Kroeger (1973), it was decided that the maximal effect of adrenoceptor agonists on the rat isolated uterus could be achieved within 30 seconds. However, in some smooth muscles the time course for effects on β-adrenoceptors may be longer period than 30 seconds since equilibration at the β-receptor site required several minutes (Sir J.W. Black, personal communication). If indeed a longer time course was required for the full development of the

inhibitory effect of the adrenoceptor agonists in the rat uterus, any factor altering the time scale of their action would affect the measurements which were made in the early phase of the response and thus, could influence interpretation of the observed changes in uterine response to the adrenoceptor agonists.

APPENDIX

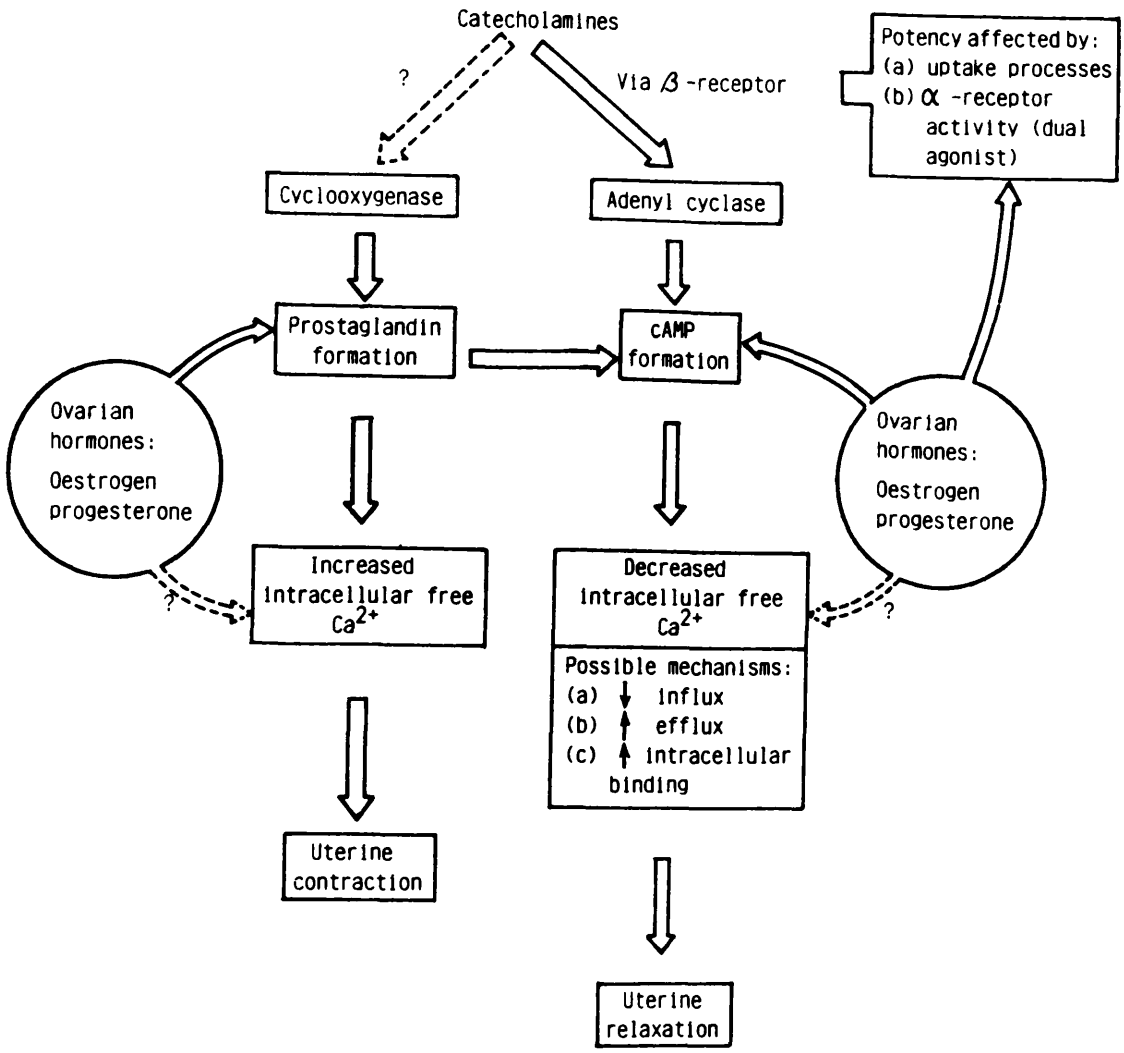


Fig. 56: Schematic representation of processes involved in catecholamine inhibitory responses in the rat uterus. Solid lines = known pathways; broken lines = hypothetical pathways. Stimulation of cyclo-oxygenase by catecholamines may be achieved either by a direct effect on the enzyme, and/or by an indirect action via stimulation of adrenoceptors. Ovarian hormones appear to alter intracellular free calcium levels by an effect on calcium movements.

REFERENCES

ABDEL-AZIZ, A. & BAKRY, N. (1973)
The reactivity of the rat uterus to sympathomimetics during the oestrous cycle and pregnancy and after parturition.
J. Reprod. & Fert. 35, 217-223.

ACRITOPOULOU-FOURCROY, S. CLABAUT, M. & SCHRUB, J. (1985)
Myometrial responses in situ to nicergoline, acebutolol, phentolamine and noradrenaline of the rat in pro estrus.
Eur. J. Pharmacol. 107, 59-64.

ADHAM, N. & SCHENK, E.A. (1969)
Autonomic innervation of the rat vagina, cervix and uterus and its cyclic variation.
Am. J. Obstet. Gynec. 104, 508-516.

AHLQUIST, R.P. (1948)
A study of the adrenotropic receptors.
Am. J. Physiol. 153, 586-600.

AHLQUIST, R.P. (1962)
The adrenoceptor receptor-detector.
Archs. int. Pharmacodyn. Thér. 139, 38-41.

ASH, A.S.F. & SCHILD, H.O. (1966)
Receptors mediating some actions of histamine.
Br. J. Pharmac. Chemother. 27, 427-439.

BARNEA, A. & SHELESNYAK, M.C. (1965)
Studies on the mechanism of nidation.
XV. The effect of cervical gangliectomy.
J. Endocr. 32, 199-204.

BATRA, S. (1972)
The role of calcium binding by subcellular particles in the contraction and relaxation of the myometrium.
Am. J. Obstet. Gynec. 112, 851-856.

BATRA, S.C. (1973)
Effect of some estrogens and progesterone on calcium uptake and calcium release by myometrial mitochondria.
Biochem. Pharmac. 22, 803-809.

BATRA, S. (1982)
Uptake and energy-dependent extrusion of calcium in the rat uterus.
Acta physiol. scand. 114, 447-452.

BATRA, S. (1985)
Characterization of [^{3}H]-nitrendipine binding to uterine smooth muscle plasma membrane and its relevance to inhibition of calcium entry.
Br. J. Pharmac. 85, 767-774.

BATRA , S. & BENGTSSON, B. (1978)
Effect of diethylstilboestrol and ovarian steroids on contractile responses and calcium movements in rat uterine smooth muscle.
J. Physiol. 276, 329-342.

BATRA, S. & SJÖGREN , C. (1983)
Effect of estrogen treatment on calcium uptake by the rat uterine smooth muscle.
Life Sci. 32, 315-319.

