MECHANISMS OF INHIBITION AND CONTRACTION IN UTERINE SMOOTH MUSCLE

A thesis presented for the degree of Doctor of Philosophy in the University of Glasgow

by

K.H. AL-ZADJALI, B.Sc., M.B.B.S. (Karachi)

Department of Pharmacology,
University of Glasgow,
Glasgow G12 8QQ.

December 1988

TABLE OF CONTENTS

Page No.

Page No.

LIST OF TABLES

LIST OF FIGURES

PLATE

ACKNOWLEDGEMENTS

I am very thankful to a number of well meaning people who made the completion of this project possible. First of all, thanks to Professor J.S. Gillespie, who gave me the opportunity to work in this prestigious Pharmacology Department. I am also grateful to my direct supervisor, Dr Frances Boyle for initiating this project.

Other members of the academic staff were also helpful: Dr D. Pollock, for his advice, and all members of the technical staff were exceedingly helpful to me in both personal and material terms. I thank Mr J. Thomson, Ian and Robert for the technical assistance. I also acknowledge my thanks to Mr Keith Morrison and Stephan Boyle.

I am equally grateful to my entire family for their support during my stay in Glasgow.

I will seize this opportunity to thank Mrs Lorna Peedle of the Anatomy Department for her excellent secretarial assistance.

PUBLICATIONS

(a) BOYLE, F.C. & AL-ZADJALI, K.H. (1988)
Involvement of prostaglandins in uterine inhibitory responses to adrenoceptor agonists in pregnant and post-partum rats.
Br. J. Pharmac., 691P.

SUMMARY

SUMMARY

(1) The possible mechanisms involved in the variation in rat uterine response to adrenoceptor agonists in preparations under different hormonal conditions have been investigated during the natural oestrous cycle and in pregnant and post-partum animals using pharmacological and biochemical techniques.

(2) Uterine tone was induced with acetylcholine (Ach) in isolated preparations from non-pregnant and pregnant animals. Tension developed to Ach was more than two-fold higher in uteri from 20-day and 1-day post-partum animals than in those from the four phases of the oestrous cycle. The observed variation in uterine response to Ach may reflect changes that occur in the thickness of the myometrium under the different hormonal states.

(3) Noradrenaline (NA), adrenaline (ADR), isoprenaline (ISO) and isoxsuprine (ISOX) produced inhibitory responses in uteri from non-pregnant and pregnant animals. During the oestrous cycle, the effects elicited by NA, ADR and SAL varied with the phase of the cycle. This variation could be partially due to the activation of α -excitatory receptors (NA and ADR) and/or to alterations in agonists removal mechanisms during the oestrous cycle.

(4) Pre-treatment of animals with oestradiol-17β increased the uterine response to SAL when compared to effects

observed in natural oestrus indicating a role for the ovarian hormones in regulating adrenoceptor function in this preparation.

(5) Inhibition of cyclo-oxygenase activity with flurbiprofen (FBF) enhanced uterine inhibitory response to the adrenoceptor agonists in preparations from non-pregnant, pregnant and post partum animals suggesting that intramurally generated prostaglandins were involved in their response. The effect elicited by FBF was, however, not reversed by excess exogenous arachidonic acid. Inhibition of phospholipase A_2 activity with quinacrine also enhanced uterine inhibitory response to the adrenoceptor agonists.

(6) Removal of the endometrium slightly reduced uterine response to ADR and SAL with the effects being greater in 1-day post-partum than in 20-day pregnant animals. In endometrium-free preparations, FBF had no significant effect on adrenoceptor agonists responses which would tend to suggest that the endometrium may play a role as a major source of PGs in the interaction between these agonists and the cyclo-oxygenase pathway leading to prostaglandin production.

(7) Biochemical measurements of uterine adenosine 3',5' cyclic monophosphate (cAMP) were made in uteri from non-pregnant and pregnant animals. During the oestrous cycle, basal cAMP levels were similar in uteri from the four phases and cyclo-oxygenase inhibition with FBF had

no effect on these levels nor on the ability of SAL to increase tissue cAMP content. Thus it would appear that cAMP may not be involved in the variation in uterine response to adrenoceptor agonists during the oestrous cycle.

(8) Removal of the endometrium in uteri from 20-day pregnant animals did not alter basal tissue cAMP content neither did it affect the ability of SAL to increase cAMP levels. An effect on tissue cAMP metabolism may, therefore, not account for the observed changes in uterine response to adrenoceptor agonists in endometrium-free preparations.

(9) In conclusion, presence of a heterogeneous population of adrenoceptors, an avid agonist removal mechanism, ovarian hormones and intramurally generated prostaglandins appear to contribute to the observed variations in uterine responses to adrenoceptor agonists during the oestrous cycle. cAMP is not the source of the variation. Adrenoceptor agonists effect on intramural prostaglandin production could play an important role in their overall response in the rat uterus since such an interaction was also present in preparations from pregnant and post-partum animals.

ABBREVIATIONS

Ach	Acetylcholine
A.A.	Arachidonic acid
ADR	Adrenaline
AZA	Azapetine
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
ISO	Isoprenaline
ISOX	Isoxsuprine
NA	Noradrenaline
pD_2	Negative logarithm to the base 10 of the EC_{50}
QUIN	Quinacrine
SAL	Salbutamol
TCA	Trichloroacetic acid

CHAPTER 1

INTRODUCTION

A. HORMONAL CONTROL OF UTERINE FUNCTION DURING THE OESTROUS CYCLE, PREGNANCY AND IN PARTURITION

The ovarian hormones, oestrogen and progesterone exert profound changes in uterine anatomy and physiology during the oestrous cycle, pregnancy and parturition (Brody & Wiqvist, 1961; Silva, 1966; Naftalin, Phear & Goldberg, 1973). The uterotrophic effects of the ovarian hormones are manifested in terms of hypertrophy and hyperplasia of cells. During the oestrous cycle, the cyclical production of the ovarian hormones (Yoshinaga, Hawkins & Stocker, 1969; Butcher, Collins & Fugo, 1974; Brenner & West, 1975) is accompanied by either an increase (as in oestrus) or a decrease (as in dioestrus) in the size of the myometrium (Naftalin et al., 1973; Digges, 1980).

There is evidence that the changes in the ovarian hormones that precede labour initiate gap junctions formation in the myometrium (Garfield, Kannan & Daniel, 1980; Mackenzie, Puri & Garfield, 1983). The presence of gap junctions between uterine muscle cells has been shown to be limited exclusively to the period just before, during and immediately following normal and premature parturition (Garfield et al., 1980; Mackenzie et al., 1983). It is possible that the absence of gap junctions between smooth muscle cells throughout gestation may maintain pregnancy by limiting electrical or metabolic communications between cells, thereby preventing coordinated contractions of the uterus (Garfield, 1985). Prostaglandins may also play a role in the formation of gap junctions since inhibitors of

prostaglandin synthesis such as indomethacin have been shown to prevent their development (Garfield, Kannan & Daniel, 1980).

The adrenergic and cholinergic autonomic neurones which innervate the rat uterus appear to be under hormonal control. During the oestrous cycle, the density of autonomic innervation is greater in oestrus than in dioestrus, whilst an intermediate number of these nerves occur in proestrus and metoestrus (Adham & Schenk, 1969).

The transmitter content of myometrial adrenergic neurones can also be influenced by the ovarian hormones resulting in its cyclical variation during the oestrous cycle and pregnancy (Sjoberg, 1967; Hervonen, Kanvera & Lietzen, 1973). Marshall (1981) found that the ovarian hormones also affected neurotransmitter turnover of uterine adrenergic neurones and their overall neuronal activity. The capacity of the rat uterus to accumulate catecholamines from the circulation can be influenced by the ovarian hormones. Wurtman, Chu and Axelrod (1963) found that after systemic injections of [^{3}H]-labelled catecholamines, uteri isolated from animals during the oestrus phase of the oestrous cycle bound more [^{3}H]-adrenaline than those in dioestrus, whilst the binding of [^{3}H]-noradrenaline was reduced. Green and Miller (1966a,b) confirmed and extended these findings leading to their proposal that the cyclic variation in uterine adrenaline content could result from differences in the ratio of adrenaline to noradrenaline in

the plasma, and in the altered ability of the uterus, to bind these amines. By affecting the capacity of the uterus to accumulate catecholamines, the ovarian hormones may indirectly modulate stores of these amines. Uterine adrenaline, but not noradrenaline stores appear to be sensitive to regulation by oestrogen and progesterone (Rudzik & Miller, 1962; Falck, Gardmark, Nybell, Owman, Rosengren & Sjoberg, 1974).

In summary, the ovarian hormones appear to produce marked effects on uterine anatomy and physiology during the oestrous cycle, pregnancy and parturition.

B. UTERINE RESPONSE TO CATECHOLAMINES DURING THE OESTROUS CYCLE, PREGNANCY AND IN PARTURITION

Responses of the uterus to adrenoceptor agonists vary with species and, in some species, with different states of hormonal influence (Marshall, 1970). In his first classification of the adrenoceptors in the body, Ahlquist found excitation to be generally associated with activation of α -adrenoceptors, and inhibition with activation of β -adrenoceptors (Ahlquist, 1948, 1962). Ahlquist (1962) classified rat uterine adrenoceptors as belonging to the β-subtype. The classification of rat uterine adrenoceptors is, however, no longer tenable in view of subsequent demonstration by other workers of the presence of α -excitatory receptors in this organ (Diamond & Brody, 1966; Krall, Mori, Tuck, Le Shon & Korenman, 1978; Boyle & Digges, 1982; Boyle & Ohia, 1984; Acritopoulou-Fourcroy,

Clabaut & Schrub, 1985). Evidence, however, exists which suggests that the α -adrenoceptor population in the rat uterus may subserve an inhibitory function (Jensen & Vennerod, 1961; Rudzik & Miller, 1962). However the adrenoceptor antagonists employed in these studies have since been reported to produce non-specific excitatory effects on uterine smooth muscle (Tothill, 1967; Paton, 1968). In the circular muscle of the pregnant rat myometrium, α -receptors predominate and noradrenaline increases spontaneous contractions (Chow & Marshall, 1981). In the preparturient rat myometrium, the relative population of α_1 and α_2-subtypes was assessed by studying competition between [^{3}H]-dihydroergocryptine and selective agonists and antagonists, or by using [^{3}H]-prazosin and [^{3}H]-rauwolscine (Maltier & Legrand, 1985). The results indicated that the membrane fraction obtained from a mixture of longitudinal and circular muscles contained 45% α_1-receptors and 55% α_2-receptors. Thus it would appear that uterine preparations from rats, as in those from other species, contains α -adrenoceptors which subserve an excitatory function.

The occurrence of β -inhibitory receptors in uteri from non-pregnant and pregnant rats has been generally accepted (Diamond & Brody, 1966; Miller, 1967; Krall et al, 1978; Chow & Marshall, 1981; Boyle & Digges, 1982a,b; Acritopoulou-Fourcroy et al, 1985). Rat uterine β -adrenoceptors were further subclassified by Lands, Ludena

and Bazzo (1967) into the β_1 and β_2-category. However, β_1 and β_2-adrenoceptors have been shown to co-exist in the rat uterus depending on the hormonal condition (Richardson & Nahorski, 1978; Nahorski, 1981; Morrison, Nimmo & Whitaker, 1987; Ohia & Boyle, 1987).

(i) EFFECT OF THE OVARIAN HORMONES ON UTERINE RESPONSE TO CATECHOLAMINES

The ovarian hormones, oestrogen and progesterone can influence uterine response to catecholamines via an effect on adrenoceptor activity.

The overall effect elicited by the hormones depends on the species studied. For example, in the longitudinal muscle of the virgin cat uterus, β-inhibition is dominant, whereas in the same muscle of pregnant cat α-excitation is dominant (Dale, 1906; Bulbring, Casteels and Kuriyama, 1968). In contrast, pregnant rat uterus has few α-adrenoceptors and β-inhibition is dominant (Marshall, 1967). During the middle stage of gestation, the longitudinal muscle cells of the rat uterus possess mainly β-adrenoceptors, while in the circular muscle cells, α-adrenoceptors predominate (Kawarabayashi & Osa, 1976).

However, at the last stage of gestation, activation of α-adrenoceptors in the circular muscle cells is apparent (Osa & Watanabe, 1978). In the longitudinal muscle, activation of β-adrenoceptors occurs during mid-pregnancy and α-adrenoceptors at term (Marshall, 1970).

Thus, a phenomenon akin to the "adrenaline reversal" in the cat occurs in the myometrium of various species (Dale, 1906; Bulbring et al, 1968; Abe, 1970).

In non-pregnant rats Diamond and Brody (1966) found that an oestrogen treated muscle was excited by catecholamines (an α -adrenoceptor effect), while a progesterone treated one was inhibited (a β -adrenoceptor effect). Similarly, Marshall (1967) showed that noradrenaline relaxed uteri from ovariectomized rats treated with a combination of oestrogen and progesterone, but contracted those treated with oestrogen alone. Taken together, these findings suggest that oestrogen may increase the sensitivity of the α -adrenoceptors to catecholamines (Marshall, 1967, 1970). During the oestrous cycle, α -adrenoceptors have been shown to be associated with the proestrus and oestrus phases (Butterworth & Randall, 1970; Abdel-Aziz & Bakry, 1973; Butterworth & Jarman, 1974; Boyle & Digges, 1982a; Boyle & Ohia, 1984; Acritopoulou-Foucroy et al, 1985), when the concentration of circulating oestrogen is highest (Yoshinaga et al, 1969; Butcher et al, 1974; Brenner & West, 1975). Using the radioligand binding method, Krall et al (1978) found that α -adrenoceptor density was increased in proestrus and oestrus, while β -adrenoceptor density was raised only in proestrus. These findings led Krall et al (1978) to suggest that changing receptor number might be one way through which the hormones regulated targets organ

function.

Recently, Morrison et al (1987) showed that there was no change in β -adrenoceptor density (as measured by [^{125}I]-iodocyanopinolol binding) in the rat uterus during the oestrous cycle. Thus there is good evidence that the ovarian hormones increase the α adrenoceptor population, contributing to "the adrenaline reversal". The situation is less clear with the β -adrenoceptors which do not appear to be altered by the ovarian hormones.

(ii) EFFECT OF AGONISTS REMOVAL MECHANISMS ON UTERINE RESPONSE TO CATECHOLAMINES

The importance of amine removal mechanisms in terminating the physiological effects of catecholamines is well documented (Gillespie, 1973; Iversen, 1973). Uptake into neuronal (Uptake $_1$) and extraneuronal (Uptake $_2$) sites also have pharmacological relevance in that they can be modulated by drugs (O'Donnell & Wanstall, 1976; Kenakin, 1983, 1984). Since steroids such as 17β -oestradiol and progesterone have been shown to be potent inhibitors of the uptake$_2$ process (Iversen & Salt, 1970; Salt, 1972), attempts have been made to correlate ovarian hormonal level during the oestrous cycle with uterine sensitivity to catecholamines during the oestrous cycle (Digges & Boyle, 1979; Boyle & Digges, 1982a,b). Boyle & Digges (1982a) found that the variations in the ovarian hormones in proestrus, oestrus, metoestrus and dioestrus were accompanied by differences in uterine uptake of the

catecholamines. On the basis of these observations, Boyle & Digges (1982a) concluded that the extraneuronal uptake process was a major metabolic route for the disposal of catecholamines in the rat uterus and that this mechanism was in turn controlled by oestrogen and progesterone. Blockade of amine removal mechanism into neuronal and extraneuronal sites has also been shown to increase the maximum degree of inhibition produced by adrenoceptor agonists in the rat uterus in all phases of the oestrous cycle (Ohia, 1986). Although amine removal mechanisms appear to play an important role in the disposal of catecholamines in the rat uterus (Boyle & Digges, 1982a; Boyle & Ohia, 1984; Ohia, 1986), the possibility that this process could be regulated by the ovarian hormones is yet to be fully determined.

(C) INTRACELLULAR MECHANISMS INVOLVED IN UTERINE RESPONSE TO CATECHOLAMINES

Uterine response to catecholamines is associated with changes in a number of intracellular processes such as ionic conductance and ion channel activity and cyclic nucleotide levels.

(i) ROLE OF ION CONDUCTANCE AND CHANNEL ACTIVITY

There is evidence that alterations in membrane permeability to both cations and anions by catecholamines could be one way through which they produce their effect in

the rat uterus (Goto & Csapo, 1959; Marshall, 1962, 1968). Stimulation of α -adrenoceptors in the circular muscle of the pregnant rat myometrium has been shown to cause depolarization accompanied by a decrease in membrane resistance which would suggest an increase in calcium and/or sodium conductance (Osa & Watanabe, 1978). In the guinea pig uterus, Bulbring and her co-workers (Szurszewski & Bulbring, 1973; Bulbring & Szurszewski, 1974; Bulbring, Ohashi & Tomita, 1981) demonstrated that depolarization caused by catecholamines was probably mainly due to an increase in chloride conductance because it was abolished by replacing chloride ions with glutamate, but not by replacing sodium with Tris. Thus, there may be species difference in the type of ionic changes induced by α -adrenoceptor activation in uterine smooth muscle.

Relaxation of rat myometrium due to stimulation of the β -adrenoceptor is characterized by cessation of spontaneous action potential discharge, hyperpolarization of the membrane and relaxation of the muscle (Diamond & Marshall, 1969a,b; Kroeger & Marshall, 1973; Marshall & Kroeger, 1973). The hyperpolarization and reduction of the membrane resistance are not much affected by removal of external sodium or chloride ions (Marshall, 1968). However, the hyperpolarization appeared to be dependent on the potassium ion concentration in the extracellular fluid (Marshall, 1968, 1970). Marshall and Kroeger (1973) showed that catecholamine-induced hyperpolarization was dependent on the calcium concentration in the external medium and was

reduced by ouabain and low temperature (10°C). These findings led Marshall and her co-workers (Kroeger, Marshall & Bianchi, 1975; Marshall, 1977) to suggest that an outwardly directed calcium current could underlie the relaxation produced by catecholamines in the rat uterus. However there is evidence that hyperpolarization may not be a prerequisite for relaxation because Meisheri, McNeill and Marshall (1979) found that isoprenaline produced an inhibitory response in the pregnant rat myometrium without a change in membrane potential. On the whole, both uterine excitatory or inhibitory response to catecholamines appear to involve alterations in membrane permeability to ions which in most cases leads to a change in membrane potential.

(ii) ROLE OF CYCLIC NUCLEOTIDES

Adrenoceptors of the β_1- and β_2-subtype are positively coupled to adenylate cyclase, while activation of α_2-adrenoceptors inhibits this enzyme (review by Stiles, Caron & Lefkowitz, 1984). The relaxation caused by β-adrenoceptor agonists is thought to involve an increase in adenosine 3',5' cyclic monophosphate (cAMP) formation following stimulation of adenylate cyclase, because:

(a) The relaxation is correlated with a concomitant increase in tissue cAMP content (Dobbs & Robison, 1968; Triner, Nahas, Vulliemoz, Overweg, Verosky, Habif & Ngai, 1971),

(b) cAMP (applied as the dibutyryl compound) mimicks the affect of β -adrenoceptor agonists (Mitznegg, Heim & Meythaler, 1970; Triner et al, 1971; Vesin & Harbon, 1974), and

(c) inhibitors of phosphodiesterase also have a relaxing effect (Triner et al, 1971).

In the rat uterus, cAMP appears to have satisfied the criteria for its role as a "second messenger" (Krall & Korenman, 1979; Kroeger, 1979; Kishikawa, 1981; Fortier & Krall, 1983). The mediatory role for cAMP has, however, been disputed by some workers (Polacek & Daniel, 1971; Harbon & Clauser, 1971; Diamond & Holmes, 1975; Meisheri & McNeill, 1979a,b; Marshall & Fain, 1985). Harbon and her co-workers found that adrenaline caused relaxation and increased rat uterine cAMP, both effects being antagonized by propranolol, but, in the same preparation, prostaglandins caused contraction and also increased cAMP which was not blocked by propranolol (Harbon & Clauser, 1971; Vesin & Harbon, 1974). It is possible that while cAMP can mediate relaxation in the rat uterus, under certain conditions, the nucleotide may not be an obligatory mediator.

An intermediary role for cAMP in the action of oestrogens in the rat uterus has been suggested by some workers (Marshall, 1973; Downing & Panter, 1980; Kishikawa, 1981).

The molecular mechanism involved in cAMP-mediated effects has been determined biochemically. Fortier, Chase,

Korenman and Krall (1983) suggested that stimulation of adenylate cyclase may require the presence of guanyl-nucleotides, since isoprenaline-induced effects on the enzyme in cultured myometrial cells was dependent on the presence of GTP. Fortier et al (1983) also found that adenylate cyclase was activated directly by calcium without GTP indicating the existence of another, receptor-independent mechanism for cAMP production which may play a role in autoregulation of intracellular calcium in the rat myometrium. cAMP may also affect uterine calcium function by increasing calcium binding in the sarcoplasmic reticulum (Krall, Sorensen & Korenman, 1976). Phosphorylation of a specific protein in the microsome is likely to be responsible for cAMP-dependent accumulation in microsomes prepared from rat myometrium (Nishikori & Maeno, 1979). The effects produced by cAMP appear to be mediated by cAMP-dependent protein kinases (Dokhac, D'Albis, Janmot & Harbon, 1986; Bulbring & Tomita, 1987). In summary the molecular mechanisms involved in cAMP mediated effects following catecholamine induced activation is currently under investigation.

Although guanosine 3',5' cyclic monophosphate (cGMP) and its protein kinase have been demonstrated in the plasma membrane and cytoplasm of rat myometrial cells (Flandroy, Cheung & Steiner, 1983), there are few reports so far as to the possibility of an interaction between catecholamines and cGMP. However, the ovarian hormones have been shown to

affect cGMP levels in the rat uterus indicating a possible role for this nucleotide in the regulation of uterine motility (Flandroy & Galand, 1978, 1980; Kishikawa, 1981). Kishikawa (1981) showed that at the early stage of pregnancy, cGMP levels were high in both longitudinal and circular muscles of the rat myometrium. cGMP levels, however, declined with the progress of pregnancy. Phenylephrine had no effect on cGMP levels in the pregnant rat myometrium. Thus, it would appear that catecholamines do not interact with the processes leading to cGMP production in the rat uterus. The physiological significance of alterations in cGMP levels during pregnancy is unclear and merits further studies.

(D) INVOLVEMENT OF PROSTAGLANDINS IN THE EXCITATORY AND INHIBITORY EFFECTS OF CATECHOLAMINES

Prostaglandins have been shown to play an important role in the physiological control of uterine function and in parturition (as reviewed by Harbon & Poyser, 1976; Novy & Liggins, 1980). In pregnant rats, Vane and Williams (1973) showed that uterine prostaglandin release from isolated preparations increased until it reached a maximum on the expected day of delivery. Post-partum, a decrease in uterine prostaglandin release was observed by Harney, Sneddon and Williams (1974). During the oestrous cycle, uteri from the four phases can generate prostaglandins of the E and F series (Poyser & Scott, 1980; Van Orden, Goodale, Baker, Barley & Chatnagar, 1980; Franchi, Chand,

Borda, Gimeno, Lazzari & Gimeno, 1981; Gimeno & Gimeno, 1984; Brown & Poyser, 1985). In the rat uterus, while both the myometrium and endometrium can synthesize prostaglandins, the endometrium appears to be the major source (Campos, Liggins & Seamark, 1980; Brown & Poyser, 1985).

Uterine prostaglandin production has been shown to be under the influence of the ovarian hormones (Castracane & Jordan, 1975; Carinmate, Lazzani, Soffientini & Larner, 1975; Kogo, Yamada & Aizawa, 1977; Sterin-Speziale, Gimeno, Bonacossa & Gimeno, 1980). Oestrogen appears to be the main ovarian steroid involved in the regulation of uterine prostaglandin synthesis, but a prior period of exposure to progesterone may be necessary (Castracane & Jordan, 1976; Gimeno & Gimeno, 1984). Both uterine prostaglandin E and F (Sharma & Garg, 1977; Wilson, 1983) or prostaglandin F alone (Ham, Girillo, Zanetti & Kuehl, 1975; Sterin-Speziale et al, 1980) have been demonstrated to be regulated by oestrogen. Ovarian hormonal effects on uterine prostaglandin production may play a role in implantation and decidualization in the rat (Malathy, Cheng & Dey, 1986; Tawfik, Huet, Malathy, Johnson & Dey, 1987).

There is evidence that prostaglandins may modulate both neurotransmitter release from adrenergic neurons and the responsiveness of the post-synaptic cell to noradrenaline (Hedqvist, 1977; Malik, 1978). Prostaglandins of the E series inhibit the release of

noradrenaline from sympathetic nerves, while inhibition of prostaglandin synthesis results in an increase in noradrenaline release (Samuelsson & Wennhalm, 1971). In the rat uterus, very low concentrations of prostaglandins have been shown to antagonize catecholamine-induced inhibitory responses (Clegg, 1966). A similar interaction between prostaglandins and catecholamines has also been reported by other workers (Vesin & Harbon, 1974; Krall, Banett, Jamgotchian & Korenman, 1984). However, catecholamines have been shown to stimulate prostaglandin biosynthesis and release from various tissues including the spleen (Gilmore, Vane & Wyllie, 1968; Bedwani & Millar, 1975; Bruckner-Schmidt, Jackish & Hertting, 1981), seminal vesicles (Egan, Humes & Kuehl, 1978), kidney (Davis & Harton, 1972; Needleman, Douglas, Staecklein & Johnson, 1974), cultured cells (Levine & Moskowitz, 1979), brain (Hillier & Templeton, 1982; Yohai & Danon, 1987), iris-ciliary body (Yohai & Danon, 1987), vas deferens (Trachte, 1987) and heart (Wennhalm & Brundin, 1978; Weis & Malik, 1985, 1986; Molderings & Schumann, 1987).

Stimulation of uterine prostaglandin biosynthesis by adrenoceptor agonists has been demonstrated in humans (Quaas & Zahradnik, 1985; Wikland, Lindblom & Wigvist, 1985; Quaas, Goppinger & Zahradnik, 1987), sheep (Lye, Christopher & Casper, 1987), guinea-pigs (Takei & Moritoki, 1987) and rats (Boyle & Ohia, 1985a,b; Chaud, Franchi, Gonzalez, Gimeno & Gimeno, 1986; Ohia, 1986; Ohia & Boyle, 1988).

The mechanism involved in catecholamine-induced prostaglandin biosynthesis is not well understood. Evidence from biochemical studies reveal that catecholamines can directly stimulate cyclo-oxygenase activity (Egan et al, 1978 , Banmann, Von Bruchhausen & Wurm, 1979). Activation of prostaglandin synthesis by catecholamines appears to be associated with an effect on adrenoceptors of the α -(Ojeda, Negro-Vilar & McCann, 1982; Ritta & Cardinali, 1982; Borda, Peredo & Gimeno, 1983; Molderings & Schumann, 1987), β -(Dusling & Nolan, 1981; Qmini, Folco, Sautebin, Nara, Mandelli & Feruccio, 1981; Shaffer & Malik, 1982; Lye et al 1987) and α - and β -subtypes (Boyle & Ohia, 1985a,b; Ohia, 1986; Chand et al, 1986; Trachte, 1987). In general, the prostaglandins generated by catecholamines produced excitatory effects which have been ascribed to either a direct action of prostaglandins on intracellular calcium binding sites leading to a decrease in calcium storage (Carsten, 1974; Reiner & Marshall, 1976; Carsten & Miller, 1977) or an inhibition of plasma membrane Ca^{2+}/Mg^{2+}-ATPase resulting in an overall inhibition of calcium efflux (Deliconstantinos & Fotiou, 1986).

Uterine response to catecholamines appears to be under the influence of several factors which could affect the types of adrenoceptors present (e.g. ovarian hormones), potency at receptor sites (e.g. agonists removal mechanisms) and the intracellular processes involved in its action (e.g. prostaglandins). As discussed above, some of

these factors may interact with each other and at different levels of the catecholamine induced response.

The aim of the present study was three-fold (i) to further investigate the mechanism responsible for the variation in uterine response to adrenoceptor agonists in the four phases of the oestrous cycle, (ii) to examine the possible involvement of prostaglandins in adrenoceptor agonists effects in uteri from non-pregnant, pregnant and post-partum animals in order to fully assess the role of prostaglandins in the observed variation in response, and (iii) to determine if changes in tissue cAMP metabolism could play a role in the altered uterine response to adrenoceptor agonists in non-pregnant and pregnant rats.

CHAPTER 2

MATERIALS AND METHODS

A. ANIMALS

Female Wistar strain rats were used in all experiments. There were four groups of animals:-

i) Virgin rats in the weight range 180-220 g.

ii) Oestrogen pretreated virgin rats in the weight range 180-220 g.

iii) 20-day pregnant rats in the weight range 200-280 g.

iv) 1-day post-partum rats in the weight range 200-280 g.

B. VIRGIN RATS - 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 - prooestrus, 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 thereafter 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 as shown in Table I. Vaginal smears were taken immediately after the animals were 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% (w/v) Methylene blue and examined under a microscope. Vaginal smears from each phase of the oestrus cycle are shown in Plate I.

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 smaller and thin	Nucleated epithelial cells and leucocytes

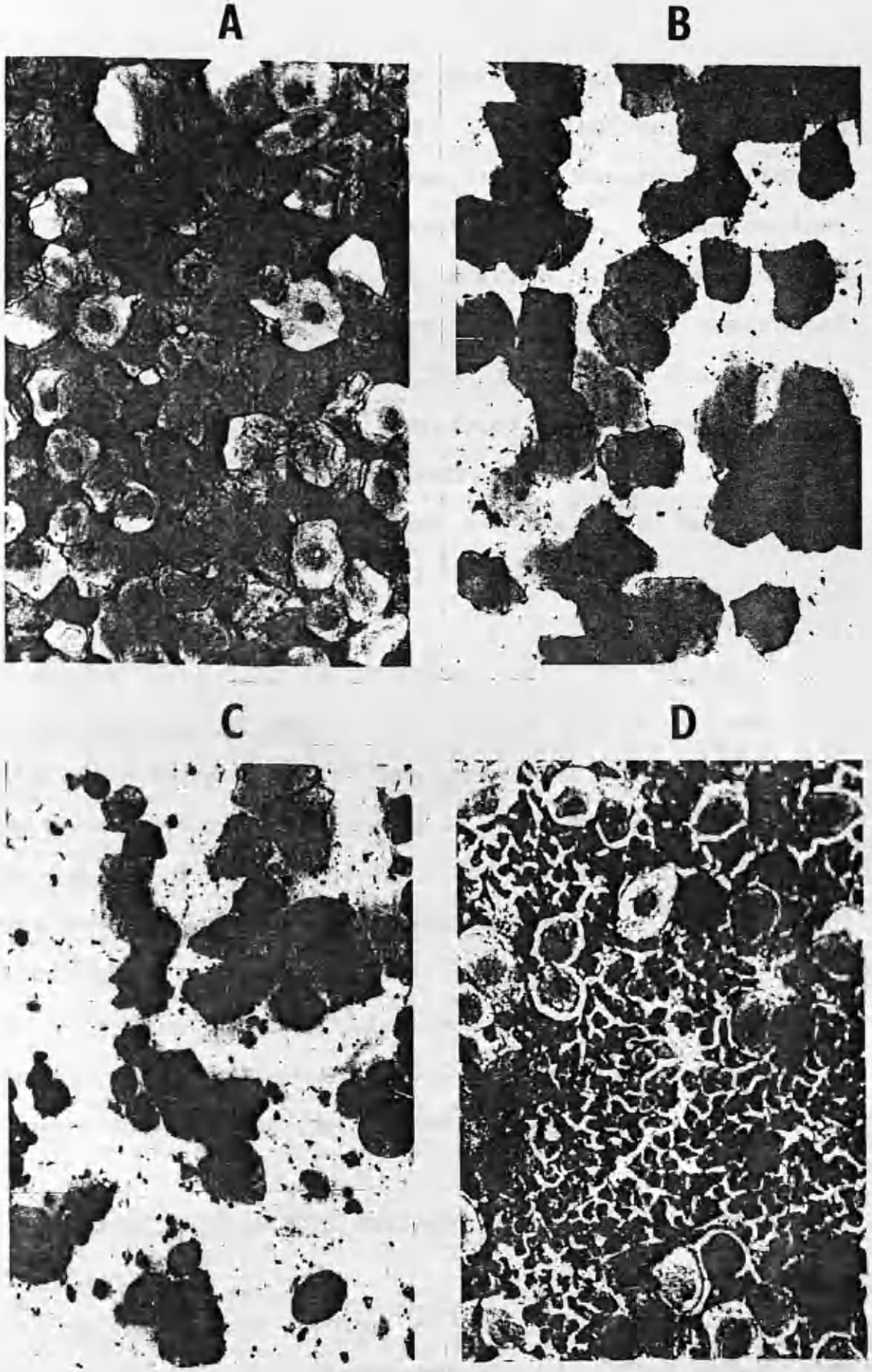
Plate I: Vaginal Smears:-

A. Proestrus - Nucleated epithelial cells only.

B. Oestrus - Cornified squamous cells only.

C. Metoestrus - Cornified cells, nucleated epithelial cells and polymorphonucleated leucocyte

D. Dioestrus - Nucleated epithelial cells and leucocytes.


A
B
C
D

Since there is evidence that ovariectomy enhances uterine inhibitory responses to catecholamines, suggesting a role for the ovarian hormones in the observed variation in their action during the oestrous cycle, it was decided to investigate the role of ovarian hormones using a different model. Animals were pretreated with oestradiol in order to induce oestrus. The steroid was dissolved in corn oil, and 0.2 mg/kg administered by intraperitoneal injection 24 hours prior to sacrifice. The effectiveness of this treatment in inducing oestrus, was verified by microscopic examination of vaginal smears.

C. PREGNANT AND POST-PARTUM RATS

i) 20 day pregnant rats: Females were placed in cages with males in a ratio of 1:1. The appearance of a vaginal plug was taken as day one of pregnancy. The gestation period in the rat is of approximately 21 days duration and animals were sacrificed at 20 days into gestation, i.e. prior to parturition.

ii) 1 day post-partum rats: Rats were mated as described for the 20 day pregnant animals. Uteri were taken from animals on the first day following parturition.

D. ISOLATED UTERINE HORN PREPARATIONS

i) Virgin rats

All animals were killed by stunning and exsanguination. The uterus was exposed after a mid line

abdominal incision and isolated from the surrounding mesentery.

Two to three 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 aerated with 95% O_2 5% CO_2 mixture. Isometric tension was recorded via Grass displacement transducers. 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 first being mounted. However, the spontaneous activity disappeared after exposure of the tissues to acetylcholine (AcH).

ii) 20 Day pregnant rats and 1 day post-partum rats

The uteri from 20 day pregnant and 1 day post-partum rats were cut open along the mesenteric border and the foetuses expelled in the pregnant animal. Longitudinal strips were prepared (2-3 cm in length) and set up for isometric recording, in the same way as with the horn preparations from the non-pregnant rats. In a series of experiments, the endometrial layer was removed by gently scraping the inner surface of the opened uterus under a dissecting microscope, a longitudinal strip of the remaining myometrium was then set up for isometric recording. One strip was prepared from each uterine horn. Uteri from 20 day pregnant animals exhibited spontaneous activity on mounting. The spontaneous activity disappeared after exposure of the tissues to Ach.

TABLE 2:

Composition of Tyrode solution.

Compound	Concentration (mmol/l)
NaCl	136.90
KCl	2.68
$CaCl_2.6H_2O$	1.80
$MgCl_2.6H_2O$	1.05
$NaHCO_3$	11.90
$NaH_2.PO_4 2H_2O$	0.42
Glucose	5.55

E. MOTOR RESPONSES TO ACETYLCHOLINE

Since the rat uterus does not possess intrinsic tone, it was necessary to induce tone in order to measure the relaxation produced by drugs.

In the present study, acetylcholine was used to induce tone in uteri from the four phases of the oestrous cycle, during pregnancy, and post-partum.

Concentration-response curves were constructed to 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.

F. EXPERIMENTS ON UTERI FROM NON-PREGNANT RATS

i) Experiments on uteri in the four stages of the oestrous cycle

1) Inhibitory responses to adrenoceptor agonists

In all experiments, the inhibitory responses to the adrenoceptor agonists, noradrenaline (NA), adrenaline (ADR), salbutamol (SAL) and isoxsuprine (ISOX) were examined in uteri in which tone was induced with Ach as described earlier. In each experiment, only one adrenoceptor agonist was used.

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.

Preliminary experiments had shown that the inhibitory effects of the adrenoceptor agonists were fully developed within 30 seconds. After inhibition with the agonists, the standard Ach response was allowed to recover fully before addition of the next dose of agonist.

Experiments in which only the adrenoceptor agonists were present are referred to as Control I studies.

In all subsequent experiments, control responses to Ach and the adrenoceptor agonist under investigation were obtained, before the addition of any antagonists.

2) Inhibitory response to adrenoceptor agonists in the presence of an α adrenoceptor 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 agonists would then be the algebraic sum of contraction plus relaxation.

In a series of experiments, α -receptor activity was abolished by using the alpha receptor antagonist Azapetine (AZA). AZA (10^{-6} M) was added to the reservoir of tyrode solution, and was present throughout the remainder of the experiment. Tissues were exposed to AZA for at least 30

minutes before addition of Ach and the adrenoceptor agonists.

3) Inhibitory responses to adrenoceptor agonists 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 I) 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 I and normetanephrine (NMN) to block uptake 2. When used both DMI (10^{-6} M) and NMN (10^{-6} M) were added to the reservoir and 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 Ach and the adrenoceptor agonists.

4) Inhibitory response to adrenoceptor agonists 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 all three drugs AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M) were present. As before the antagonists were added to the tyrodes reservoir and were present throughout the remainder

of the experiment. Such experiments are referred to as Control II studies.

5) Inhibitory responses to adrenoceptor agonists in the presence of a cyclo-oxygenase inhibitor flurbiprofen

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 Fi.1) while in the second series, controls were of the Control II type i.e. all the three antagonists were present (see Section Fi.4).

The adrenoceptor agonist used was SAL because of its selectivity for β -adrenoceptors. After obtaining control responses, FBF (10^{-5} M) was added to the tyrodes reservoir and was present in the tyrode solution for the remainder of these experiments. Tissues were exposed to (FBF) for at least 1 hour before addition of the adrenoceptor agonists.

ii) Experiments on uteri from oestrogen pretreated rats

1) Inhibitory responses to salbutamol, isoprenaline and isosuprine

The inhibitory responses to the adrenoceptor agonists Salbutamol (SAL), Isoprenaline (ISO), and Isosuxprine (ISOX) were studied in uteri exposed to oestrogen in which

tone was induced with Ach as in those from animals during the natural oestrous cycle. Control I studies were performed for SAL, ISO, and ISOX and no antagonists were present.

2) Inhibitory responses to adrenoceptor agonists in presence of (FBF)

The possibility that intramural prostaglandin production may influence adrenoceptor agonist inhibitory responses in uteri from animals pre-treated with oestrogen was investigated. The effects of SAL, ISO and ISOX were examined in the presence of (FBF) as described in Section Fi.5.

3) Inhibitory responses to adrenoceptor agonists in the presence of a phospholipase A_2 inhibitor, Quinacrine

The concentration of free-fatty acids within cells is low and thus the initial and rate limiting step in the biosynthesis of prostaglandins is the enzymic liberation of free arachidonic acid from ester pools. Arachidonic acid is located predominantly in the 2-position of phospholipids and its release occurs by hydrolysis controlled by phospholipase A_2.

Thus in order to further examine the role of intramurally generated prostaglandins in uterine response to adrenoceptor agonists, a series of experiments was performed in the presence of the phospholipase A_2

inhibitor quinacrine. Control I studies were performed for SAL and quinacrine (10^{-5} M) was present in the tyrode solution for the remainder of these experiments.

Tissues were exposed to quinacrine for at least 45 minutes before the addition of SAL.

4) Inhibitory responses to adrenoceptor agonists in the presence of a cyclo-oxygenase inhibitor (FBF) and arachidonic acid

A series of experiments was carried out to determine if the effects elicited by the cyclo-oxygenase inhibitor, FBF, can be reversed in the presence of exogenous arachidonic acid. FBF was present in the tyrodes solution from the start of the experiment, and concentration curves for SAL were obtained. Arachidonic acid (10^{-6} m) was then added to the tyrode's reservoir and was present for the remainder of the experiment.

Tissues were exposed to FBF and arachidonic acid for at least 1 hour before the addition of SAL.

G) EXPERIMENTS ON UTERI FROM 20 DAY PREGNANT AND ONE-DAY POST-PARTUM RATS

Two series of experiments were performed to investigate the role of prostaglandins in uterine response to adrenoceptor agonists in late pregnancy and early post-partum. In the first series, adrenoceptor agonist effects were examined in uteri with the endometrium present while in the second series, endometrium-free preparations were

employed.

The effects of the cyclo-oxygenase inhibitor, FBF, were examined in each of the series.

i) Experiments on uterine preparations with endometrium present

Inhibitory responses to adrenoceptor agonists in pregnant uteri in the presence of a cyclo-oxygenase inhibitor.