BAUMANN, J., v. BRUCHHAUSEN, F. & WURM , G. (1979)
A structure-activity study of the influence of phenolic compounds and bioflavonoids on rat renal prostaglandin synthetase.
Naunyn-Schmiedeberg's Arch. Pharmacol. 307, 73-78.

BEDWANI, J.R. & MILLAR, G.C. (1975)
Prostaglandin release from cat and dog spleen.
Br. J. Pharmac. 54, 499-505.

BELL, C. (1972)
Autonomic nervous control of reproduction: circulatory and other factors.
Pharmac. Rev. 24 , 657-736.

BENGTSSON, B. (1978)
Estrogen and the relaxant effect of intramural noradrenaline on calcium induced contractives in depolarized rat uterus.
Acta physiol. scand. 102, 368-373.

BENGTSSON, B. (1982)
Factors of importance for regulation of uterine contractile activity.
Acta Obstet. Gynecol. Scand. Suppl. 108, 13-16.

BERTACCINI, G., MOLINA, E., VITALI, T. & ZAPPIA, L. (1979)
Action of histamine receptor agonists and antagonists on the rat uterus.
Br. J. Pharmac. 66, 13-17.

BHALLA, R.C., SANDBORN, B.M. & KORENMAN, S.G. (1972)
Hormonal interactions in the uterus: inhibition of isoproterenol-induced accumulation of adenosine 3':5'-cyclic monophosphate by oxytocin and prostaglandins.
Proc. natn. Acad. Sci. U.S.A. 69, 3761-3764.

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972)
Definition and antagonism of histamine H_2-receptors.
Nature 236, 385-390.

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

BOYLE, F.C. & DIGGES, K.G. (1982a)
Responses to catecholamines of the rat isolated uterus throughout the natural oestrous cycle.
Naunyn-Schmiedeberg's Arch. Pharmacol. 321, 56-62.

BOYLE, F.C. & DIGGES, K.G. (1982b)
Effects of catecholamines on pregnant rat uterus.
J. Auton. Pharmac. 2, 97-102.

BRENNER, R.M. & WEST, N.B. (1975)
Hormonal regulation of the reproductive tract in female mammals.
A. Rev. Physiol. 37, 273-302.

BRODY, M.J., EDVISNNON, L. & SJÖBERG, N.O. (1975)
Preservation of estrogen-induced increase of uterine blood volume following catecholamine and mast cell histamine depletion.
Proc. Soc. exp. Biol. Med. 149, 120-123.

BROOKS, J.R., SCHAEPPI, U. & PINCUS, G. (1965)
Evidence for the presence of alpha adrenergic excitatory receptors in the rat uterus.
Life Sci. 4, 1817-1821.

BROWN, C.G. & POYSER, N.L. (1985)
Further studies on prostaglandin and thromboxane production by the rat uterus during the oestrous cycle.
J. Reprod. & Fert. 73, 391-399.

BRUCKNER-SCHMIDT, R., JACKISH, R. & HERTTING, G. (1981)
Stimulation of prostaglandin E_2-synthesis by noradrenaline in primary cell cultures from rabbit splenic pulpa is mediated by atypical α-adrenoceptors.
Naunyn-Schmiedeberg's Arch. Pharmacol. 316, 1-7.

BULBRING, E., OHASHI, H. & TOMITA, T. (1981)
Adrenergic mechanisms. In Smooth Muscle: an assessment of current knowledge ed. Bulbring E. Brading, A.F., Jones, A.W. & Tomita, T. pp.219-248.
London: Arnold.

BULBRING, E. & SZURSZEWSKI, J.H. (1974)
The stimulant action of noradrenaline (α-action) on guinea-pig myometrium compared with that of acetylcholine.
Proc. R. Soc. B. 185, 225-262.

BUTCHER, R.L., COLLINS, W.E. & FUGO, N.W. (1974)
Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat.
Endocrinology 94, 1704-1708.

BUTTERWORTH, K.R. & JARMAN, D.A. (1974)
Effect of temperature on changes induced by oestrus in α- and ρ-adrenoceptor activities of the rat uterus.
Br. J. Pharmac. 51, 462-464.

BUTTERWORTH, K.R. & RANDALL, M.J. (1970)
The effects of α- and ρ-adrenoceptor blocking agents on the responses of the rat uterus to catecholamines throughout the oestrous cycle.
Br. J. Pharmac. 40, 160P.

CAMPOS, G.A., LIGGINS, G.C. & SEAMARK, R.F. (1980)
Differential production of PGF and 6-keto-$PGF_{1\alpha}$ by the rat endometrium and myometrium in response to oxytocin, catecholamines and calcium ionophore.
Prostaglandins 20, 297-310.

CARMINATI, P., LUZZANI, F., SOFFIENTINI, A. & LERNER, L.J. (1975)
Influence of day of pregnancy on rat placental uterine and ovarian PG synthesis and metabolism.
Endocrinology 97, 1071-1079.

CARRAHER, R., HAHN, D.W., RITCHIE, D.M. & McGUIRE, J.L. (1983)
Involvement of lipoxygenase products in myometrial contractions.
Prostaglandins 26, 23-32.

CARSTEN, M.E. (1969)
Role of calcium binding by sarcoplasmic reticulum in the contraction and relaxation of uterine smooth muscle.
J. gen. Physiol. 53, 414-426.

CARSTEN, M.E. (1974)
Prostaglandins and oxytocin: their effects on uterine smooth muscle.
Prostaglandins 5, 33-40.

CARSTEN, M.E. & MILLER, J.D. (1977)
Effects of prostaglandins and oxytocin on calcium release from a uterine microsomal fraction. Hypothesis for ionophoretic action of prostaglandins.
J. biol. Chem. 252, 1576-1581.

CASTRACANE, V.D. & JORDAN, V.C. (1975)
The effect of estrogen and progesterone on uterine prostaglandin biosynthesis in the ovariectomized rat.
Biol. Reprod. 13, 587-596.

CASTRACANE, V.D. & JORDAN, V.C. (1976)
Considerations into the mechanism of estrogen-stimulated uterine prostaglandin synthesis.
Prostaglandins 12, 243-251.

CHATURVEDI, A.K., FOX, S., RAMA SASTRY, B.V. & LANDON, E.J. (1979)
Uptake and release of calcium by microsomes of nonvascular smooth muscle.
Biochem. biophys. Res. Commun. 88, 1132-1139.

CHOW, E.H.M. & MARSHALL, J.M. (1981)
Effects of catecholamines on circular and longitudinal uterine muscle of the rat.
Eur. J. Pharmacol. 76, 157-165.

CLEGG, P.C. (1966)
Antagonism by prostaglandins of the responses of various smooth muscle preparations to sympathomimetics.
Nature 209, 1137-1139.

CSAPO, I.A. & KURIYAMA, H.A. (1963)
Effect of ions and drugs on cell membrane activity and tension in the postpartum rat myometrium.
J. Physiol. 165, 575-592.

DANIEL, E.E. (1982)
Pharmacology of adrenergic, cholinergic and drugs acting on other receptors in gastro-intestinal muscle. In Handbook of Exp. Pharmac. 59 (11), ed. Bertaccini, G. pp.249-322. Berlin: Springer-Verlag.

DAVIS, H.A. & HORTON, E.W. (1972)
Output of prostaglandins from the rabbit kidney, its increase on renal nerve stimulation and its inhibition by indomethacin.
Br. J. Pharmac. 46, 658-675.

DETH, R.C. (1978)
Effect of lanthanum and reduced temperature on ^{45}Ca efflux from rabbit aorta.
Am. J. Physiol. 234, C139-C145.

DIAMOND, J. & BRODY, T.M. (1966)
Hormonal alteration of the response of the rat uterus to catecholamines.
Life Sci. 5, 2187-2193.

DIAMOND, J. & HARTLE, D.K. (1974)
Cyclic nucleotide levels during spontaneous uterine contractions.
Can. J. Physiol. Pharmacol. 52, 763-767.

DIAMOND, J. & HOLMES, T.G. (1975)
Effects of potassium chloride and smooth muscle relaxants on tension and cyclic nucleotide levels in rat myometrium.
Can. J. Physiol. Pharmacol. 53, 1099-1107.

DIAMOND, J. & MARSHALL, J.M. (1969a)
A comparison of the effects of various smooth muscle relaxants on the electrical and mechanical activity of rat uterus.
J. Pharmac. exp. Ther. 168, 21-30.

DIAMOND, J. & MARSHALL, J.M. (1969b)
Smooth muscle relaxants: dissociation between resting membrane potential and resting tension in the rat myometrium.
J. Pharmac. exp. Ther. 168, 13-20.

DIGGES, K.G. (1980)
Adrenoceptors in rat uterus.
Ph.D. Thesis. Department of Pharmacology, University of Glasgow.

DIGGES, K.G. & BOYLE, F.C. (1979)
The effects of catecholamines on the rat isolated uterus.
Br. J. Pharmac. 67, 466P.

DOBBS, J.W. & ROBISON, G.A. (1968)
Functional biochemistry of beta receptors in the uterus.
Fedn. Proc. 27, 352.

DOWNING, S.J. & PORTER, D.G. (1980)
Oestrogen-induced myometrial quiescence in the post-partum rat is not mediated by adrenaline or by α- or β-adrenoceptor activation.
J. Endocr. 85, 405-413.

DUBIN, N.H., GHODGAONKAR, R.B. & KING, T.M. (1979)
Role of prostaglandin production in spontaneous and oxytocin-induced uterine contractile activity in in vitro pregnant rat uteri.
Endocrinology 105, 47-51.

DUJOVNE, A.R., DE LABORDE, N.P., CARRIL, L.M., CHEVIAKOFF, S., PEDROZA, E. & ROSNER, J.M. (1976)
Correlation between catecholamine content of human Fallopian tube and the uterus and plasma levels of estradiol and progesterone.
Am. J. Obstet. Gynec. 124, 229-233.

EGAN, R.W., HUMES, J.L. & KUEHL, F.A. (1978)
Differential effects of prostaglandin synthetase stimulators on inhibition of cyclooxygenase.
Biochemistry 17, 2230-2234.

FALCK, B., GARDMARK, S., NYBELL, G., OWMAN, C., ROSENGREN, E. & SJÖBERG, N. (1974)
Ovarian influence on the content of norepinephrine transmitter in guinea-pig and rat uterus.
Endocrinology 94, 1475-1479.

FEHER, I. & GIDALI, J. (1974)
Prostaglandin E_2 as stimulator of haemopoietic stem cell proliferation.
Nature 247, 550-551.

FEINSTEIN, M.B. (1966)
Inhibition of contraction and calcium exchangeability in rat uterus by local anaesthetics.
J. Pharmac. exp. Ther. 152, 516-524.

FLANDROY, L., CHEUNG, W.Y. & STEINER, A.L. (1983)
Immunofluorescent localization of cGMP, cGMP-dependent protein kinase, calmodulin and cAMP in the rat uterus. Effects of oestrogen treatment.
Cell Tissue Res. 233, 639-646.

FLANDROY, L. & GALAND, P. (1978)
Changes in cGMP and cAMP content in the oestrogen-stimulated rat uterus: temporal relationship with other parameters of hormonal stimulation.
J. Cyclic Nucleotide Res. 4, 145-158.

FLANDROY, L. & GALAND, P. (1980)
In vitro stimulation by estrogen of guanosine 3'5'-monophosphate accumulation in incubated rat uterus.
Endocrinology 106, 1187-1192.

FLEMING, W.W. (1975)
Supersensitivity in smooth muscle. Introduction and historical perspective.
Fedn. Proc. 34, 1969-1970.

FORTIER, M. & KRALL, J.F. (1983)
Adenylate cyclase activity of circular and longitudinal muscle layers of rat myometrium.
Biochem. Pharmac. 32, 2118-2120.

FRANCHI, A.M., CHAUD, M., BORDA, E.S., GIMENO, M.F., LAZZARI, M.A. & GIMENO, A.L. (1981)
Spontaneous motility and prostaglandin generation in rat uterine horns isolated during the oestrous cycle.
Prostaglandins 22, 637-649.

GARFIELD, R.E., KANNAN, M.S. & DANIEL, E.E. (1980)
Gap junction formation in myometrium: control by estrogens, progesterone and prostaglandins.
Am. J. Physiol. 238, C81-C89.

GILLESPIE, J.S. (1973)
Uptake of noradrenaline by smooth muscle.
Br. med. Bull. 29(2), 136-141.

GIMENO, A.L. & GIMENO, M.F. (1984)
Prostaglandins and the regulation of motility in non-pregnant isolated uterus.
Trends Pharmacol. Sci. 5, 28-30.

GIMENO, M.F., STERIN-SPEZIALE, N., LANDA, A. BONACOSSA, A. & GIMENO, A.L. (1979)
Is the spontaneous motility of isolated rat uterus controlled by prostaglandin E?
Prostaglandins 17, 673-683.

GOLDBERG, V.J. & RAMWELL, P.W. (1975)
Role of prostaglandins in reproduction.
Physiol. Rev. 55, 325-351.

GOTO, M. & CSAPO, A. (1959)
The effect of the ovarian steroids on the membrane potential of uterine muscle.
J. gen. Physiol. 43, 455-466.

GRANGER, S.E., HOLLINGSWORTH, M., WESTON, A.H. (1986)
Effects of calcium entry blockers on tension development and calcium influx in rat uterus.
Br. J. Pharmac. 87, 147-156.

GREEN, R.D. & MILLER, J.W. (1966a)
Evidence for the active transport of epinephrine and norepinephrine by the uterus of the rat.
J. Pharmac. exp. Ther. 152, 42-50.

GREEN, R.D. & MILLER, J.W. (1966b)
Catecholamine concentrations: changes in plasma of rats during estrous cycle and pregnancy.
Science 151, 825-826.

GUIMARAES, S. & TRENDELENBURG, U. (1985)
Deviation supersensitivity and inhibition of saturable sites of loss.
Trends Pharmacol. Sci. 6, 371-374.

HAM, E.A., CIRILLO, V.J., ZANETTI, M.E. & KUEHL, F.A. (1975)
Estrogen directed synthesis of specific prostaglandins in uterus.
Proc. natn. Acad. Sci. U.S.A. 72, 1420-1424.