The role of the prostaglandins in adrenoceptor induced inhibition in pregnant and post-partum rats was investigated by examining the effects of FBF on the responses to ADR and SAL. After obtaining control responses to ADR and SAL, FBF (10^{-5} M) was added to the tyrode solution for the remainder of these experiments. Tissues were exposed to FBF for at least 1 hour before the addition of the adrenoceptor agonists.

ii) Experiments on uterine preparations with endometrium removed

Inhibitory responses to adrenoceptor agonists in pregnant and post-partum myometrium in the presence of a cyclo-oxygenase inhibitor

As with experiments on pregnant and post-partum uteri with intact endometrium (Section G(i)), both Control I and Control II studies were carried out for ADR and SAL before and after the addition of FBF (10^{-5} M).

H) Measurement of adenosine 3'5' cyclic monophosphate content in the uterus

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

i) 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 dry and weighed. Tissues were incubated in Tyrodes solution containing a phosphodiesterase inhibitor, theophylline (10^{-3} M) at 37^{o}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 (TCA), which were left in a cold room (2-4^{o}C) for 24 hours.

0.5 ml of the TCA 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^{o}C. Some crystals of calcium carbonate were added to each tube to neutralize any remaining TCA and to maintain a constant sample pH.

ii) 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.

1) 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, 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.

2) Assay procedure

(1) Fourteen 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 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 (5 µCi) 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 analyzer (Model 2000 CA).

3) Preparation of standard curve for cAMP assay

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

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

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

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

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

(6) From the Co/Cx 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.

iii) Measurement of tissue cAMP in the four phases of the oestrous cycle.

1) Measurement of basal tissue cAMP

Since variation in the ovarian hormones may affect cAMP generation, basal cAMP levels were measured in uteri from rats in proestrus, oestrus, metoestrus and dioestrus phases.

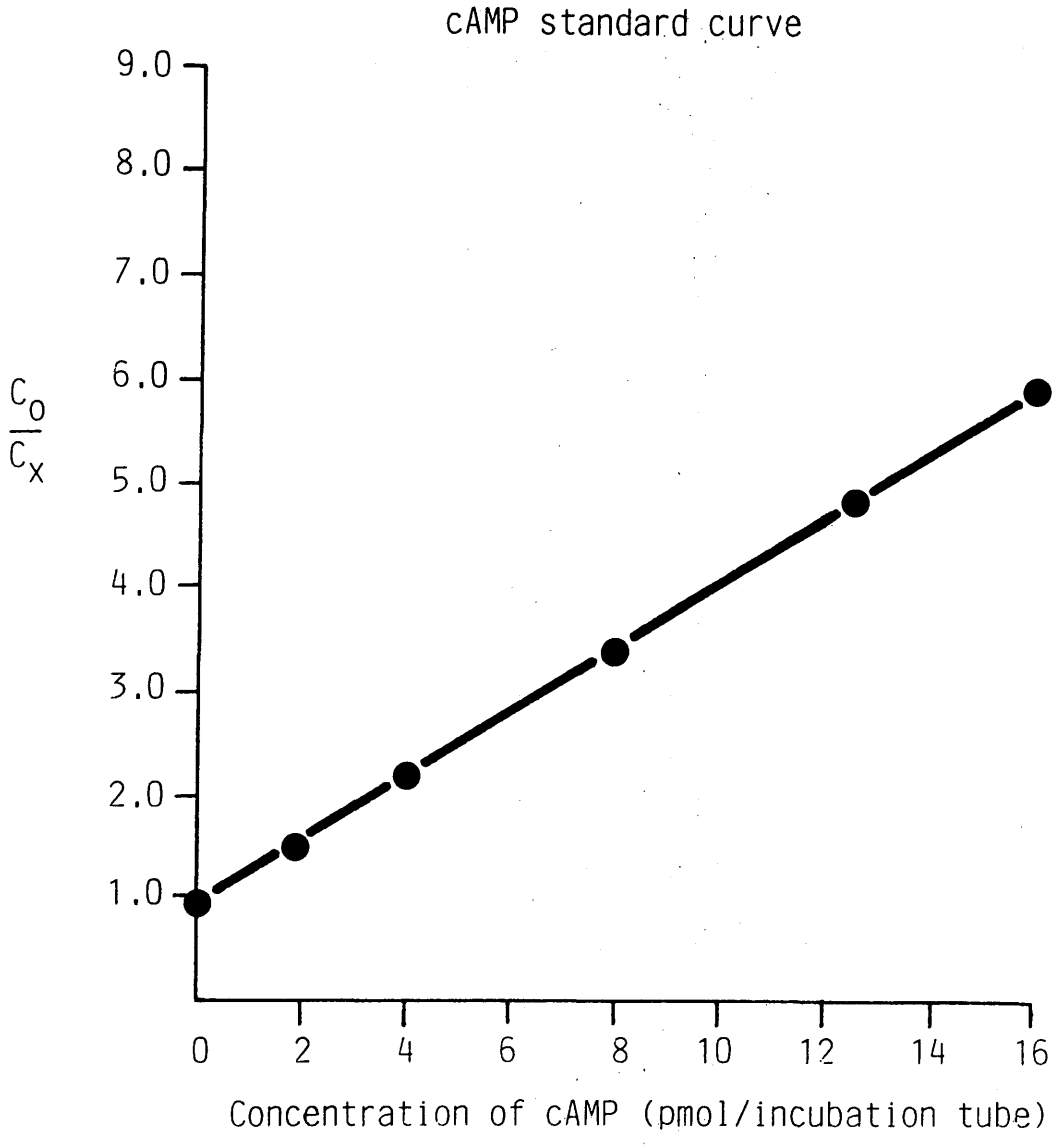


Fig 1: STANDARD CURVE FOR determination of unknown cAMP concentration in samples. Co = counts per minute (c.p.m.) of labelled compound bound in the absence of unlabelled cAMP: Cx = c.p.m. of labelled compound bound in the presence of standard unlabelled cAMP.

2) Measurement of the effect of a β -adrenoceptor agonist on cAMP levels

The effect of the β -receptor agonist SAL on cAMP levels was investigated in a series of experiments. The SAL concentration used was in the range 10^{-9}M to 10^{-5}M).

Tissues were incubated in Tyrode solution at 37°C for 1 hour. After incubation, tissues were transferred to Tyrode solution containing various concentrations of SAL for 1 minute, and then immediately subjected to cAMP analysis.

3) Measurement of the effect of a cyclo-oxygenase inhibitor on cAMP levels

In a series of experiments, the effect of intramurally generated prostaglandins on cAMP levels was examined in uteri from the four phases of the oestrous cycle. Tissues were incubated in Tyrode solution containing the cyclo-oxygenase inhibitor FBF (10^{-5}M) at 37°C for 1 hour, and then immediately subjected to cAMP analysis.

4) Measurement of the effect of a β -adrenoceptor agonist on cAMP levels in the presence of a cyclo-oxygenase inhibitor

The effect of SAL (10^{-9}M - 10^{-5}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^{-5}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.

iv) Measurement of tissue cAMP in uteri from 20-day pregnant rats

1. Measurement of basal tissue cAMP levels

Basal tissue cAMP was measured in uterine preparations from 20-day pregnant animals. Two series of experiments were carried out. In the first, the uterus was intact, i.e. the endometrium was present, while in the second, the endometrium was removed (as described in Section D.ii).

2. Measurement of the effect of a β-adrenoceptor agonist on basal cAMP levels

The effect of SAL (10^{-9}M - 10^{-5}M) on cAMP levels was investigated in uteri with endometrium present and in uteri from which the endometrium was removed. Tissues were exposed to SAL for 1 minute as described in Section H.iii, 2 and then immediately subjected to cAMP analysis.

I. CHEMICALS AND DRUGS

Acetylcholine	-	Sigma
Arachidonic acid	-	Sigma
DL-Adrenaline bitartrate	-	Sigma
Ascorbic acid	-	B.D.H.
Azapetine phosphate	-	Roche
Desmethylimipramine hydrochloride	-	Geigy
Glucose	-	Formachem
Isprenaline hydrochloride	-	Amersham
Isoxsuprine hydrochloride	-	Sigma
Magnesium chloride	-	M & B
Methylene blue	-	B.D.H.
L-Noradrenaline bitartrate	-	Sigma
DL-Normetanephrine hydrochloride	-	Sigma
Oestradiol 17 β	-	Sigma
Potassium chloride	-	Koch-Light
Quinacrine dihydrochloride	-	Sigma
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

J. STATISTICS

All values given are mean ± standard error of the mean. Student's 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 concentration response curves. pD_2 values = negative $\log_{10} EC_{50}$.

CHAPTER 3

RESULTS

(1) 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 concentration-related contraction of the non-pregnant, pregnant and post-partum rat uteri.

The mean maximum tension developed to Ach under these conditions varied significantly (Table 3). Within the oestrous cycle, the highest, maximum tension was achieved in oestrus and the lowest in dioestrus while in pregnant and post-partum animals a considerably higher tension was developed when compared to non-pregnant preparations. These differences were significant at $P < 0.001$. No variation in potency as estimated from pD_2 values to Ach was observed in uteri from non-pregnant, pregnant and post-partum rats (Table 3).

Since the tension induced by Ach varied, a concentration-response curve was required in each experiment and the dose which produced approximately 60-70% of the maximum was then used as the standard, for the remainder of the experiment. 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.

(B) RESPONSES TO ADRENOCEPTOR AGONISTS IN THE FOUR PHASES OF THE OESTROUS CYCLE

The criteria used for the choice of the agonists were

Table 3:

Maximum tension developed to Acetylcholine (Ach) and potency in nonpregnant, pregnant and post partum rat uteri.

Hormonal state of animal	Maximum tension (g)	Potency (pD2 values)	(n)
Proestrus	4.09 ± 0.56	7.86 ± 0.13	(16)
Oestrus	4.90 ± 0.57	8.29 ± 0.20	(14)
Metoestrus	3.63 ± 0.66	8.05 ± 0.21	(14)
Dioestrus	3.58 ± 0.80	8.20 ± 0.31	(14)
20-day pregnant	12.00 ± 0.35	7.91 ± 0.31	(6)
Post partum	9.83 ± 0.30	7.72 ± 0.39	(6)

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

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.

(a) Noradrenaline

Concentration response curves and pD_2 values for NA in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 2 to 5 and Table 4, respectively. NA (10^{-9} M - 10^{-5} M) produced concentration-related inhibition of the standard Ach-induced contraction in the four phases of the oestrus cycle. The maximum degrees of inhibition produced during the cycle were approximately: proestrus, 35%; oestrus, 30%; metoestrus, 40% and dioestrus, 30%. Uteri from the oestrus phase were most sensitive to the inhibitory effects of NA (Table 4).

(b) Adrenaline

Concentration-response curves and pD_2 values for ADR in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 6 to 9 and Table 5, respectively. ADR (3 x 10^{-10} M - 10^{-6} M) elicited concentration dependent inhibition of the standard Ach-induced contraction in all phases of the oestrus cycle. The maximum degrees of inhibition produced during the cycle were approximately:

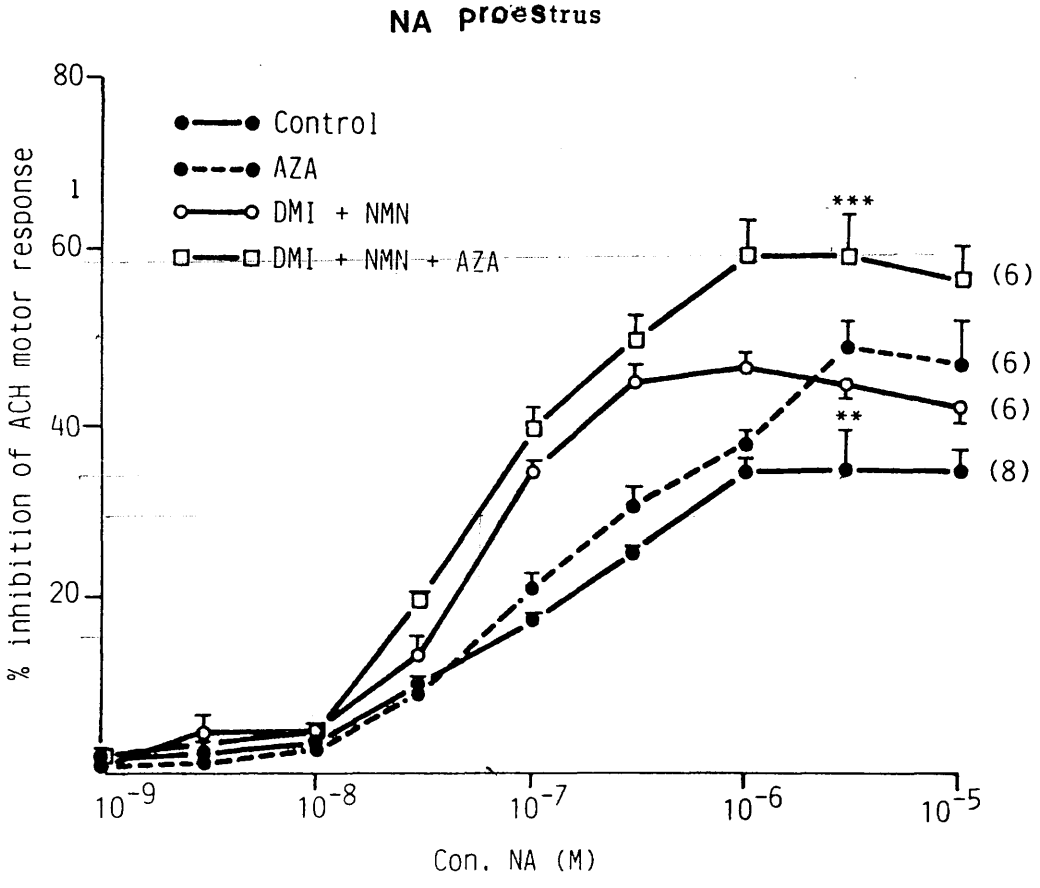


FIGURE 2: Log. concentration response curves to noradrenaline, in the rat isolated uterus in proestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6}); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

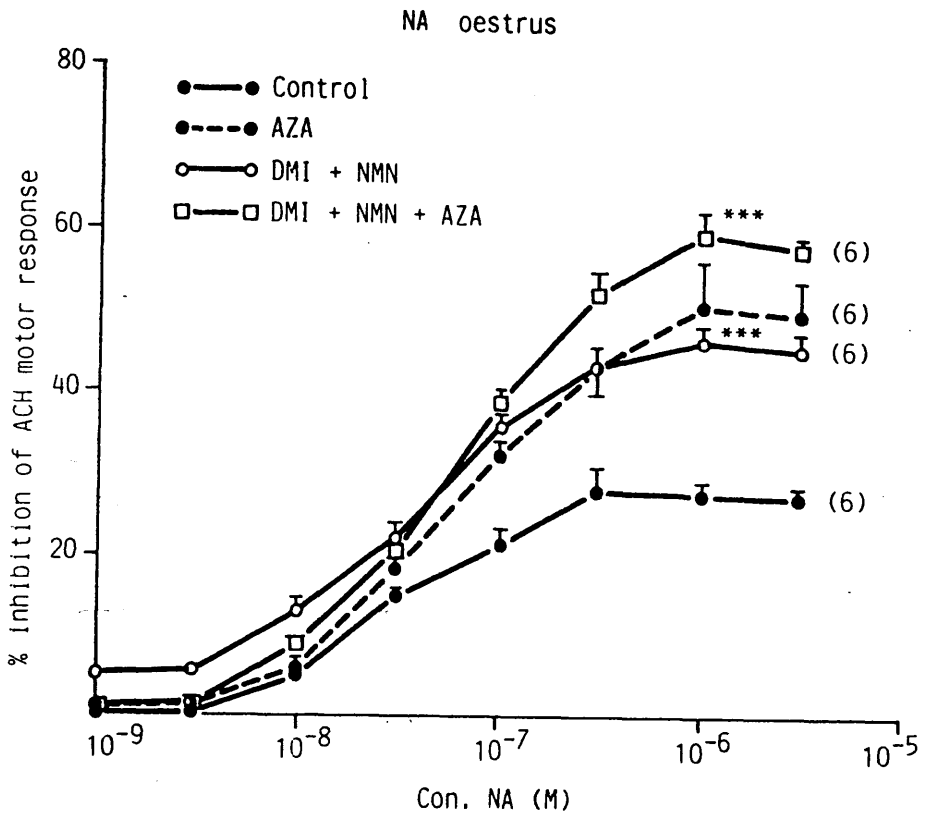


FIGURE 3: Log. concentration response curves to noradrenaline, in the rat isolated uterus in oestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

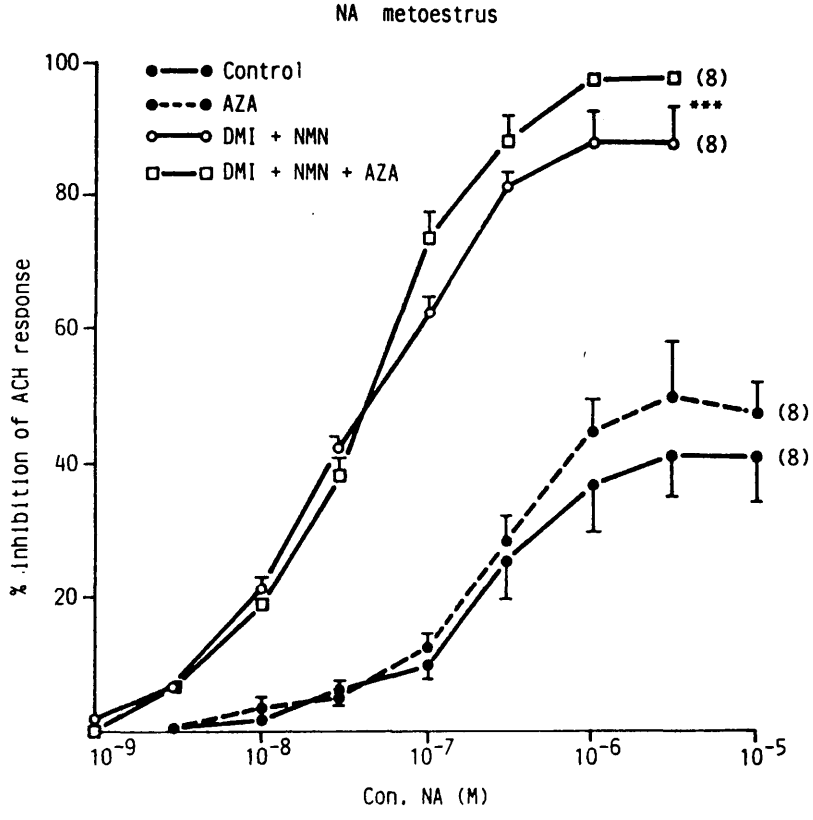


FIGURE 4: Log. concentration response curves to noradrenaline, in the rat isolated uterus in metoestrus; Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

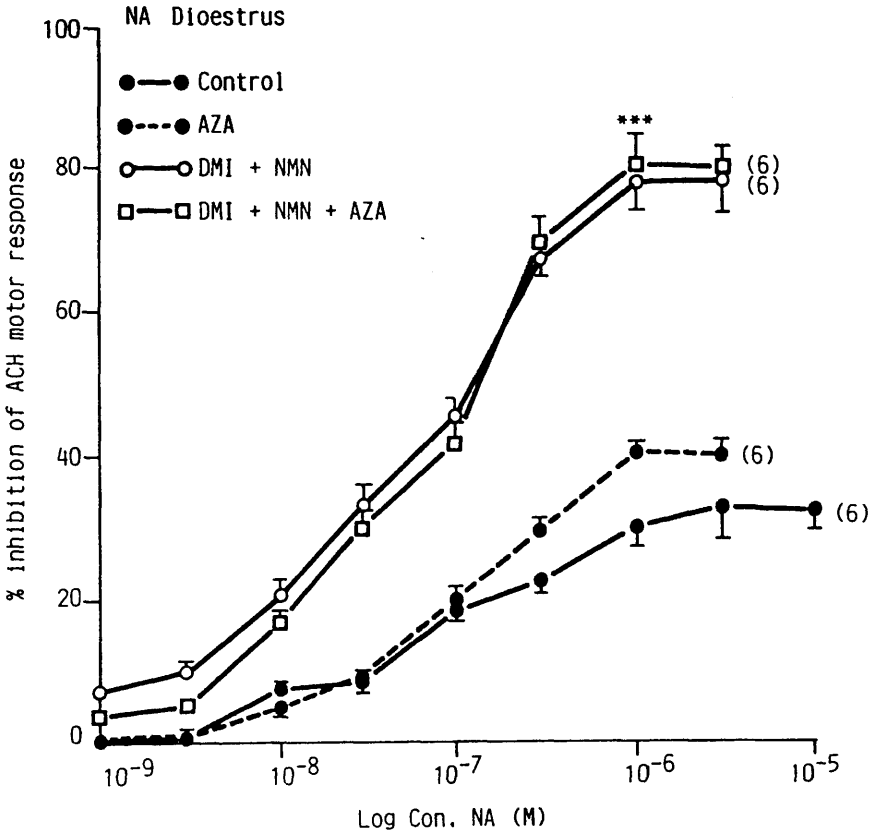


FIGURE 5: Log. concentration response curves to noradrenaline, in the rat isolated uterus in dioestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