HARBON, S. & CLAUSER, H. (1971)
Cyclic adenosine 3',5'-monophosphate levels in rat myometrium under the influence of epinephrine, prostaglandins and oxytocin. Correlation with uterus motility.
Biochem. biophys. Res. Commun. 44, 1496-1503.

HARNEY, P.J., SNEDDON, J.M. & WILLIAMS, K.I. (1974)
The influence of ovarian hormones upon the motility and prostaglandin production of the pregnant rat uterus in vitro.
J. Endocr. 60, 343-351.

HAYE, B., CHAMPION, S. & JACQUEMIN, C. (1974)
Existence of two pools of prostaglandins during stimulation of the thyroid by TSH.
FEBS Lett. 41, 89-93.

HERVONEN, A., KANERVA, L. & LIETZEN, R. (1973)
Histochemically demonstrable catecholamines and cholinesterases in the rat uterus during estrus cycle, pregnancy and after estrogen treatment.
Acta physiol. Scand. 87, 283-288.

HODGSON, B.J. (1976)
Effect of isoproterenol on uptake of calcium-45 by pregnant human and rat myometrium.
Res. Commun. Chem. Pathol. Pharmacol. 14, 141-151.

HODGSON, B.J. & DANIEL, E.E. (1973)
Studies concerning the source of calcium for contraction of rat myometrium.
Can. J. Physiol. Pharmacol. 51, 914-932.

HOGARTH, P.J. (1978)
In Biology of Reproduction, pp.54-76
Glasgow, Blackie & Sons Ltd.

HORTON, E.W. & POYSER, N.L. (1976)
Uterine luteolytic hormone: A physiological role for prostaglandin $F_{2\alpha}$.
Physiol. Rev. 56, 595-651.

IVERSEN, L.L. (1973)
Catecholamine uptake processes.
Br. med. Bull. 29(2), 130-135.

IVERSEN, L.L. & SALT, P.J. (1970)
Inhibition of catecholamine uptake by steroids in the isolated rat heart.
Br. J. Pharmac. 40, 528-530.

JANIS, R.A. & DIAMOND, J. (1979)
Relationship between cyclic nucleotide levels and drug-induced relaxation of smooth muscle.
J. Pharmac. exp. Ther. 211, 480-484.

JENSEN, K.B. & VENNEROD, A.M. (1961)
Reversal of the inhibitory action of adrenaline and histamine on rat uterus.
Acta pharmacol. et toxicol. 18, 298-306.

JOHANSSON, S.R. & ANDERSSON, R.G. (1978)
Effects of isoprenaline on the time course of the cyclic AMP level in rat uterus.
Acta pharmacol. et toxicol. 42, 347-353.

JOHANSSON, S.R.M., ANDERSSON, R.G.G. & WIKBERG, J.E.S. (1980)
Comparison of β_1- and β_2-receptor stimulation in oestrogen or progesterone dominated rat uterus.
Acta pharmacol. et toxicol. 47, 252-258.

KAMM, K.E. & STULL, J.T. (1985)
The function of myosin and myosin light chain kinase phosphorylation in smooth muscle.
Ann. Rev. Pharmacol. Toxicol. 25, 593-620.

KAWARABAYASHI, T. & OSA, T. (1976)
Comparative investigations of alpha- and beta-effects on longitudinal and circular muscles of the pregnant rat myometrium.
Jpn. J. Physiol. 26, 403-416.

KENAKIN, T.P. (1980)
On the importance of agonist concentration-gradients within isolated tissues. Increased maximal response of the rat vasa deferentia to (-)-noradrenaline after blockade of neuronal uptake.
J. Pharm. Pharmac. 32, 833-838.

KENAKIN, T.P. (1983)
Receptor classification by selective agonists: coping with circularity and circumstantial evidence.
Trends Pharmacol. Sci. 4, 291-295.

KENAKIN, T.P. (1984)
The classification of drugs and drug receptors in isolated tissues.
Pharmac. Rev. 36, 165-222.

KISHIKAWA, T. (1981)
Alteration in the properties of the rat myometrium during gestation and post partum.
Jpn. J. Physiol. 31, 515-536.

KLINGMAN, G.I. (1965)
Catecholamine levels and dopa-decarboxylase activity in peripheral organs and adrenergic tissues in the rat after immunosympathectomy.
J. Pharmac. exp. Ther. 148, 14-21.

KOGO, H., YAMADA, K. & AIZAWA, Y. (1977)
Effect of estradiol on prostaglandin metabolism in rat uterus.
Prostaglandins 13, 785-794.

KRALL, J.F., BARRETT, J.D., JAMGOTCHIAN, N. & KORENMAN, S.G. (1984)
Interaction of prostaglandin E_2 and β-adrenergic catecholamines in the regulation of uterine smooth muscle motility and adenylate cyclase in the rat.
J. Endocr. 102, 329-336.

KRALL, J.F. & KORENMAN, S.G. (1979)
Regulation of uterine smooth muscle cell beta-adrenergic catecholamine-sensitive adenylate cyclase by Mg^{2+} and guanyl nucleotide.
Biochem. Pharmac. 28, 2771-2775.

KRALL, J.F., MORI, H., TUCK, M.L., LE SHON, S.L. & KORENMAN, S.G. (1978)
Demonstration of adrenergic catecholamine receptors in rat myometrium and their regulation by sex steroid hormones.
Life Sci. 23, 1073-1082.

KRALL, J.F., SWENSEN, J.L. & KORENMAN, S.G. (1976)
Hormonal control of uterine contraction. Characterization of cAMP-dependent membrane properties in the myometrium.
Biochim. Biophys. Acta 448, 578-588.

KREJCI, I. & DANIEL, E.E. (1970a)
Effect of contraction on movements of calcium 45 into and out of rat myometrium.
Am. J. Physiol. 219, 256-262.

KREJCI, I. & DANIEL, E.E. (1970b)
Effect of altered external calcium concentrations on fluxes of calcium 45 in rat myometrium.
Am. J. Physiol. 219, 263-269.

KROEGER, E.A. (1979)
Effect of tissue adenosine 3':5'-monophosphate on the potassium contracture of myometrium: role of endogenous norepinephrine.
J. Pharmac. exp. Ther. 210, 82-86.

KROEGER, E.A. & MARSHALL, J.M. (1974)
Beta-adrenergic effects on rat myometrium: role of cyclic AMP.
Am. J. Physiol. 226, 1298-1303.

KROEGER, E.A., MARSHALL, J.M. & BIANCHI, C.P. (1975)
Effect of isoproterenol and D-600 on calcium movements in rat myometrium.
J. Pharmac. exp. Ther. 193, 309-316.

LABELLA, F.S. (1985)
Neurotransmitter uptake and receptor-ligand internalization - are they two distinct processes?
Trends Pharmacol. Sci. 6, 319-322.

LANDS, A.M., LUDENA, F.P. & BUZZO, H.J. (1967)
Differentiation of receptors responsive to isoproterenol.
Life Sci. 6, 2241-2249.

LAYCHOCK, S.G., WARNER, W. & RUBIN, R.P. (1977)
Further studies on the mechanisms controlling prostaglandin biosynthesis in the cat adrenal cortex: The role of calcium and cyclic AMP.
Endocrinology 100, 74-81.

LEE, T.P., KUO, J.F. & GREENGARD, P. (1972)
Role of muscarinic cholinergic receptors in regulation of guanosine 3'5' cyclic monophosphate content in mammalian brain, heart muscle and intestinal smooth muscle.
Proc. natn. Acad. Sci. U.S.A. 69, 3287-3291.

LEIBER, D., VESIN, M.F. & HARBON, S. (1978)
Regulation of guanosine 3'5'-cyclic monophosphate levels and contractility in rat myometrium.
FEBS. Lett. 86, 183-187.

LEVINE, L. & MOSKOWITZ, M.A. (1979)
α- and β-adrenergic stimulation of arachidonic acid metabolism in cells in culture.
Proc. natn. Acad. Sci. U.S.A. 76, 6632-6636.

LEVY, G.P. & APPERLEY, G.H. (1978)
Recent advances in the pharmacological sub-classification of β-adrenoceptors. In
Recent Advances in the Pharmacology of Adrenoceptors
ed. Szabadi, E., Bradshaw, C.M. & Bevan, P.
pp.201-208. Amsterdam: Elsevier.

LEVY, B. & TOZZI, S. (1963)
The adrenergic receptive mechanism of the rat uterus.
J. Pharmac. exp. Ther. 142, 178-184.

LEVY, B. & WILKENFELD, B.E. (1968)
The potentiation of rat uterine inhibitory responses to noradrenaline by theophylline and nitroglycerin.
Br. J. Pharmac. 34, 604-612.

MACKENZIE, L.W., PURI, C.P. & GARFIELD, R.E. (1983)
Effect of estradiol-17β and prostaglandins on rat myometrial gap junctions.
Prostaglandins 26, 925-941.

MANN, M. (1949)
Sympathin and the rat uterus.
J. Physiol. 110, 11P.

MARSHALL, J.M. (1962)
Regulation of activity in uterine smooth muscle.
Physiol. Rev. 42, Suppl. 5, 213-227.

MARSHALL, J.M. (1968)
Relation between the ionic environment and the action of drugs on the myometrium.
Fedn. Proc. 27, 115-119.

MARSHALL, J.M. (1970)
Adrenergic innervation of the female reproductive tract: anatomy, physiology and pharmacology.
Ergebn. Physiol. 62, 6-67.

MARSHALL, J.M. (1973)
Effects of catecholamines on the smooth muscle of the female reproductive tract.
A. Rev. Pharmac. 13, 19-32.

MARSHALL, J.M. (1977)
Modulation of smooth muscle activity by catecholamines.
Fedn. Proc. 36, 2450-2455.

MARSHALL, J.M. (1981)
Effect of ovarian steroids and pregnancy on adrenergic nerves of uterus and oviduct.
Am. J. Physiol. 240, C165-C174.

MARSHALL, J.M. & FAIN, J.N. (1985)
Effects of forskolin and isoproterenol on cyclic AMP and tension in the myometrium.
Eur. J. Pharmacol. 107, 25-34.

MARSHALL, J.M. & KROEGER, E.A. (1973)
Adrenergic influences on uterine smooth muscle.
Phil. Trans. R. Soc. B. 265, 135-148.

MEISHERI, K.D., DIAMOND, J. & McNEILL, J.H. (1981)
High - K^+-induced inhibition of cAMP accumulation in smooth muscle.
Eur. J. Pharmacol. 73, 81-83.

MEISHERI, K.D. & McNEILL, J.H. (1979a)
Mg^{2+} -Ca^{2+}-Na^+ interactions in uterine smooth muscle: studies with cAMP.
Can. J. Physiol. Pharmacol. 57, 1177-1182.

MEISHERI, K.D. & McNEILL, J.H. (1979b)
Role of Ca in isoproterenol-induced increases in cAMP levels in rat uterus.
Am. J. Physiol. 237, C257-C263.

MILLER, J.W. (1967)
Adrenergic receptors in the myometrium.
Ann. N.Y. Acad. Sci. 139, 788-798.

MITZNEGG, P., HEIM, F. & MEYTHALER, B. (1970)
Influence of endogenous and exogenous cyclic 3',5'-AMP on contractile responses induced by oxytocin and calcium in isolated rat uterus.
Life Sci. 9, 121-128.

MITZNEGG, P., SCHUBERT, E. & FUCHS, W. (1975)
Relations between the effects of histamine, pheniramin and metiamide on spontaneous motility and formation of cAMP in the isolated rat uterus.
Naunyn-Schmiedeberg's Arch. Pharmacol. 287, 321-327.

MOSSMAN, R.G. & CONRAD, J.T. (1967)
In vitro blocking and oxytocic effects of water-soluble estrogens on pregnant human, mouse, and rat uteri.
Am. J. Obstet. Gynec. 99, 539-545.

MUELLER, E. & VAN BREEMEN, C. (1979)
Role of intracellular Ca^{2+} sequestration in β-adrenergic relaxation of a smooth muscle.
Nature 281, 682-683.

NAFTALIN, N.J., PHEAR, W.P.C. & GOLDBERG, A.H. (1973)
Isometric contractions of isolated rat uterine muscle: Relation to estrous cycle and pregnancy.
Proc. Soc. exp. Biol. Med. 143, 884-888.

NAHORSKI, S.R. (1981)
Identification and significance of beta-receptor subtypes. In Towards Understanding Receptors. Current Reviews in Biomedicine 1 ed. Lamble, J.W. pp.71-77. Amsterdam: Elsevier.

NORBERG, K.A. & FREDERICSSON, B. (1966)
Cellular distribution of monoamines in the uterine and tubal walls of the rat.
Acta physiol. Scand. 68, Suppl. 277, 149.

O'DONNELL, S.R., PERSSON, C.G.A. & WANSTALL, J.C. (1978)
An in vitro comparison of β-adrenoceptor stimulants on potassium-depolarized uterine preparations from guinea-pigs.
Br. J. Pharmac. 62, 227-233.

O'DONNELL, S.R. & WANSTALL, J.C. (1976)
The contribution of extraneuronal uptake to the trachea-blood vessel selectivity of β-adrenoceptor stimulants in vitro in guinea-pigs.
Br. J. Pharmac. 57, 369-373.

OHIA, S.E. & OKPAKO, D.T. (1981)
Effect of animals maturity on histamine receptor activity in the rat uterus.
Br. J. Pharmac. 74, 306P-307P.

OLSSON, O.A.T. & PERSSON, C.G.A. (1971)
Relaxing potency of terbutaline and orciprenaline on rat uterus.
J. Pharm. Pharmac. 23, 878-879.

OSA, T. & KURIYAMA, H. (1975)
Electrophysiological and mechanical investigations on the dual action of prostaglandin E_1 in the pregnant rat myometrium in vitro.
Jpn. J. Physiol. 25, 357-369.

OWMAN, C., ROSENGREN, E. & SJÖBERG, N.O. (1967)
Adrenergic innervation of the human female reproductive organs: a histochemical and chemical investigation.
Obstet. Gynecol. 30, 763-773.

OWMAN, C. & SJÖBERG, N. (1967)
Difference in rate of depletion and recovery of noradrenaline in "short" and "long" sympathetic nerves after reserpine treatment.
Life Sci. 6, 2549-2556.

PATERSON, G. (1965)
The nature of inhibition of the rat uterus by relaxin.
J. Pharm. Pharmac. 17, 262-264.