Table 4:

Noradrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	(n)
Proestrus	6.92 ± 0.51	(7)
Oestrus	7.40 ± 0.09	(16)
Metoestrus	6.70 ± 0.08	(13)
Dioestrus	7.00 ± 0.51	(9)

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

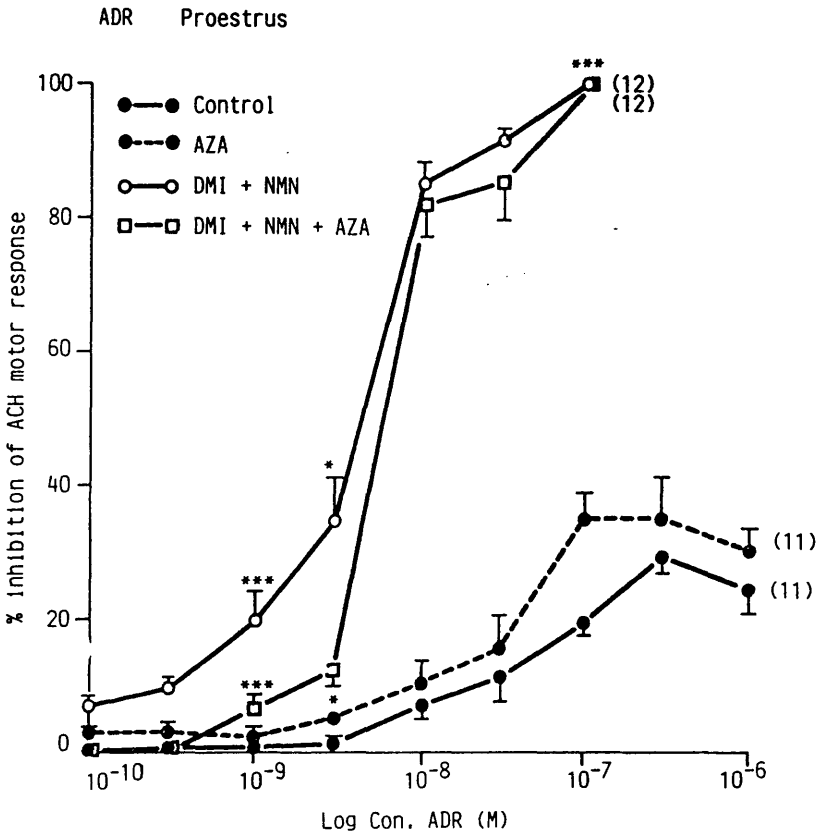


FIGURE 6: Log. concentration response curves to adrenaline, in the rat isolated uterus in proestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

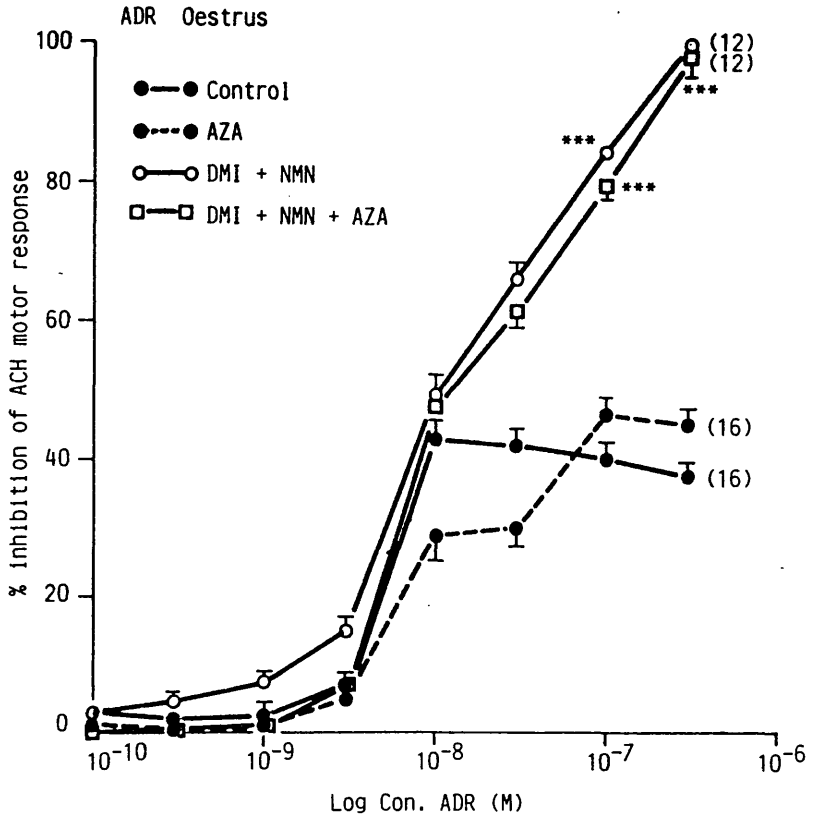


FIGURE 7: Log. concentration response curves to adrenaline, in the rat isolated uterus in oestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

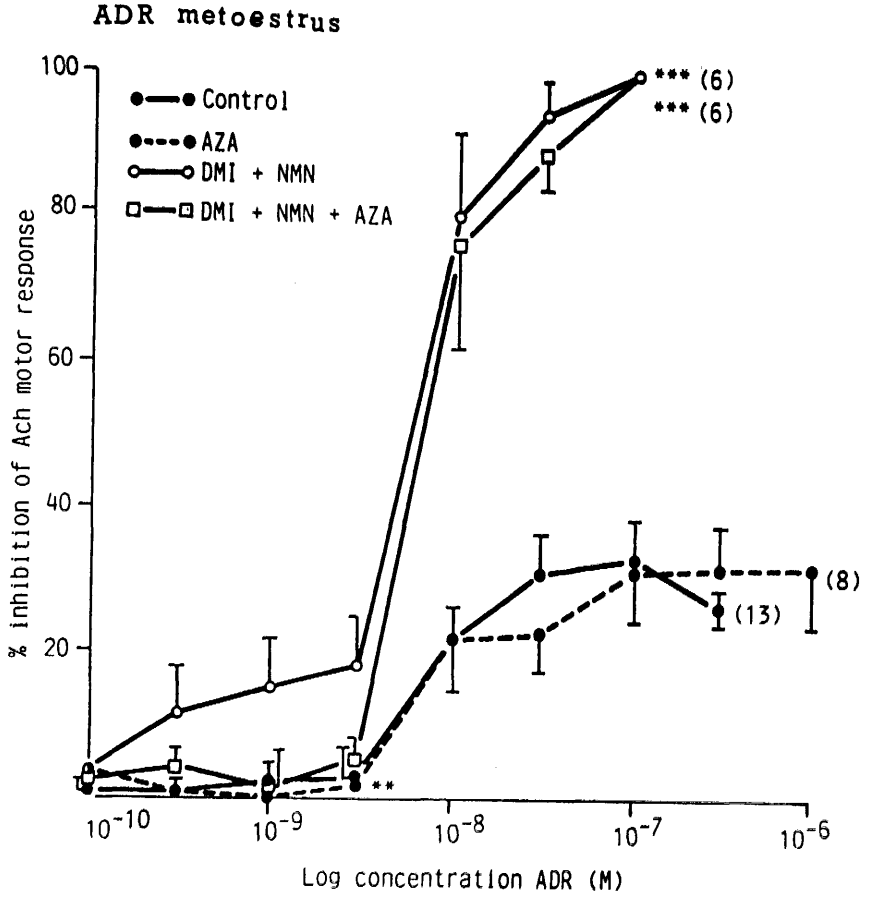


FIGURE 8: Log. concentration response curves to adrenaline, in the rat isolated uterus in metoestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

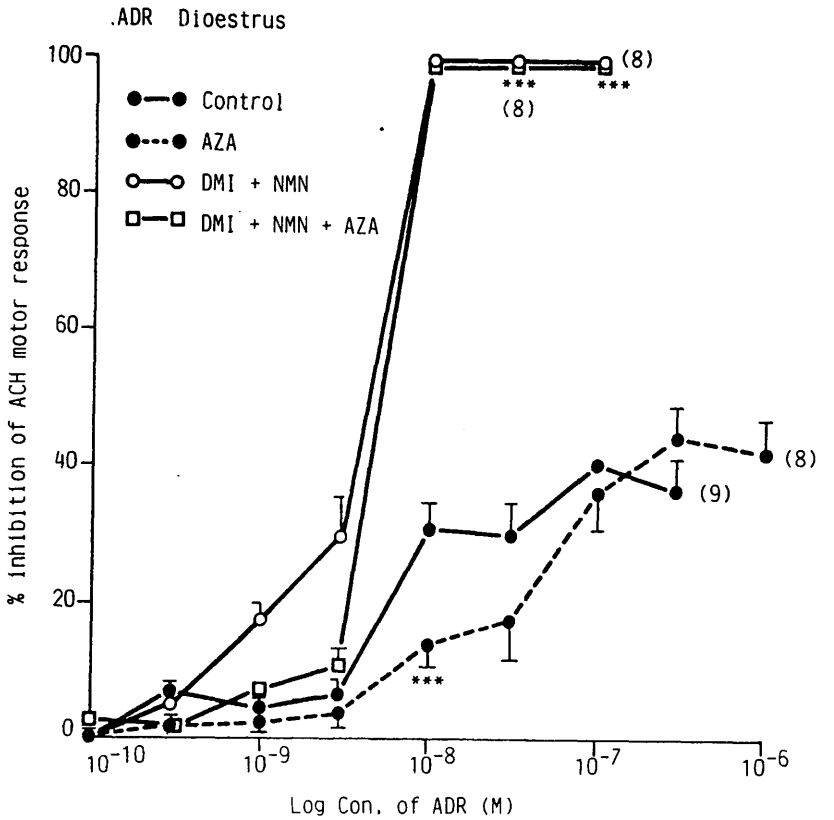


FIGURE 9: Log. concentration response curves to adrenaline, in the rat isolated uterus in dioestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

Table 5:

Adrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	(n)
Pro estrus	7.22 ± 0.30	(7)
Oestrus	8.22 ± 0.15	(16)
Metoestrus	8.10 ± 0.17	(13)
dioestrus	8.16 ± 0.18	(9)

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

proestrus, 30%; oestrus, 45%; metoestrus, 30% and dioestrus, 40%. Uteri from the oestrus phase were most sensitive to the inhibitory effects of ADR (Table 5).

(c) Salbutamol

Concentration-response curves and pD_2 values for SAL in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 10 to 13, and Table 6, respectively. SAL (10^{-9}M - 10^{-5}M) produced concentration-related inhibition of the standard Ach evoked contraction in the four phases of the oestrus cycle. The maximum degrees of inhibition produced during the oestrous cycle was approximately: proestrus, 35%; oestrus, 65%; metoestrus, 100% and dioestrus, 40%. Uteri in the oestrus phase were the least sensitive to the inhibitory effects of SAL (Table 6).

The degrees of inhibition produced by NA, ADR and SAL, and their corresponding pD_2 values varied throughout the oestrous cycle. While the maximum response to NA and ADR never exceeded 45% inhibition of the ACH induced contraction, that produced by SAL reached 65% in oestrus and 100% in metoestrus. During the oestrous cycle, uteri from the oestrus phase were most sensitive to the inhibitory effects of NA and ADR and least sensitive to SAL. Interestingly, SAL displayed a lower potency in oestrus when compared to the other 3 phases, in which salbutamol and adrenaline were significantly more potent than noradrenaline as indicated by the pD_2 values.

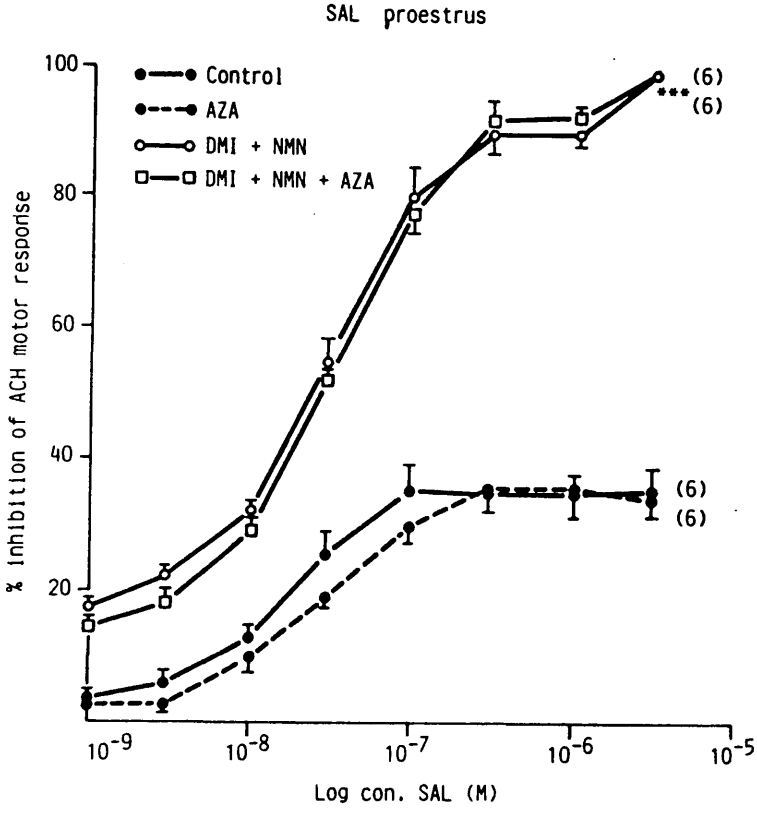


FIGURE 10: Log. concentration response curves to salbutamol, in the rat isolated uterus in proestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

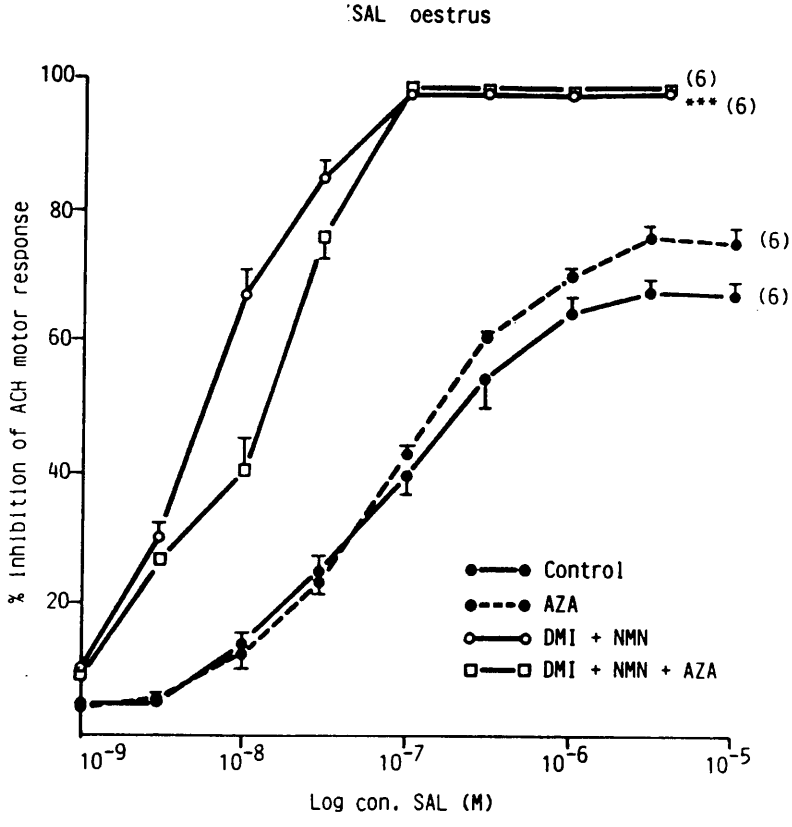


FIGURE 11: Log. concentration response curves to Salbutamol, in the rat isolated uterus in oestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

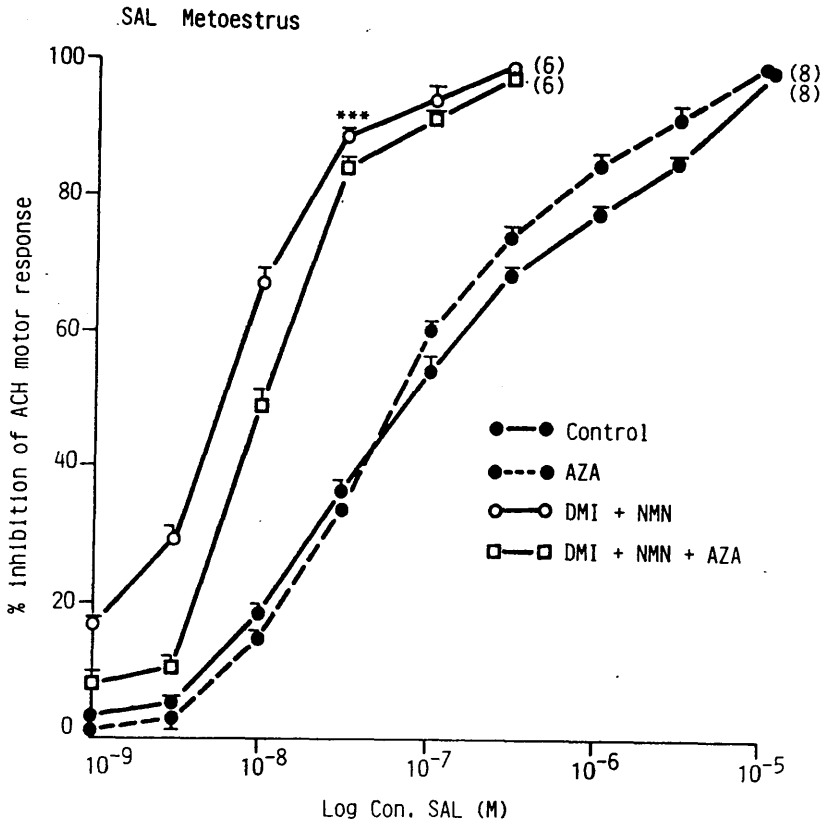


FIGURE 12: Log. concentration response curves to Salbutamol, in the rat isolated uterus in metoestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

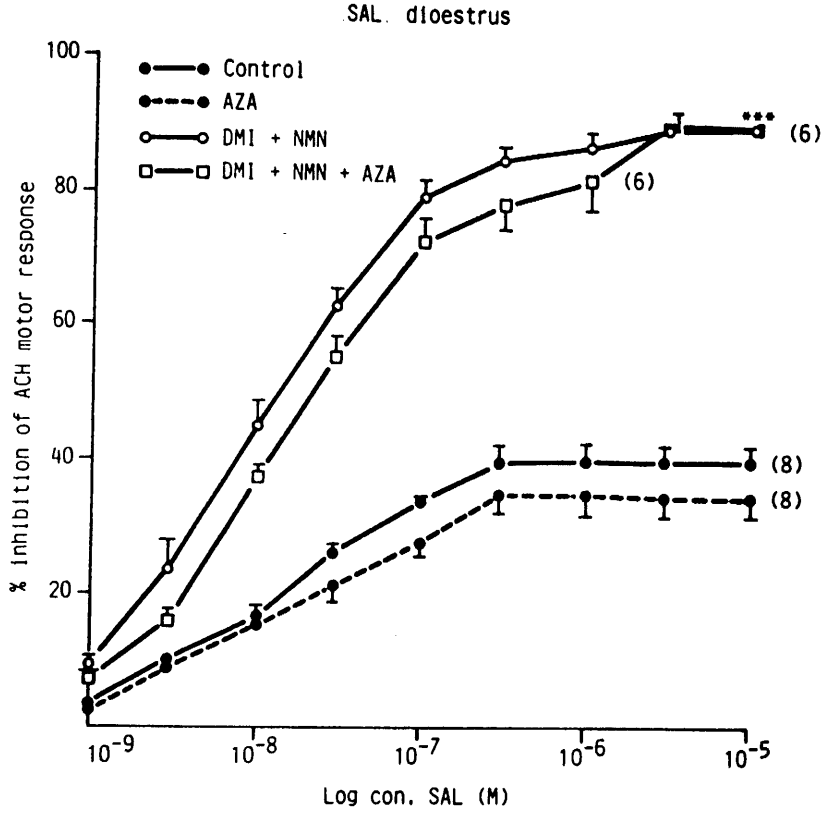


FIGURE 13: Log. concentration response curves to Salbutamol, in the rat isolated uterus in dioestrus: Controls; in presence of azapetine (AZA, 10^{-6} M); in presence of desipramine (DMI, 10^{-6} M) and normetanephrine (NMN, 10^{-6} M); in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

Table 6:

Salbutamol potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD2)	(n)
Proestrus	7.82 ± 0.20	(7)
Oestrus	7.16 ± 0.04	(16)
Metoestrus	7.10 ± 0.23	(13)
Dioestrus	7.82 ± 0.04	(9)

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

(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 RESPONSES

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 and showed very rapid tachyphylaxis. The motor responses were not

concentration-related, and were abolished by the α-receptor antagonist, Azapetine (10^{-6} M).

(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; Ohia, 1986).

(a) Noradrenaline

Concentration-response curves and pD_2 values for NA in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 2 to 5, and Table 7 respectively. AZA enhanced significantly ($P < 0.01$) the maximum degree of inhibition produced by NA in proestrus, oestrus, and dioestrus. In contrast, AZA had no significant effect on the NA response in the metoestrus phase. Compared with control values, there were no significant differences in the pD_2 values for NA after AZA treatment. (Table 7).

(b) Adrenaline

Concentration-response curves and pD_2 values for ADR in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 6 to 9 and Table 8 respectively. Unlike NA, AZA had no significant effect on the maximum degree of inhibition produced by ADR during the oestrus cycle. However, there was a significant ($P < 0.001$) shift in the ADR concentration-response curves to the right, at the

Table 7:

Effect of Azapetine (AZA, 10^{-6}M) on noradrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	
	Control (n)	After Aza(n)
Proestrus	6.92 ± 0.51 (7)	6.82 ± 0.25 (8)
Oestrus	7.40 ± 0.09 (16)	7.15 ± 0.12 (4)
Metoestrus	6.70 ± 0.08 (13)	6.70 ± 0.21 (8)
Dioestrus	7.00 ± 0.51 (9)	6.96 ± 0.04 (4)

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

Table 8:

Effect of Azapetine (AZA, 10^{-6}M) on Adrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD2)	
	Control (n)	After Aza(n)
Proestrus	7.22 ± 0.30 (7)	7.40 ± 0.24 (11)
Oestrus	8.22 ± 0.15 (16)	8.10 ± 0.15 (17)
Metoestrus	8.10 ± 0.17 (13)	8.16 ± 0.25 (11)
Dioestrus	8.16 ± 0.18 (9)	7.22 ± 0.80 (8)

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

Table 9:

Effect of Azapetine (AZA, 10^{-6}M) on Salbutamol potency in uteri from the four phases of the oestrous cycle (pD_2 values).

	Potency (pD_2)	
Phase	Control (n)	After AZA (h)
Proestrus	7.82 ± 0.20 (7)	7.52 ± 0.22 (12)
Oestrus	7.16 ± 0.04 (16)	7.10 ± 0.04 (12)
Metoestrus	7.10 ± 0.23 (13)	7.15 ± 0.76 (6)
Dioestrus	7.82 ± 0.04 (9)	7.82 ± 0.36 (8)

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

lower concentrations, in oestrus and dioestrus. Compared with control values, there were no significant differences in the pD_2 values for ADR after AZA treatment (Table 8).

(c) Salbutamol

Concentration-response curves and pD_2 values for SAL in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 10 to 13 and Table 9, respectively. AZA had no significant effect on SAL concentration-response curves in any phase of the oestrous cycle and there were no significant differences in the pD_2 values for SAL after AZA treatment (Table 9).

α-receptor blockade increased the maximum degree of inhibition produced by NA in proestrus, oestrus and dioestrus, but had no effect on ADR and SAL responses. Shifts in the ADR concentration-response curves to the right were observed at the lower concentration in oestrus and dioestrus. α-receptor antagonism had no effect on NA, ADR and SAL potency throughout the oestrous cycle.

(3) INHIBITORY RESPONSES TO NA, ADR AND SAL IN THE PRESENCE OF INHIBITORS OF NEURONAL AND EXTRA-NEURONAL UPTAKE MECHANISMS

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

(a) Noradrenaline

Concentration-response curves and pD_2 values for NA in proestrus, oestrus, metoestrus and dioestrus are shown in

Table 10:

Effect of Desipramine (DMI, 10^{-6}M) and Normetanephrine (NMN, 10^{-6}M) on noradrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	
	Control (n)	After DMI + NMN (n)
Proestrus	6.92 ± 0.51 (7)	7.22 ± 0.40 (12)
Oestrus	7.40 ± 0.09 (16)	7.52 ± 0.26 (12)
Metoestrus	6.70 ± 0.08 (13)	7.46 ± 0.14 (12)***
Dioestrus	7.00 ± 0.51 (9)	7.15 ± 0.04 (8)

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

Table 11:

Effect of Desipramine (DMI, 10^{-6}M) and normetanephrine (NMN, 10^{-6}M) on Adrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	
	Control (n)	After DMI + NMN (n)
Proestrus	7.22 ± 0.30 (7)	8.30 ± 0.08 (12)
Oestrus	8.22 ± 0.15 (16)	8.00 ± 0.29 (12)
Metoestrus	8.10 ± 0.17 (13)	8.22 ± 0.33 (6)
Dioestrus	8.16 ± 0.18 (9)	8.30 ± 0.16 (8)

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

Figures 2 to 5 and Table 10, respectively. DMI and NMN shifted NA concentration-response curves to the left, and enhanced significantly ($P < 0.001$) its maximum degree of inhibition. Compared with the control value, there was a significant increase ($P < 0.001$) in the pD_2 value for NA in metoestrus, in the presence of DMI and NMN (Table 10).

(b) Adrenaline

Concentration-response curves and pD_2 values for ADR in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 6 to 9 and Table 11, respectively. DMI and NMN shifted ADR concentration-response curves to the left, and enhanced significantly ($P < 0.001$) its maximum degree of inhibition. ADR now produced a complete inhibition of the Ach-induced contraction in all phases. Compared with control values, there were no significant differences in the pD_2 value for ADR in the presence of DMI and NMN (Table 11).

(c) Salbutamol

Concentration-response curves and pD_2 values for SAL in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 10 to 13 and Table 12, respectively. DMI and NMN shifted SAL concentration-response curves to the left (all phases), and enhanced significantly ($P < 0.001$) its maximum degree of inhibition in proestrus, oestrus and dioestrus. SAL now produced a complete inhibition of the Ach contraction in all phases of the oestrous cycle. Compared with the control value, there was a significant

Table 12:

Effect of Desipramine (DMI, 10^{-6}M) and Normetanephrine (NMN, 10^{-6}M) on Salbutamol potency in uteri from the four phases of the oestrous cycle. (pD_2 values).

Phase	Potency (pD_2)	
	Control	After DMI + NMN (n)
Proestrus	7.82 ± 0.30 (7)	7.60 ± 0.06 (12)
Oestrus	7.16 ± 0.04 (16)	8.30 ± 0.12 (12)***
Metoestrus	7.10 ± 0.23 (13)	8.22 ± 0.11 (6)
Dioestrus	7.82 ± 0.04 (9)	7.82 ± 0.29 (8)

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

increase ($P < 0.001$) in the pD_2 value for SAL in oestrus, in the presence of DMI and NMN (Table 12).

Blockade of agonist uptake processes shifted concentration-response curves produced by all three agonists to the left and enhanced their maximum degrees of inhibition throughout the oestrous cycle. Furthermore, there were significant increases in NA and SAL potency in metoestrus and oestrus phases, respectively. However, shifts in agonist concentration-response curves were not always reflected in increase in pD_2 values.

(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.

(a) Noradrenaline

Concentration-response curves and pD_2 values for NA in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 2 to 5 and Table 13, respectively. The combined antagonists shifted NA concentration-response curves to the left, and enhanced significantly ($P < 0.001$) its maximum degree of inhibition. Compared with the control value, there was a significant increase ($P < 0.001$) in the pD_2 value for NA in metoestrus, in the presence of the combined

Table 13:

Effect of Azapetine (AZA, 10^{-6}M), Desipramine (DMI, 10^{-6}M) and Normetanephrine (NMN, 10^{-6}M) on Noradrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	
	Control	After DMI + NMN + AZA (n)
Proestrus	6.92 ± 0.51 (7)	7.22 ± 0.29 (12)
Oestrus	7.40 ± 0.09 (16)	7.15 ± 0.10 (12)
Metoestrus	6.70 ± 0.08 (13)	7.40 ± 0.11 (6) ***
Dioestrus	7.00 ± 0.51 (9)	7.15 ± 0.7 (8)

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

antagonists (Table 13).

(b) Adrenaline

Concentration-response curves and pD_2 values for ADR in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 6 to 9, and Table 14, respectively. The combined antagonists enhanced significantly ($P < 0.001$) ADR maximum degree of inhibition in all phases. ADR produced a complete inhibition of the Ach evoked contraction throughout the oestrous cycle. Compared with controls, there were no significant differences in the pD_2 values for ADR in the presence of the combined antagonists (Table 14).

(c) Salbutamol

Concentration-response curves and pD_2 values for SAL in proestrus, oestrus, metoestrus and dioestrus are shown in Figures 10 to 13 and Table 15, respectively. The combined antagonists shifted SAL concentration-response curves to the left and enhanced significantly ($P < 0.001$) its maximum degree of inhibition. SAL gave a complete inhibition of the Ach induced contraction in all phases. Compared with control values, there were no significant differences in the pD_2 values for SAL in the presence of the combined antagonists (Table 15).

In the presence of combined -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. Except for ADR, agonists concentration-response curves were also shifted to the left. The effects produced

Table 14:

Effect of Azapetine (AZA, 10^{-6}M), Desipramine (DMI, 10^{-6}M) and Normetanephrine (NMN, 10^{-6}M), on Adrenaline potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	
	Control	After DMI + NMN + AZA (n)
Proestrus	7.22 ± 0.30 (7)	8.22 ± 0.12 (12)
Oestrus	8.22 ± 0.15 916)	8.00 ± 0.29 (12)
Metoestrus	8.10 ± 0.17 (13)	8.16 ± 0.36 (6)
Dioestrus	8.16 ± 0.18 (9)	8.22 ± 0.04 (8)

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

Table 15:

Effect of Azapetine (AZA, 10^{-6}M) Desipramine (DMI, 10^{-6}M) and Normetanephrine (NMN, 10^{-6}M) on salbutamol potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)	
	Control	After DMI + NMN + AZA (n)
Proestrus	7.82 ± 0.30 (7)	7.52 ± 0.20 (4)
Oestrus	7.16 ± 0.04 (16)	7.82 ± 0.29 (4)
Metoestrus	7.10 ± 0.23 (13)	8.00 ± 0.41 (4)
Dioestrus	7.82 ± 0.04 (9)	8.00 + 0.02 (4)

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

by the combined receptor and uptake antagonists were no greater than those achieved in the presence of neuronal and extraneuronal blockade.

(D) RESPONSES TO β -ADRENOCEPTOR AGONISTS IN THE PRESENCE OF A CYCLOOXYGENASE INHIBITOR

An effect leading to the generation of excitatory prostaglandins by β -adrenoceptor agonists may functionally antagonize their inhibitory response. In two series of experiments, prostaglandin biosynthesis was prevented using FBF, a cyclo-oxygenase inhibitor. FBF (10^{-5} M) had no effect on the Ach induced contraction. Concentration-response curves were constructed for SAL during the oestrous cycle in the absence of uptake blockers, and AZA (control I, see Methods, Section Fi.5) and in the presence of AZA (10^{-6} M), DMI (10^{-6} M), and NMN (10^{-6} M) (control II, see Methods, Section Fi.4).

(E) INHIBITORY RESPONSES TO SALBUTAMOL IN THE PRESENCE OF A CYCLO-OXYGENASE INHIBITOR IN THE FOUR PHASES OF THE OESTROUS CYCLE

Concentration-response curves and pD_2 values for SAL in the absence and presence of FBF are shown in Figures 15 to 22 and Table 16, respectively.

PROESTRUS:

FBF shifted significantly ($P < 0.001$) the control I concentration-response curve to the left (Figure 15). While, in control II experiments, FBF produced no further

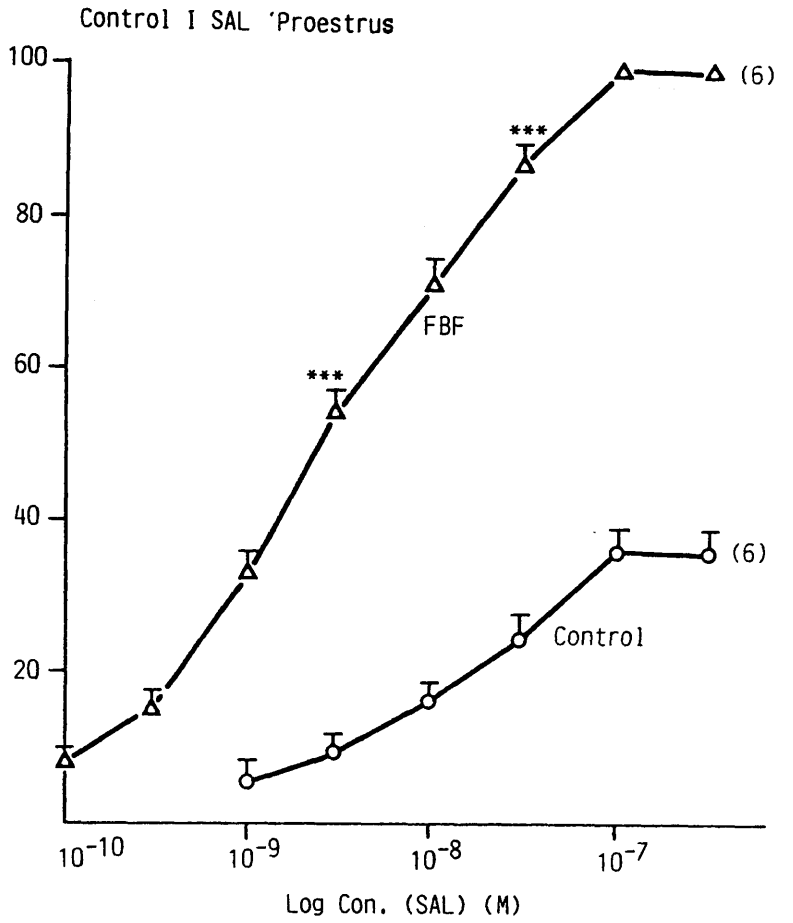


FIGURE 15: Log. concentration response curves to Salbutamol in the rat isolated uterus in proestrus: Controls; in presence of flurbiprofen (FBF, 10^{-5} M). Control I = SAL alone. Number of observations in brackets.

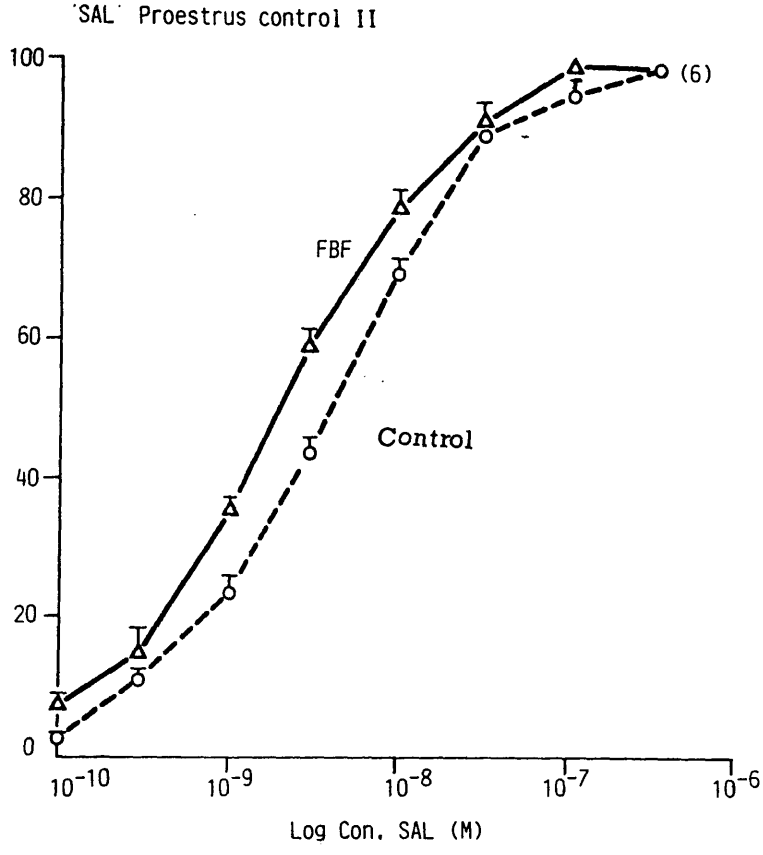


FIGURE 16: Log concentration response curves to Salbutamol in the rat isolated uterus in proestrus: Control II = SAL, in presence of AZA (10^{-6} M); DMI (10^{-6} M) and NMN (10^{-6} M); in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

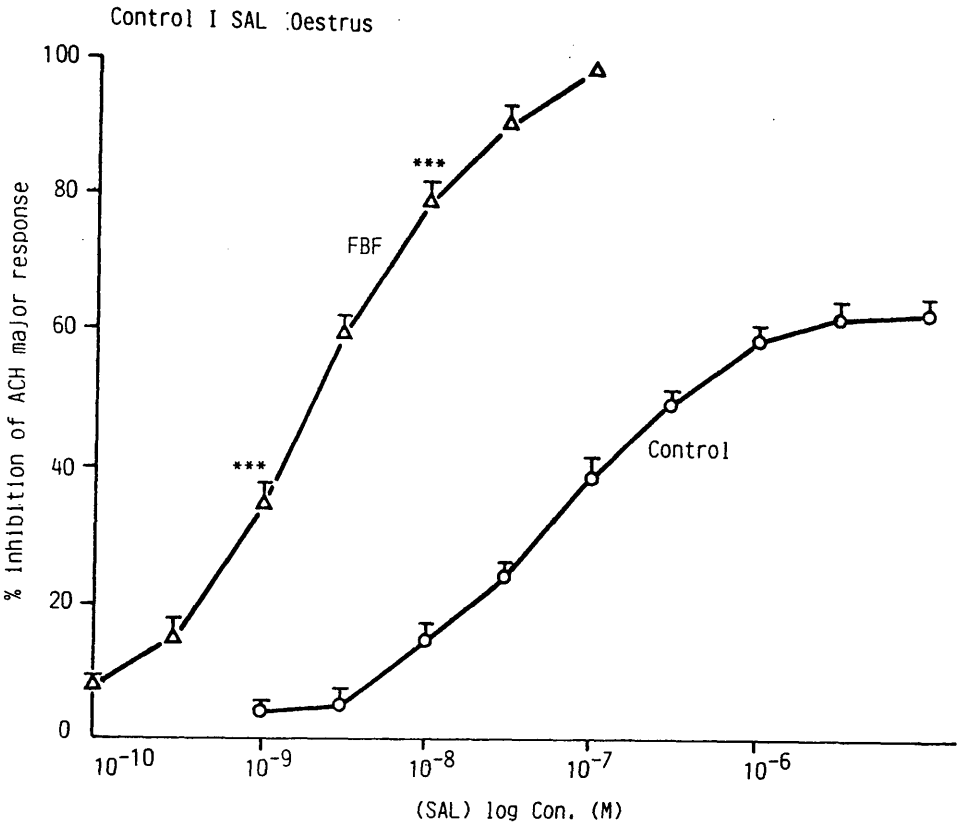


FIGURE 17: Log. concentration response curves to Salbutamol in the rat isolated uterus in oestrus: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Control I = SAL alone. Number of observations in brackets.