PATON, D.M. (1968)
The contractile response of the isolated rat uterus to noradrenaline and 5-hydroxytryptamine.
Eur. J. Pharmacol. 3, 310-315.

PENG, C.T. (1977)
In Sample preparation in liquid scintillation counting. Review 17, pp.32-33. Amersham: The Radiochemical Centre.

PHARRISS, B.B. & RUSSELL, R.L. (1968)
A comparison of progesterone and epinephrine inhibition on the myometrium of the rat.
Biochem. Pharmac. 17, 355-362.

POLACEK, I., BOLAN, J. & DANIEL, E.E. (1971)
Accumulation of adenosine 3'5' monophosphate and relaxation in the rat uterus in vitro.
Can. J. Physiol. Pharmacol. 49, 999-1004.

POLACEK, I. & DANIEL, E.E. (1971)
Effect of α- and β-adrenergic stimulation on the uterine motility and adenosine 3'5' monophosphate level.
Can. J. Physiol. Pharmacol. 49, 988-998.

POYSER, N.L. & SCOTT, F.M. (1980)
Prostaglandin and thromboxane production by the rat uterus and ovary in vitro during the oestrous cycle.
J. Reprod. & Fert. 60, 33-40.

QAYUM, A. & YUSUF, S.M. (1977)
Effect of azapetine phosphate on the isolated rat ureter preparation.
Life Sci. 21, 1617-1620.

QUAAS, L. & ZAHRADNIK, H.P. (1985)
The effects of α- and β-adrenergic stimulation on contractility and prostaglandin (prostaglandins E_2 and $F_{2\alpha}$ and 6-Keto-prostaglandin $F_{1\alpha}$) production of pregnant human myometrial strips.
Am. J. Obstet. Gynec. 152, 852-856.

RAINSFORD, K.D. (1985)
New approaches to antirheumatic drugs.
Trends Pharmacol. Sci. 6, 95-97.

RANGACHARI, P.K., PERNOLLET, M.G. & WORCEL, M. (1976)
Calcium uptake by myometrial membranes: effect of A23187, a calcium ionophore.
Eur. J. Pharmacol. 40, 291-294.

REINER, O. & MARSHALL, J.M. (1976)
Action of prostaglandin $PGF_{2\alpha}$ on the uterus of the pregnant rat.
Naunyn-Schmiedeberg's Arch. Pharmacol. 292, 243-250.

RICHARDSON, A. & NAHORSKI, S.R. (1978)
Direct identification of the β-adrenoceptor in rat uterus. In Recent Advances in the Pharmacology of Adrenoceptors ed. Szabadi, E., Bradshaw, C.M. & Bevan, P. pp.335-336. Amsterdam: Elsevier.

ROBISON, G.A. (1970)
Cyclic AMP as a second messenger.
J. Reprod. & Fert. Suppl. 10, 55-74.

ROSENFELD, M.G. & O'MALLEY, B.W. (1970)
Steroid hormones: Effects on adenyl cyclase activity and adenosine 3'5'-monophosphate in target tissues.
Science 168, 253-255.

RUBANYI, G. & VANHOUTTE, P.M. (1985)
Inhibitors of prostaglandin synthesis augment β-adrenergic responsiveness in canine coronary arteries.
Cir. Res. 56, 117-125.

RUDZIK, A.D. & MILLER, J.W. (1962)
The mechanism of uterine inhibitory action of relaxin-containing ovarian extracts.
J. Pharmac. exp. Ther. 138, 82-87.

SALDIVAR, J.T. & MELTON, C.E. (1966)
Effects of *in vivo* and *in vitro* sex steroids on rat myometrium.
Am. J. Physiol. 211, 835-843.

SALT, P.J. (1972)
Inhibition of noradrenaline uptake$_2$ in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines.
Eur. J. Pharmacol. 20, 329-340.

SANDBORN, B.M., BHALLA, R.C. & KORENMAN, S.G. (1973)
Use of a modified radioligand binding assay to measure the effect of estradiol on uterine adenosine 3'5'-cyclic monophosphate.
Endocrinology 92, 494-499.

SCHILD, H.O. (1966)
Calcium and the relaxant effect of isoprenaline in the depolarized rat uterus.
Pharmac. Rev. 18, 495-501.

SCHILD, H.O. (1967)
The action of isoprenaline in the depolarized rat uterus.
Br. J. Pharmac. Chemother. 31, 578-592.

SCHULTZ, G., HARDMAN, J.G., SCHULTZ, K., DAVIS, J.W. & SUTHERLAND, E.W. (1973)
A new enzymatic assay for guanosine 3'5'-cyclic monophosphate and its application to the ductus deferens of the rat.
Proc. natn. Acad. Sci. U.S.A. 70, 1721-1725.

SHARMA, S.C. & GARG, S.K. (1977)
Measurement of prostaglandins E and F in the uterine tissue and uterine fluid of ovariectomized rats treated with various doses of oestradiol-17β.
J. Reprod. & Fert. 51, 119-121.

SILVA, D.G. (1966)
The ultrastructure of the myometrium of the rat with special reference to the innervation.
Anat. Rec. 158, 21-34.

SJÖBERG, N. (1967)
The adrenergic transmitter of the female reproductive tract: distribution and functional changes.
Acta physiol. scand. suppl. 305, 5-32.

SPAZIANI, E. (1975)
Accessory reproductive organs in mammals: control of cell and tissue transport by sex hormones.
Pharmac. Rev. 27, 207-286.

SPORRONG, B., CLASE, L., OWMAN, C. & SJÖBERG, N. (1977)
Electron microscopy of adrenergic, cholinergic and "P-type" nerves in the myometrium and a special kind of synaptic contact with smooth muscle cells.
Am. J. Obstet. Gynec. 127, 811-817.

SPRATTO, G.R. & MILLER, J.W. (1968a)
The effect of various estrogens on the weight, catecholamine content and rate of contractions of rat uteri.
J. Pharmac. exp. Ther. 161, 1-6.

SPRATTO, G.R. & MILLER, J.W. (1968b)
An investigation of the mechanism by which estradiol 17-β elevates epinephrine content of the rat uterus.
J. Pharmac. exp. Ther. 161, 7-13.

STERIN-SPEZIALE, N., GIMENO, M.F., BONACOSSA, A. & GIMENO, A.L. (1980)
The effect of estradiol on isolated rat uterine motility and on prostaglandin generation.
Prostaglandins, 20, 233-243.

SZEGO, C.M. & DAVIS, J.S. (1967)
Adenosine 3', 5'-monophosphate in the uterus: Acute elevation by estrogen.
Proc. natn. Acad. Sci. U.S.A. 58, 1711-1718.

SZEGO, C.M. & DAVIS, J.S. (1969)
Inhibition of estrogen-induced elevation of cyclic 3',5'-adenosine monophosphate in rat uterus. 1. By beta-adrenergic receptor-blocking drugs.
Mol. Pharmacol. 5, 470-480.

SZURSZEWSKI, J.H. & BULBRING, E. (1973)
The stimulant action of acetylcholine and catecholamines on the uterus.
Phil. Trans. R. Soc. B. 265, 149-156.

TOTHILL, A. (1967)
Investigation of adrenaline reversal in the rat uterus by the induction of resistance to isoprenaline.
Br. J. Pharmac. Chemother. 29, 291-301.