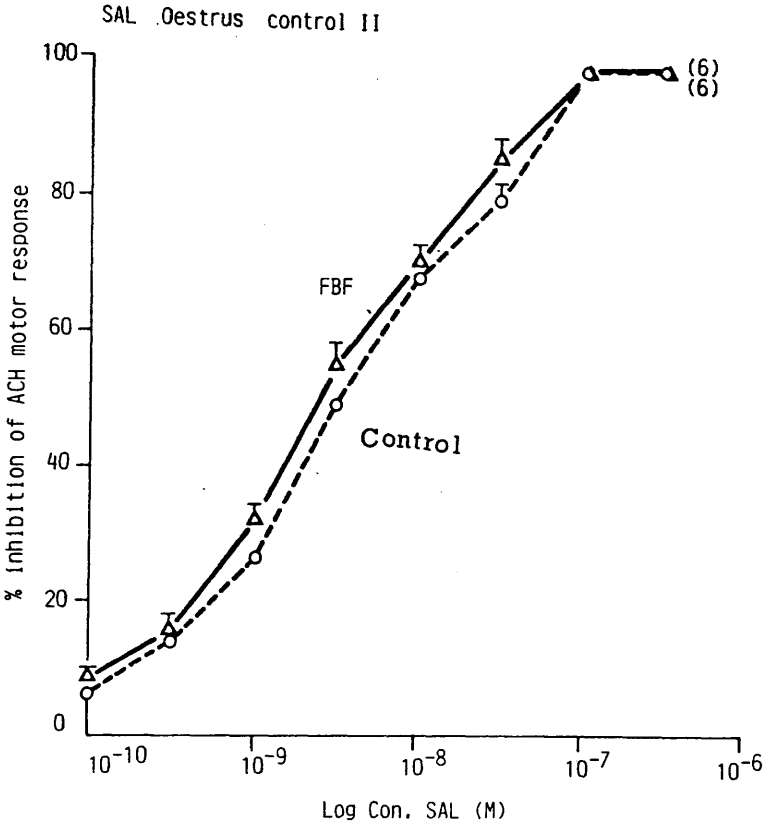


FIGURE 18: Log. concentration response curves to Salbutamol in the rat isolated uterus in oestrus: Control II = SAL, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M); in presence of AZA (10-6 M), DMI (10-6 M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

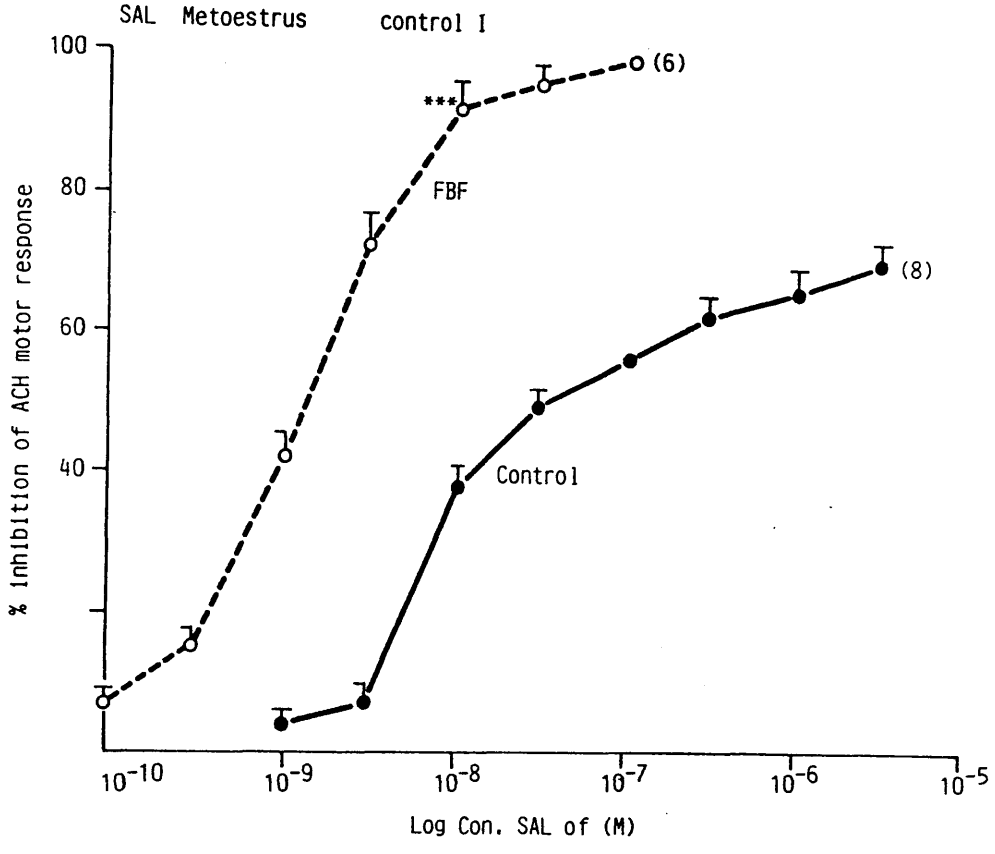


FIGURE 19: Log. concentration response curves to Salbutamol in the rat isolated uterus in metoestrus: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Control I = SAL alone. Number of observations in brackets.

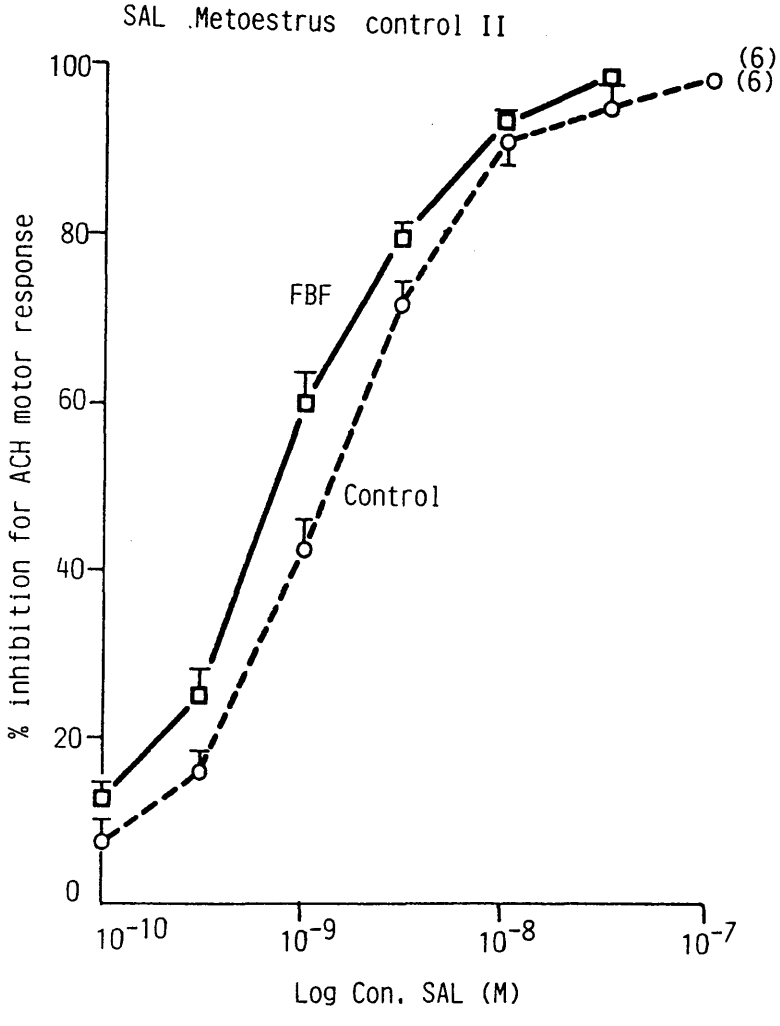


FIGURE 20: Log. concentration response curves to Salbutamol, in the rat isolated uterus in metoestrus: Control II = SAL, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M); in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

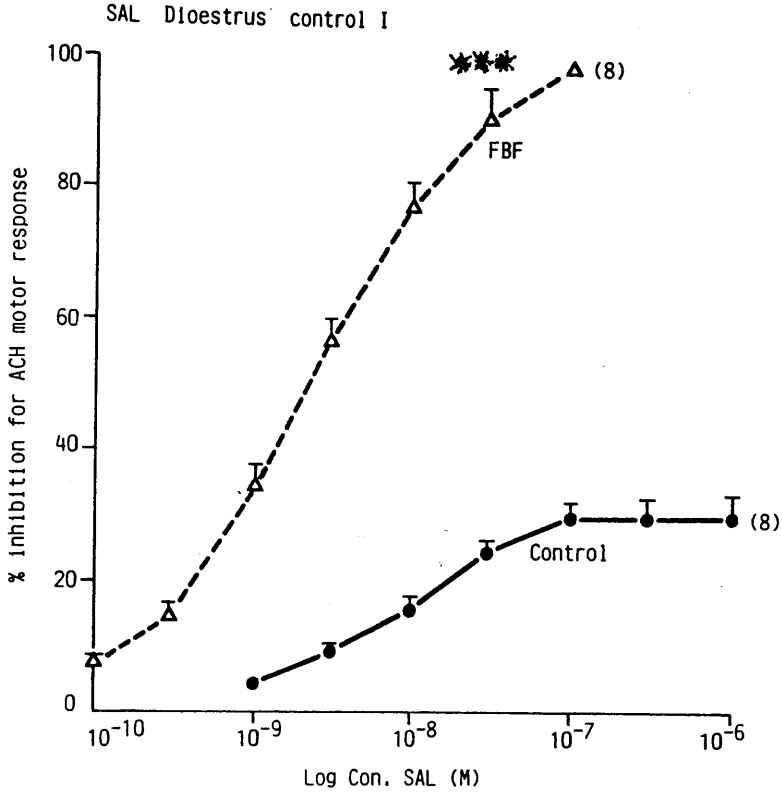


FIGURE 21: Log. concentration response curves to Salbutamol, in the rat isolated uterus in dioestrus: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Control I = SAL alone. Number of observations in brackets.

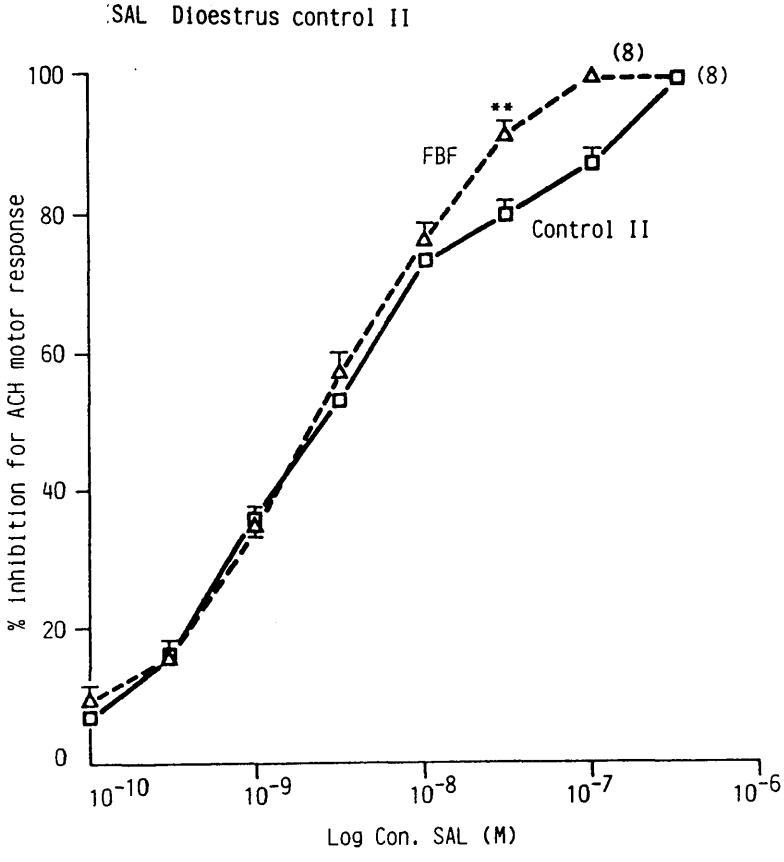


FIGURE 22: Log. concentration response curves to Salbutamol, in the rat isolated uterus in dioestrus: Control II = SAL in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M); in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

Table 16:

Effect of Flurbiprofen (FBF, 10^{-5}M), on Salbutamol potency in uteri from the four phases of the oestrous cycle (pD_2 values).

Phase	Potency (pD_2)		
	Experiment		After FBF
Proestrus	Control I	7.82 ± 0.20 (7)	8.70 ± 0.60 (6)
	Control II	8.30 ± 0.20 (4)	8.70 ± 0.03 (6)
Oestrus	Control I	7.22 ± 0.04 (16)	8.70 ± 0.09 (6) ***
	Control II	8.52 ± 0.29 (4)	8.70 ± 0.02 (6)
Metoestrus	Control I	8.00 ± 0.23 (13)	8.82 ± 0.04 (6) **
	Control II	8.82 ± 0.41 (4)	9.15 ± 0.23 (6)
Dioestrus	Control I	8.00 ± 0.04 (9)	8.70 ± 0.02 (6)
	Control II	8.52 ± 0.02 (4)	8.60 ± 0.04 (6)

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

increase in the responses to SAL (Fig. 16) and there was no change in the pD_2 values (Table 16).

OESTRUS:

FBF shifted the control I concentration-response curve to the left, and enhanced significantly ($P < 0.001$) the maximum degree of inhibition (Figure 17). In control II experiments, only a slight but non-significant shift in the concentration-response curve was achieved (Figure 18). In control I experiments, there was a significant increase ($P < 0.001$) in the pD_2 values for SAL, in the presence of FBF (Table 16). In contrast, there was no significant difference in the control II pD_2 value for SAL.

METOESTRUS:

FBF shifted significantly ($P < 0.001$) both control I and control II concentration-response curves to the left, and enhanced significantly ($P < 0.001$) the maximum degree of inhibition (Figures 19 and 20). In control I experiments, there was a significant increase ($P < 0.001$) in the pD_2 values for SAL, in the presence of FBF (Table 16). In contrast there was no significant difference in the control II pD_2 value for SAL.

DIOESTRUS:

FBF shifted the control I concentration-response curve to the left, and enhanced significantly ($P < 0.001$) the maximum degree of inhibition (Figure 21). In control II experiments (Figure 22), only responses to the higher concentrations of SAL were enhanced significantly ($P <$

0.01). There were no significant differences in the control I and control II pD_2 values for SAL, in the presence of FBF (Table 16).

In general, cyclo-oxygenase inhibition shifted the SAL concentration-response curves to the left, and enhanced its maximum degree of inhibition. However, the effects produced by cyclo-oxygenase inhibition were less under the control II situation.

(F) RESPONSES TO A β -ADRENOCEPTOR AGONIST IN OESTROGEN PRETREATED ANIMALS

In order to induce a particular phase of the oestrous cycle, animals can be pretreated with oestrogen or progesterone. Since differences in the degree of uterine inhibition produced by the adrenoceptor agonists during the oestrous cycle persisted even when α -receptors were blocked and uptake mechanisms were prevented, a series of experiments was performed to investigate the role of the ovarian hormones. Animals were pretreated with 17β -oestradiol (1 mg/kg) to induce oestrus, which was confirmed by examination of vaginal smears. In the oestrogen pretreated rats, Ach produced greater tension (5.20 $\pm$ 0.62g, n = 12) than in naturally occurring oestrus (4.90 $\pm$ 0.57g, n = 14). As in the four stages of the oestrous cycle and in the pregnant and post-partum rats, there was no significant difference between the pD_2 values for Ach in the oestrogen pretreated and natural oestrus rats, (8.36 $\pm$ 0.21, n = 12 and 8.29 $\pm$ 0.20, n = 14 respectively).

(i) INHIBITORY RESPONSES TO SALBUTAMOL, ISOPRENALINE AND ISOXSUPRINE IN UTERI FROM OESTROGEN PRETREATED ANIMALS

The effects of selective β -adrenoceptor agonists SAL (β_2), isoprenaline (β_1 β_2) and isoxsuprine (β_1 β_2) were examined in the absence and presence of the cyclo-oxygenase inhibitor, FBF.

Concentration-response curves and pD_2 values for SAL, ISO, and ISOX in the absence and presence of FBF are shown in Figures 23 to 25 and Table 17, respectively:

Salbutamol:

The SAL concentration response curves in uteri from oestrogen primed animals lay to the left of those obtained in natural oestrus, and the pD_2 values for SAL were 9.30 $\pm$ 0.43 (n = 6) in oestrogen treated animals and 8.42 $\pm$ 0.04 (n = 16) in natural oestrus. Thus uteri from oestrogen pretreated animals appear to be more sensitive to the inhibitory effect of SAL. AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M) had no significant effect on the SAL response, and in subsequent experiments these drugs were not present, i.e. the experiments were all of the control I class.

FBF increased significantly ($P < 0.01$) the responses to SAL enhancing its maximum degree of inhibition (Figure 23). Compared with the control value, there was no significant difference in SAL pD_2 value in the presence of FBF (Table 17).

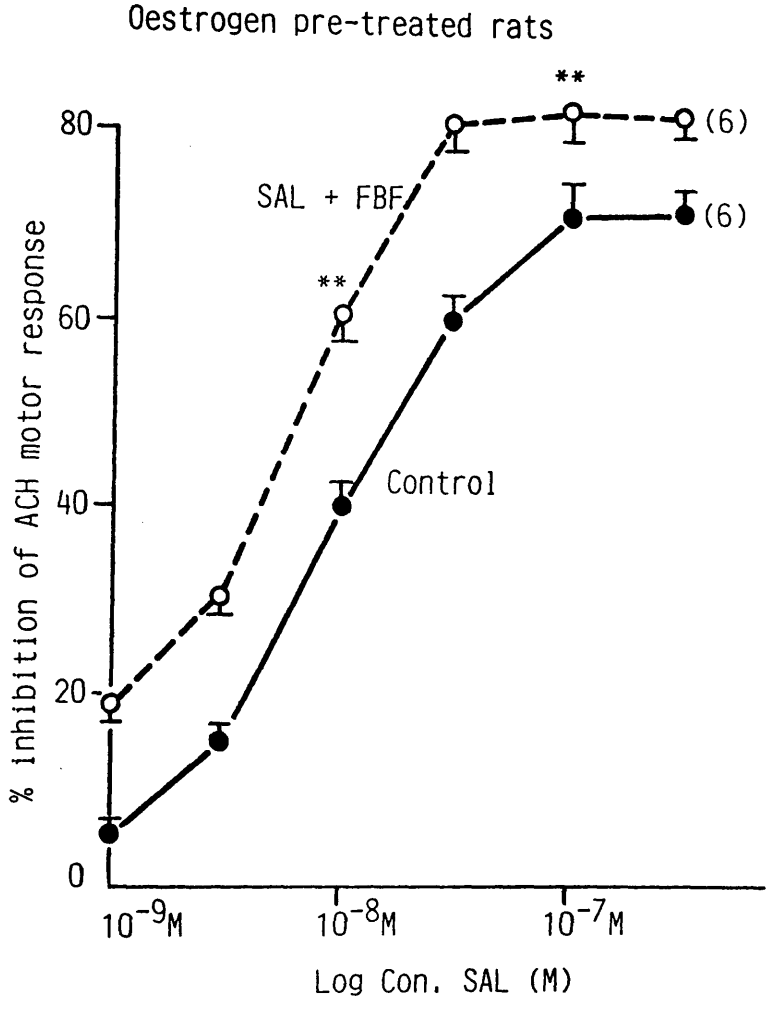


FIGURE 23: Log. concentration response curves to Salbutamol, in the rat isolated uterus in oestrogen pretreated: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

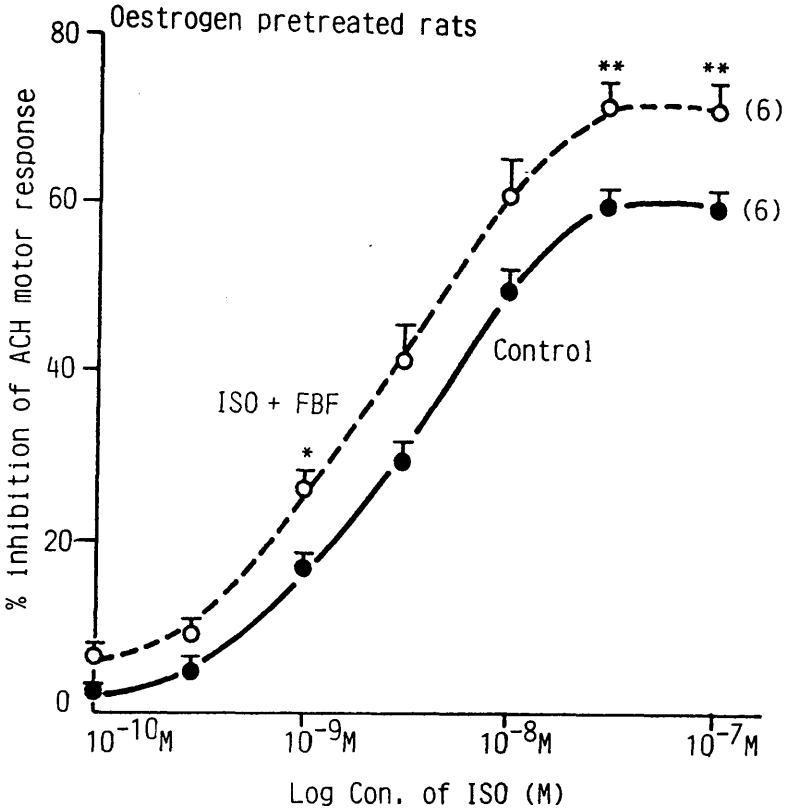


FIGURE 24: Log. concentration response curves to isoprenaline in the rat isolated uterus in oestrogen pretreated: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