TRINER, L., NAHAS, G.G., VULLIEMOZ, Y., OVERWEG, N.I.A., VEROSKY, M., HABIF, D.V. & NGAI, S.H. (1971)
Cyclic AMP and smooth muscle function.
Ann. N.Y. Acad. Sci. 185, 458-476.

TRINER, L., VULLIEMOZ, Y., VEROSKY, M. & NAHAS, G.G. (1972)
Acetylcholine and cyclic AMP system in smooth muscle.
Biochem. biophys. Res. Commun. 46, 1866-1873.

TSAI, T.H. & FLEMING, W.W. (1964)
The adrenotropic receptors of the cat uterus.
J. Pharmac. exp. Ther. 143, 268-272.

VAN BREEMEN, C., AARONSON, P. & LOUTZENHISER, R. (1979)
Sodium-calcium interactions in mammalian smooth muscle.
Pharmac. Rev. 30, 167-208.

VAN BREEMEN, C. & DANIEL, E.E. (1966)
The influence of high potassium depolarization and acetylcholine on calcium exchange in the rat uterus.
J. gen. Physiol. 49, 1299-1317.

VAN BREEMEN, C., DANIEL, E.E. & VAN BREEMEN, D. (1966)
Calcium distribution and exchange in the rat uterus.
J. gen. Physiol. 49, 1265-1297.

VAN BREEMEN, C., FARINAS, B.R., CASTEELS, R., GERBA, P., WUYTACK, F. & DETH, R. (1973)
Factors controlling cytoplasmic Ca^{2+} concentration.
Phil. Trans. R. Soc. B. 265, 57-71.

VANE, J.R. & WILLIAMS, K.I. (1973)
The contribution of prostaglandin production to contractions of the isolated uterus of the rat.
Br. J. Pharmac. 48, 629-639.

VAN ORDEN, D.E., GOODALE, D.B., BAKER, H.A., BARLEY, D.B. & BHATNAGAR, R.K. (1980)
Uterine catecholamines and prostaglandins during the oestrous cycle of the rat.
Endocrinology 106, 1650-1654.

VESIN, M.F., DO KHAC, L.D. & HARBON, S. (1978)
Modulation of intracellular adenosine cyclic 3', 5' monophosphate and contractility of rat uterus by prostaglandins and polyunsaturated fatty acids.
Mol. Pharmacol. 14, 24-37.

VESIN, M.F., DO KHAC, L.D. & HARBON, S. (1979)
Prostacyclin as an endogenous modulator of adenosine cyclic 3', 5' monophosphate levels in rat myometrium and endometrium.
Mol. Pharmacol. 16, 823-840.

VESIN, M. & HARBON, S. (1974)
The effects of epinephrine, prostaglandins and their antagonists on adenosine cyclic 3',5'-monophosphate concentrations and motility of the rat uterus.
Mol. Pharmacol. 10, 457-473.

WASSERMAN, M.A. & LEVY, B. (1972)
Selective beta adrenergic receptor blockade in the rat.
J. Pharmac. exp. Ther. 182, 256-263.

WEIS, M.T. & MALIK, K.U. (1985)
Beta adrenergic receptor-stimulated prostaglandin synthesis in the isolated rabbit heart: relationship to extra- and intracellular calcium.
J. Pharmac. exp. Ther. 235, 178-185.

WENNMALM, A. & BRUNDIN, T. (1978)
Prostaglandin-mediated inhibition of noradrenaline release: IV. Prostaglandin synthesis is stimulated by myocardial adrenoceptors differing from the α- and β-type.
Acta physiol. Scand. 102, 374-381.

WIKLAND, M., LINDBLOM, B. & WIQVIST, N. (1985)
Catecholamines and contractility of the human myometrium at term: a possible role for prostaglandins.
Acta physiologica Hungarica, 65, 331-334.

WILLEMS, J.L. & DE SCHAEPDRYVER, A.F. (1966)
Adrenergic receptors in the oestradiol and allyl-oestrenol dominated rabbit uterus.
Archs. int. Pharmacodyn. Ther. 161, 269-274.

WILLIAMS, K.I., DEMBINSKA-KIEC, A., ZMUDA, A. & GRYGLEWSKI, R.J. (1978)
Prostacyclin formation by myometrial and decidual fractions of the pregnant rat uterus.
Prostaglandins 15, 343-350.

WILSON, L. (1983)
Effects of estradiol and progesterone on uterine prostaglandin levels in the pregnant rat.
Prostaglandins 26, 47-54.

WIQVIST, N. (1959)
Immediate and prolonged effects of relaxin on the spontaneous activity of the mouse and rat uterus.
Acta endocrinologica 32, Suppl. 46, 3-14.

WURTMAN, R.J., AXELROD, J. & KOPIN, I.J. (1963)
Uterine epinephrine and blood flow in pregnant and postparturient rats.
Endocrinology 73, 501-503.

WURTMAN, R.J., CHU, E.W. & AXELROD, J. (1963)
Relation between the oestrous cycle and the binding of catecholamines in the rat uterus.
Nature 198, 547-548.

YOSHINAGA, K., HAWKINS, R.A. & STOCKER, J.F. (1969)
Estrogen secretion by the rat ovary in vivo during the estrous cycle and pregnancy.
Endocrinology 85, 103-112.

Abstracts of the 9th IUPHAR Congress, 389P.

THE EFFECTS OF UPTAKE MECHANISMS AND ALPHA RECEPTOR ACTIVITY ON RAT UTERINE RESPONSE TO CATECHOLAMINES.

F.C. Boyle & S.E. Ohia, Department of Pharmacology, Glasgow University, Gl2 8QQ, U.K.

The importance of catecholamine uptake processes and/or α-receptor activity to the overall response of the isolated rat uterus to noradrenaline (NA), adrenaline (ADR) and salbutamol (SAL) have been studied throughout the oestrous cycle. $Uptake_1$ and $Uptake_2$ were blocked with desipramine (DMI) (10^{-6}M) and normetanephrine (NMN) (10^{-6}M) respectively, whilst α-receptor activity was blocked with azapetine (AZA) (10^{-6}M). AZA potentiated the maximum degree of inhibition produced by NA (proestrus and oestrus only), SAL (oestrus only) and ADR in all stages of the cycle. DMI and NMN shifted the NA, SAL and ADR dose response curves to the left throughout the cycle. AZA, in combination with DMI and NMN produced further potentiations of the NA curves in proestrus and oestrus only, but had no effect on NA, SAL and ADR responses in the other stages. Both NA and ADR (10^{-6}M-$3x10^{-5}$M) produced small motor responses in proestrus and oestrus which were abolished by AZA (10^{-6}M). Blockade of either uptake and/or α-receptor activity produced significant changes in PD_2 values for the 3 amines in the 4 stages of the cycle. The above experiments show that both uptake mechanisms and α-receptor activity should be controlled when assessing uterine response to catecholamines. However, the importance of each factor varies with the amine.

British Journal of Pharmacology, 84, 42P.

CHANGES IN CATECHOLAMINE INHIBITORY EFFECTS IN THE RAT ISOLATED UTERUS INDUCED BY OVARIECTOMY AND CYCLO-OXYGENASE INHIBITION

F.C. Boyle & S.E. Ohia*, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ

Catecholamine induced relaxation of rat uterus is mediated mainly through β-adrenoceptors (Boyle & Digges, 1982), but β-adrenoceptor agonists produce different degrees of inhibition throughout the oestrous cycle in both controls and in uteri in which α-adrenoceptors and catecholamine-removal processes are blocked (Boyle & Ohia, 1984). The mechanisms underlying these differences are unclear. Two possible explanations lie in the presence of the ovarian hormones and the generation of prostaglandins within the tissue (Gimeno & Gimeno, 1984). The present study was designed to examine the roles of the hormones and prostaglandins, by investigating adrenoceptor agonist activity in ovariectomized rats and in rats after treatment with the cyclo-oxygenase inhibitor flubiprofen (FBF) to inhibit prostaglandin synthesis.

Virgin female untreated (180-250g) and ovariectomized (200-260g) Wistar rats were used. The four phases of the oestrous cycle were identified from vaginal smears. 2-3cm lengths of each uterine horn were set up in paired 10ml organ baths containing Tyrodes solution at 37°C and gassed with 95% O_2/5% CO_2. Since the rat uterus has no intrinsic tone, inhibitory responses to adrenoceptor agonists and to the smooth muscle relaxant papaverine (PAP) were measured as the percentage inhibition of sub-maximal (60-70%) acetylcholine (ACh)-induced contractions, measured isometrically. The agonists were added to the organ baths 30s before addition of ACh. FBF (10^{-6}M), added to the Tyrodes solution, was in contact with the tissue for at least 30 min before addition of ACh. FBF had no effect on the response to ACh in either control or ovariectomized rats.

Noradrenaline (NA), adrenaline (ADR) and salbutamol (SAL), each produced a dose dependent inhibition of ACh in both control (NA 10^{-9}-10^{-5}M; ADR 10^{-10}-10^{-7}M; SAL 5×10^{-9}-5×10^{-6}M) and in ovariectomized rats (NA 3×10^{-10}-10^{-6}M; ADR 10^{-11}-3×10^{-8}M; SAL 5×10^{-11}-5×10^{-8}M) but in the latter, the effects of the amines were enhanced significantly (e.g. in metoestrus phase, % maximal inhibition of ACh for NA is 44.6±5.4, n=6 whilst in ovariectomized rats, it is 100±0.0, n=6. $P < 0.001$) and each amine consistently abolished the ACh-induced contraction.

FBF treatment also enhanced significantly the effects of the three amines in untreated rats and each amine again abolished ACh contractions. FBF shifted the dose-response curve of each amine to the left in both untreated and ovariectomized rats.

In contrast to the adrenoceptor agonists PAP (3×10^{-7}-10^{-4}M) inhibited ACh induced contractions completely in all stages of oestrous and was unaffected by FBF.

These results show that removal of ovarian hormones and inhibition of prostaglandin synthesis enhance β-adrenoceptor agonist inhibitory effects and suggest that the presence of ovarian hormones and prostaglandin generation underlie the differences observed in the inhibitory effects of NA, ADR and SAL. The site and mechanism of the interaction is not known, but the effectiveness of PAP in inhibiting ACh contractions is consistent with an intracellular mechanism which is also linked with adrenoceptor activation.

S.E.O. receives ORS support.

Boyle, F.C. & Digges, K.G. (1982) N.S.Arch.Pharmacol. 321, 56-62
Boyle, F.C. & Ohia, S.E. (1984) Abst. 9th IUPHAR Congress, 389P
Gimeno, A.L. & Gimeno, M.F. (1984) Trends Pharmac. Sci. 5, 28-30

British Journal of Pharmacology, 86, 648P.

SPECIFICITY OF ENHANCEMENT OF SALBUTAMOL RESPONSES BY CYCLO-OXYGENASE INHIBITION IN THE RAT ISOLATED UTERUS

F.C. Boyle & S.E. Ohia*, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ

Inhibition of acetylcholine (ACh)-induced tone in the rat uterus by β-adrenoceptor agonists is enhanced after cyclo-oxygenase blockade (Boyle & Ohia, 1985). The inhibition produced by relaxants in smooth muscles which possess no intrinsic tone may be influenced differentially by the mechanisms whereby tension is increased (Daniel, 1982). The cyclo-oxygenase substrate arachidonic acid is also a substrate for lipoxygenase, and products from this pathway may be involved in uterine motility (Carraher, Hahn, Ritchie & McGuire, 1983). Thus this study investigated whether the enhancement of β-adrenoceptor responses seen after cyclo-oxygenase inhibition was dependent on the motor agent used and whether lipoxygenase inhibition affected the relaxations in the four phases of the oestrous cycle.

Virgin female Wistar rats weighing (180-250g) were used and the preparation was set up as described previously (Boyle & Ohia, 1985). Tone was induced with either ACh or potassium chloride (KCl, $5x10^{-2}$M). Since KCl produced a biphasic contraction with an initial transient phase and a secondary sustained phase lasting for more than one hour, cummulative dose response curves were constructed to the relaxants 15 min into the sustained phase until maximum inhibition of the KCl-induced tone was obtained. The cyclo-oxygenase inhibitor, flurbiprofen (FBF, 10^{-6}M), and the inhibitor of both cyclo-oxygenase and lipoxygenase, BW 755C (10^{-5}M), when used, were present in the Tyrodes solution and had no effect on KCl or ACh responses.

As with ACh-induced contractions, salbutamol (SAL, $1.5x10^{-11}$-$5x10^{-6}$M) produced dose dependent relaxations of KCl-induced tone in proestrus, oestrus, metoestrus and dioestrus. FBF increased SAL inhibitory responses and produced a significant shift in the dose response curves to the left. For instance in proestrus, SAL pD_2 values were increased significantly from 9.77±0.30, n=6 before, to 10.66±0.09, n=6 ($P<0.01$) after FBF.

BW 755C enhanced SAL relaxation of KCl-induced tone and shifted dose response curves to the left to the same degree as occurred with FBF.

When inhibition of ACh-induced tone was produced by histamine acting via H_2-receptors (HIS, 10^{-7}-10^{-4}M, in the presence of H_1-receptor antagonist mepyramine, $5x10^{-8}$M), FBF produced no enhancement in any phase of the oestrous cycle.

The FBF enhancement of SAL inhibition in uteri stimulated with both KCl and ACh suggests that its effect is not dependent on the motor agent used. Since BW 755C had no greater effect than FBF, it is likely that products of the lipoxygenase pathway were not involved in β-adrenoceptor mediated relaxation. Inhibition induced by stimulating HIS H_2-receptors was not affected by FBF and therefore enhancement of SAL responses is specific to β-adrenoceptor activation. The hormonal state of the animal did not appear to be of major importance because the same effects were observed in all four phases of the oestrous cycle. Rubanyi & Vanhoutte (1985) reported similar potentiation of β-adrenoceptive mechanisms by cyclo-oxygenase inhibition in vascular smooth muscle.

S.E.O. is an ORS award scholar.

Boyle, F.C. & Ohia, S.E. (1985) Br.J.Pharmac. 84, 42P
Carraher, R. et al (1983) Prostaglandins, 26, 23-31
Daniel, E.E. (1982) Handbook of Exp. Pharmac. 59, 249-322
Rubanyi, G. & Vanhoutte, P.M. (1985) Cir.Res. 56, 117-125