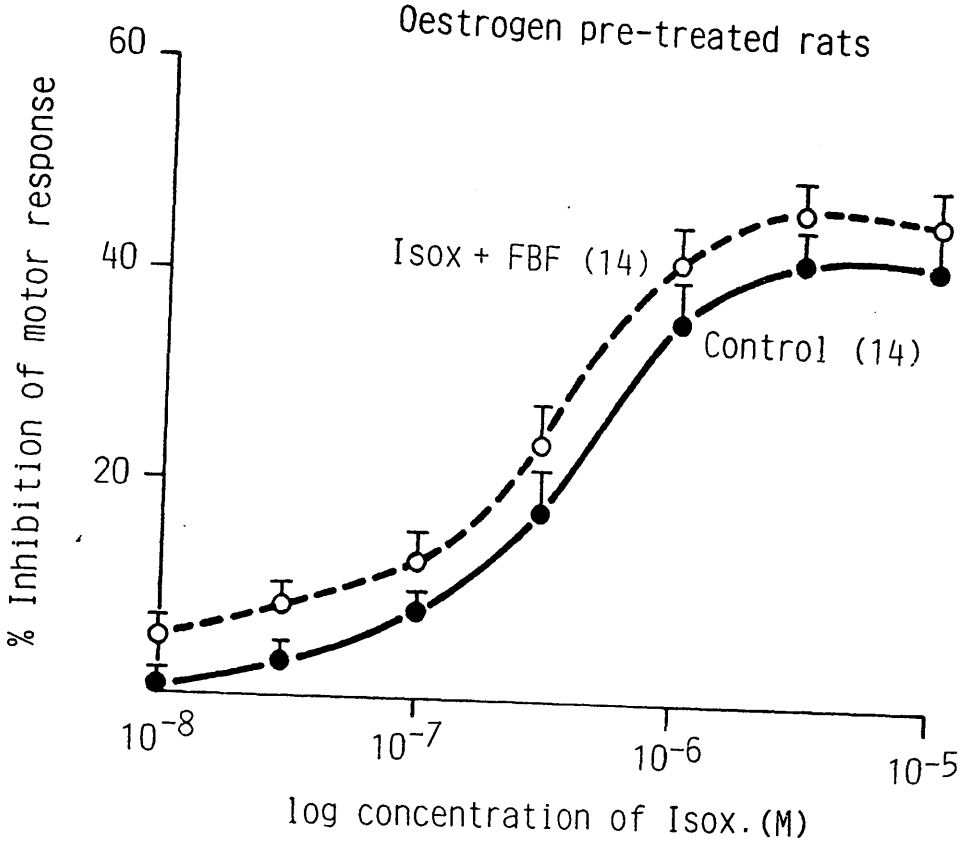


FIGURE 25: Log. concentration response curves to Isoxsuprine in the rat isolated uterus in oestrogen pretreated: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

Table 17:

Effect of Flurbiprofen (FBF, 10^{-5}M) on Sal, Iso and Isx potencies in uteri from oestrogen pretreated animals (pD_2 values).

Drug	Potency (pD_2) Control	After FBF (n)
Salbutamol	8.10 ± 0.43 (6)	8.40 ± 0.14 (6)
Isoprenaline	8.52 ± 0.22 (6)	8.70 ± 0.33 (6)
Isox suprine	6.30 ± 0.55 (6)	6.52 ± 0.47 (6)

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

Isoprenaline:

ISO was of equal potency and effectiveness with SAL in inhibiting Ach. FBF increased significantly ($P < 0.05$) isoprenaline responses, enhancing its maximum degree of inhibition (Figure 24). Compared with the control value, there was no significant difference in isoprenaline pD_2 value in the presence of FBF (Table 17).

Isoxsuprine:

ISOX was significantly less potent and effective than SAL + ISO in inhibiting Ach. FBF caused no significant changes in the ISOX concentration-response curve (Figure 25), and pD_2 value (Table 17). Thus while cyclo-oxygenase inhibition enhanced SAL, and ISO responses in uteri from oestrogen pre-treated animals, there was little or no effect on the isoxsuprine response.

(G) RESPONSES TO A β -ADRENOCEPTOR AGONIST IN THE PRESENCE OF A PHOSPHOLIPASE A_2 INHIBITOR

The possible involvement of intramurally generated prostaglandins in uterine response to β -adrenoceptor agonists was further investigated in a series of experiments in which Quinacrine was used to inhibit phospholipase A_2, the enzyme which releases arachidonic acid from ester pools. Quinacrine (10^{-5} M) had no effect on the motor response to Ach, in uteri from oestrogen pretreated animals. Quinacrine increased the SAL responses significantly ($P < 0.01$) and enhanced its maximum degree of inhibition (Figure 26).

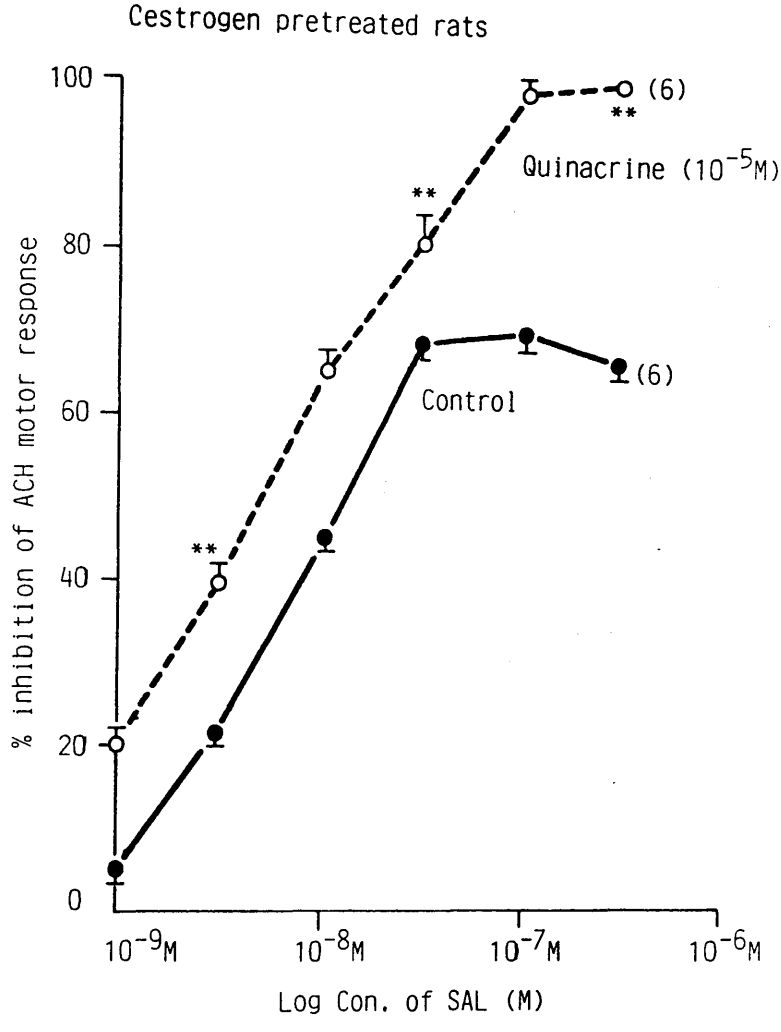


FIGURE 26: Log. concentration response curves to Salbutamol in the rat isolated uterus in oestrogen pretreated: Controls, and in presence of Quinacrine (10^{-5} M). Number of observations in brackets.

pD_2 values obtained in the absence and presence of Quinacrine was 8.16 ± 0.43 (n = 6) and 8.25 ± 0.28 (n = 6) respectively. There was no significant difference in the pD_2 value for SAL in the presence of Quinacrine.

Inhibition of phospholipase A_2 produced effects similar to that observed in the presence of FBF confirming a role for prostaglandins in the adrenoceptor agonist inhibitory response.

(H) RESPONSES TO A β-ADRENOCEPTOR AGONIST IN THE COMBINED PRESENCE OF A CYCLO-OXYGENASE INHIBITOR AND ARACHIDONIC ACID

Since arachidonic acid is the substrate for cyclo-oxygenase, a series of experiments was performed to determine if the effects produced by the cyclo-oxygenase inhibitor, FBF, can be reversed by addition of exogenous arachidonic acid. Arachidonic acid (10^{-5} M) had no effect on Ach-induced contraction in uteri from oestrogen pretreated animals. Arachidonic acid alone was also without effect on the SAL responses. FBF increased significantly ($P < 0.001$) SAL responses and enhanced its maximum degree of inhibition. The shift of the SAL concentration-response curve induced by FBF was unaffected by arachidonic acid (Figure 27).

The effect produced by cyclo-oxygenase inhibition on adrenoceptor agonists responses was not reversed in the presence of arachidonic acid.

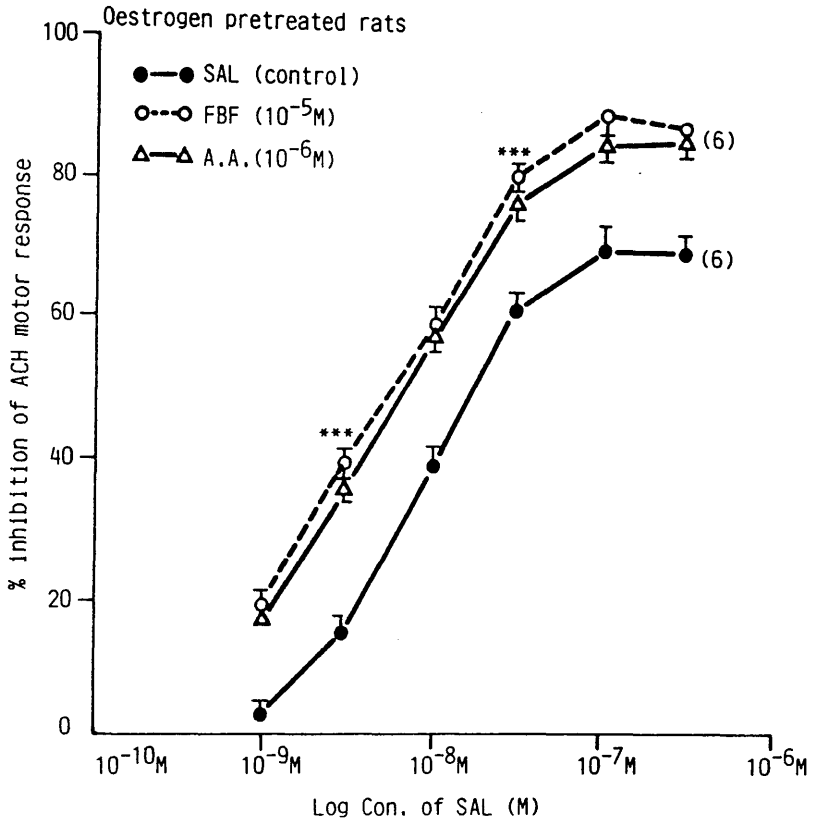


FIGURE 27: Log. concentration response curves to Salbutamol in the rat isolated uterus in oestrogen pretreated: Controls, in presence of (FBF, 10^{-5} M) and then in presence of (FBF, 10^{-5} M), and arachidonic acid (AA 10^{-6} M). Number of observations in brackets.

(I) RESPONSES TO ADRENOCEPTOR AGONISTS IN UTERI FROM 20-DAY PREGNANT ANIMALS

So far, the responses to the adrenoceptor agonists and the effects of cyclo-oxygenase inhibition have been examined in uteri from animals undergoing the natural oestrous cycle and from oestrogen pretreated rats. It was, therefore, of interest to re-examine these effects in uteri from rats in late pregnancy.

(i) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL

ADR (10^{-9} M - 10^{-6} M) and SAL (10^{-9}M - 3 x 10^{-7} M) produced concentration-related inhibition of Ach-induced contraction in both control I and control II studies (Figures 28 and 29). In both Control I and II 20 day pregnant rats, ADR and SAL were more potent, than that in the non-pregnant rats, the pD_2 values were also significantly greater. The agonists were also more effective in inhibiting the responses to ACH. ADR and SAL in Control I produced 60% inhibition, while in Control II ADR produced 80% and SAL 90% respectively. Whereas in the non-pregnant rats the maximum degree of inhibition was 45% achieved in oestrus. For both ADR and SAL, there was a significant increase ($P < 0.01$) in their maximum degrees of inhibition under the control II situation. There was, however, no significant difference between the control I and control II pD_2 values for ADR and SAL (Table 18).

(ii) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL IN THE PRESENCE OF A CYCLO-OXYGENASE INHIBITOR

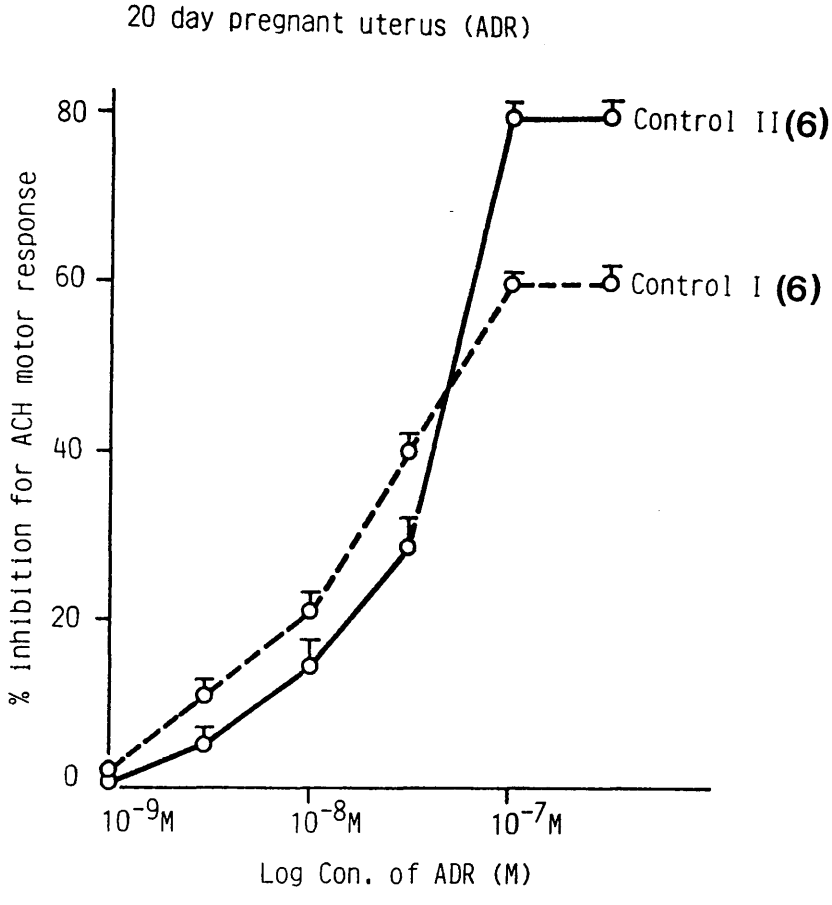


FIGURE 28: Log. concentration response curves to adrenaline in the rat isolated uterus in 20 day pregnant:
Control I = ADR alone. Control II = ADR, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M). Number of observations in brackets.

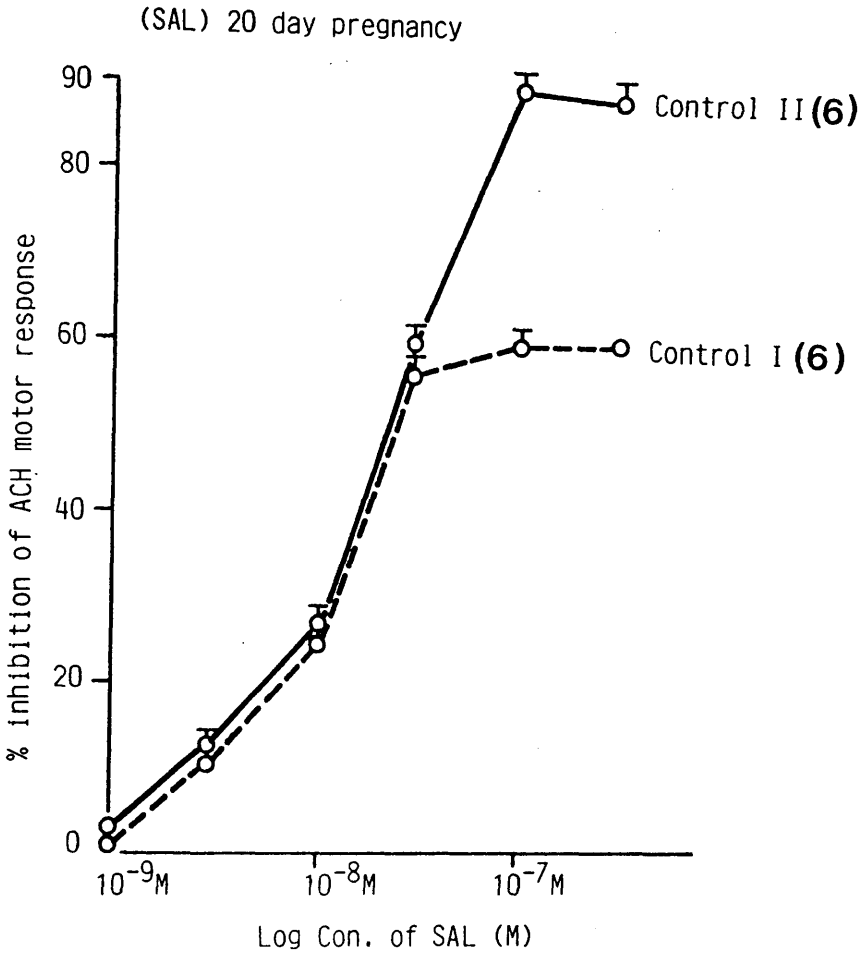


FIGURE 29: Log. concentration response curves to Salbutamol in the rat isolated uterus in 20 day pregnant: Control I = SAL alone; Control II = SAL, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M). Number of observations in brackets.

Table 18:

Adrenaline and Salbutamol potency in uteri from 20-day pregnant animals (pD_2 values).

	Potency (pD_2)	
Experiment	ADR	SAL
Control I	7.70 ± 0.19 (13)	7.82 ± 0.19 (14)
Control II	7.30 ± 0.37 (6)	7.70 ± 0.24 (6)

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

Concentration-response curves to ADR and SAL were obtained in the absence (control I experiments) and presence (control II experiments) of the combined antagonists, and cyclo-oxygenase activity was inhibited by FBF (10^{-5} M). Concentration-response curves and pD_2 values for the agonists in the absence and presence of FBF are shown in Figures 30 to 33, and Table 19 respectively.

ADRENALINE:

In the presence of FBF, the concentration response curve to ADR in control I moved to the left, and there was an increase in the maximum degree of inhibition (Figure 30). However, although there was increased sensitivity to ADR, which reached significance at some concentrations (10^{-8} M and 3 x 10^{-8} M) the pD_2 values for ADR in the absence and presence of FBF did not differ significantly. A similar situation occurred in control II (Figure 31) but FBF produced no increase in the maximum degree of inhibition which had already been increased to 100% by the uptake blockers.

SALBUTAMOL:

FBF shifted SAL concentration curves in both control I and control II experiments to the left. Sensitivity to SAL at certain concentrations was increased significantly, but there was no significant difference in the pD_2 values for SAL in the absence and presence of FBF. As with ADR, the maximum degree of inhibition was increased by FBF only in the control I experiments.

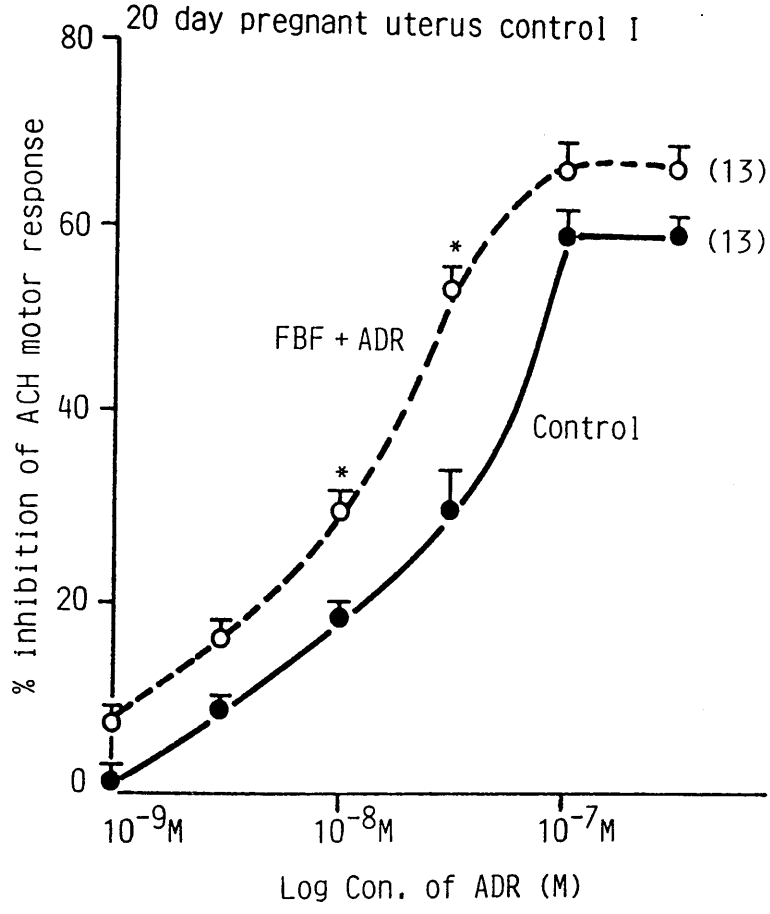


FIGURE 30: Log. concentration response curves to Adrenaline in the rat isolated uterus in 20 day pregnant: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

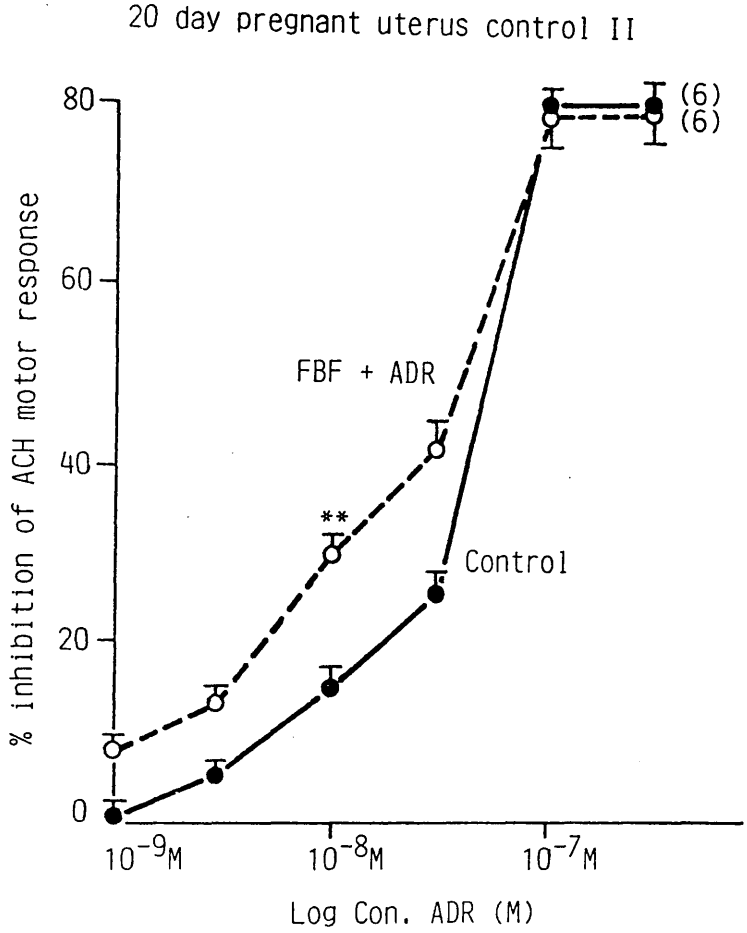


FIGURE 31: Log. concentration response curves to Adrenaline in the rat isolated uterus in 20 day pregnant:
Control II = ADR, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M): in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

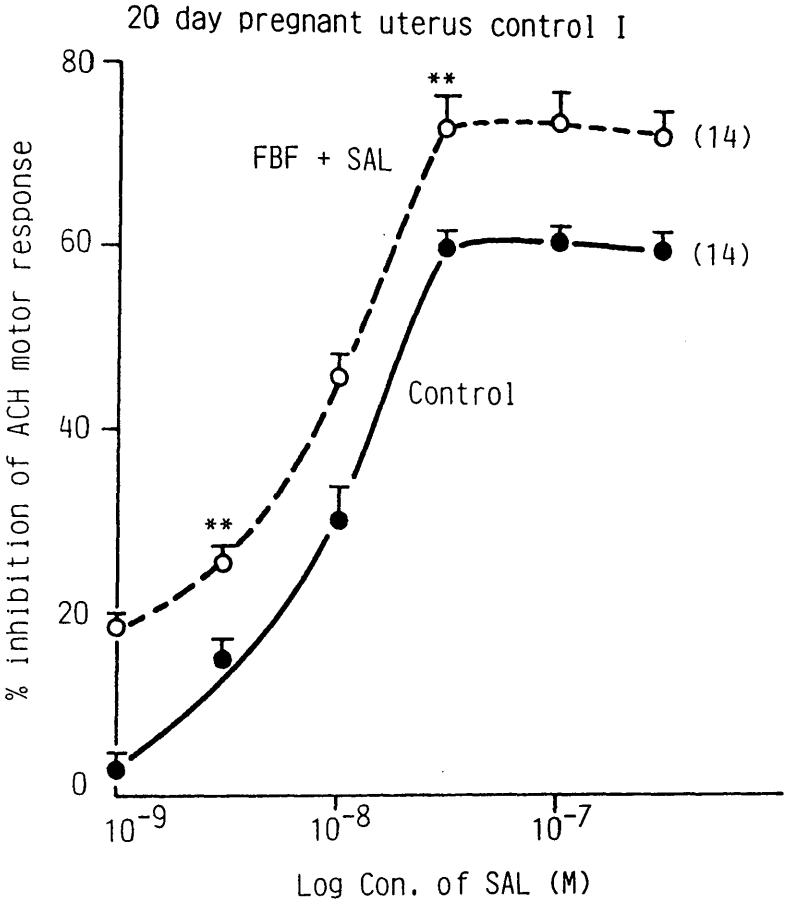


FIGURE 32: Log. concentration response curves to Salbutamol in the rat isolated uterus in 20 day pregnant: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

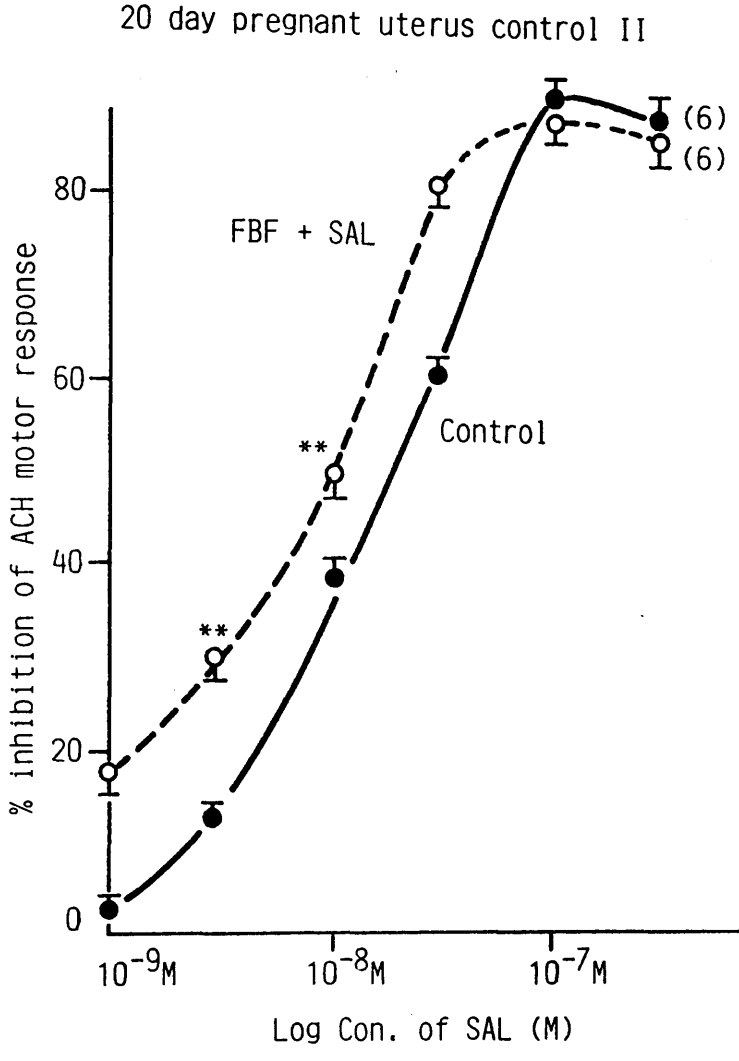


FIGURE 33: Log. concentration response curves to Salbutamol in the rat isolated uterus in 20 day pregnant: Control II = SAL in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M); in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M and FBF (10^{-5} M). Number of observations in brackets.

Table 19:

Effect of Flurbiprofen (FBF, 10^{-5}M) on Adr and Sal potency in uteri from 20-day pregnant animals (pD_2 values).

Drug	Experiment	Potency (pD_2)	After FBF
Adr	Control I	7.52 ± 0.19 (13)	7.82 ± 0.13 (13)
	Control II	7.40 ± 0.37 (6)	7.60 ± 0.16 (6)
Sal	Control I	8.00 ± 0.19 (14)	8.10 ± 0.19 (14)
	Control II	7.70 ± 0.24 (6)	8.10 ± 0.30 (6)

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

FBF enhanced the activity of the adrenoceptor agonists in late pregnancy as it did in preparations from non-pregnant and oestrogen pretreated animals but the extent of the effect was less in the pregnant animals.

(J) RESPONSES TO ADRENOCEPTOR AGONISTS IN UTERI FROM ONE-DAY POST-PARTUM ANIMALS

The possible involvement of intramurally generated prostaglandins in uterine response to adrenoceptor agonists was also investigated in post-partum animals.

(i) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL

ADR (10^{-9} M - 10^{-6} M) and SAL (10^{-9} M - 3×10^{-7} M) produced concentration-dependent inhibition of Ach-induced contraction in both control I and control II studies (Figures 34 and 35). In one day post partum rats, both ADR and SAL in Control I produced 90% inhibition, while in Control II ADR was less potent than SAL compared to non-pregnant and pregnant rats. In the non pregnant rats the maximum degree of inhibition was around 45% in oestrus, while in 20 day pregnant rats, ADR and SAL in Control I produced 60% inhibition, while in Control II, ADR produced 80% and SAL 90% inhibition respectively. There were no significant differences between control I and control II concentration-response for ADR and SAL and in their pD_2 values (Table 20).

(ii) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL IN

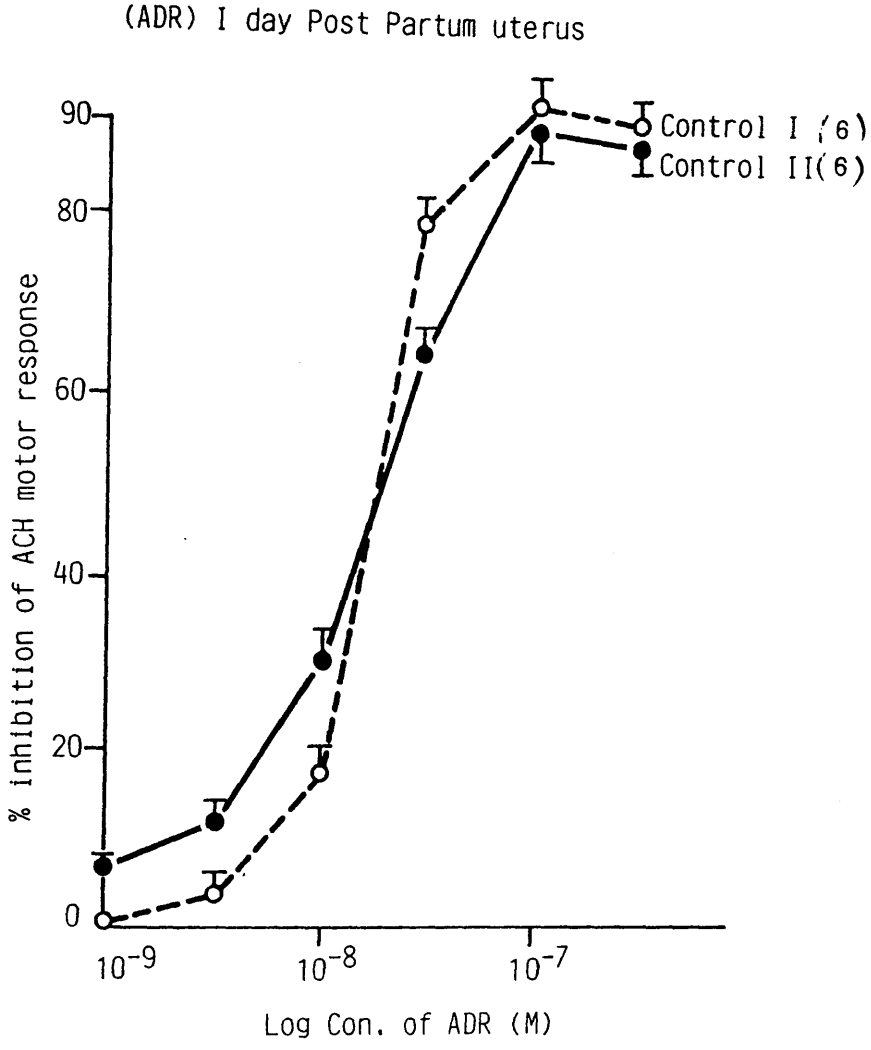


FIGURE 34: Log. concentration response curves to adrenaline in the rat isolated uterus in 1 day post partum: Control I = ADR, alone: and Control II = ADR, in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

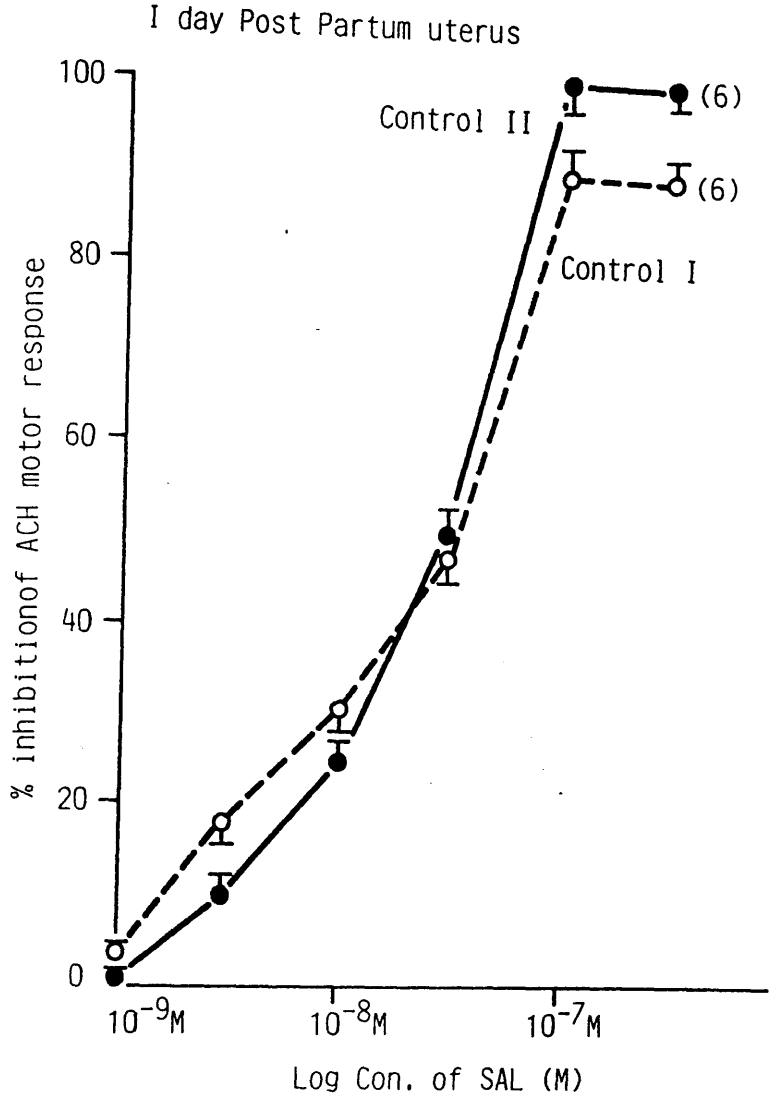


FIGURE 35: Log. concentration response curves to Salbutamol in the rat isolated uterus in 1 day post partum: Control I = SAL, alone: and Control II = SAL, in presence of AZA with DMI and NMN (all at 10^{-6} M). Number of observations in brackets.

Table 20:

Adrenaline and Salbutamol potency in uteri from one-day post partum animals (pD_2 values).

Experiment	Potency (pD_2) ADR	SAL
Control I	7.70 ± 0.03 (6)	7.60 ± 0.07 (6)
Control II	7.70 ± 0.21 (6)	7.52 ± 0.15 (4)

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

THE PRESENCE OF A CYCLO-OXYGENASE INHIBITOR

Concentration-response curves to ADR and SAL were obtained in the absence (control I experiments) and presence (control II experiments) of the combined antagonists, and cyclo-oxygenase activity was inhibited by FBF (10^{-5} M). Concentration response curves and pD_2 values for the agonists in the absence and presence of FBF are shown in Figures 36 to 39, and Table 21, respectively.

ADRENALINE:

FBF did not produce any change in the concentration curve for ADR. ADR alone produced 100% inhibition and so no further increase was possible with FBF.

SALBUTAMOL:

FBF shifted both control I and control II concentration-response curves to the left (Figures 38 and 39). In control I experiments, the shift was significant and there was a significant increase ($P < 0.001$) in the pD_2 value for SAL, in the presence of FBF (Table 21). In contrast, there was no significant difference in the control II pD_2 value for SAL (Table 21).

As with uteri from pregnant animals, cyclo-oxygenase inhibition also enhanced SAL responses in preparations from post-partum animals. However, cyclo-oxygenase inhibition did not affect ADR inhibitory responses.

(K) RESPONSES TO ADRENOCEPTOR AGONISTS IN ENDOMETRIUM-FREE UTERI FROM 20-DAY PREGNANT ANIMALS

The experiments described so far have been on

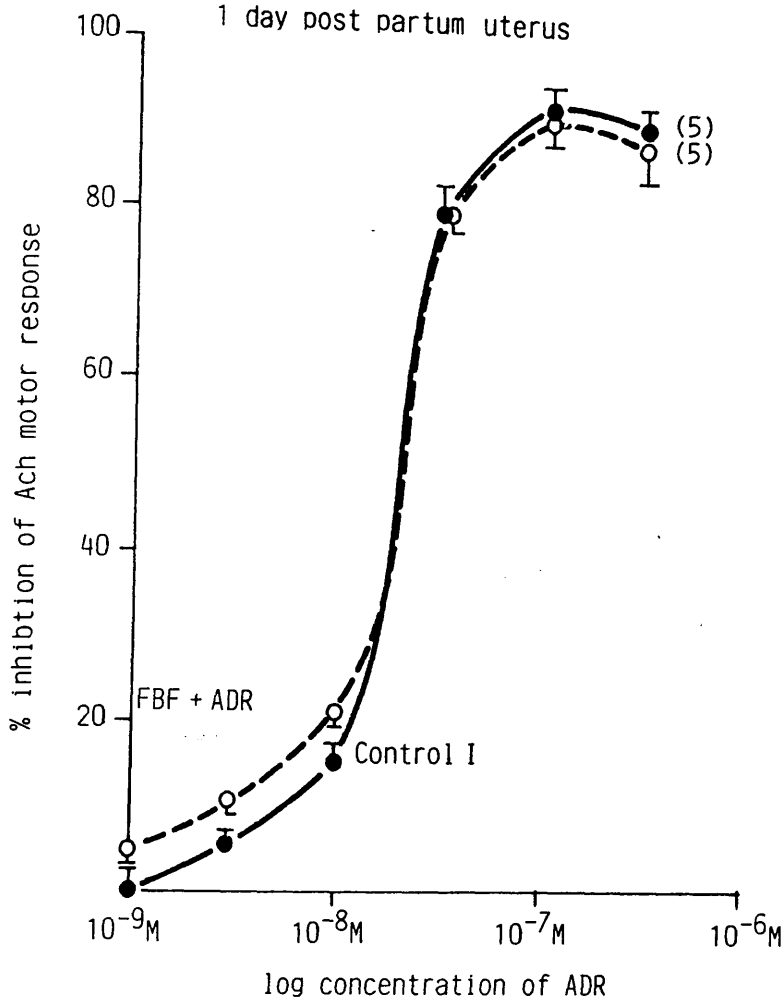


FIGURE 36: Log. concentration response curves to adrenaline in the rat isolated uterus in 1 day post partum: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

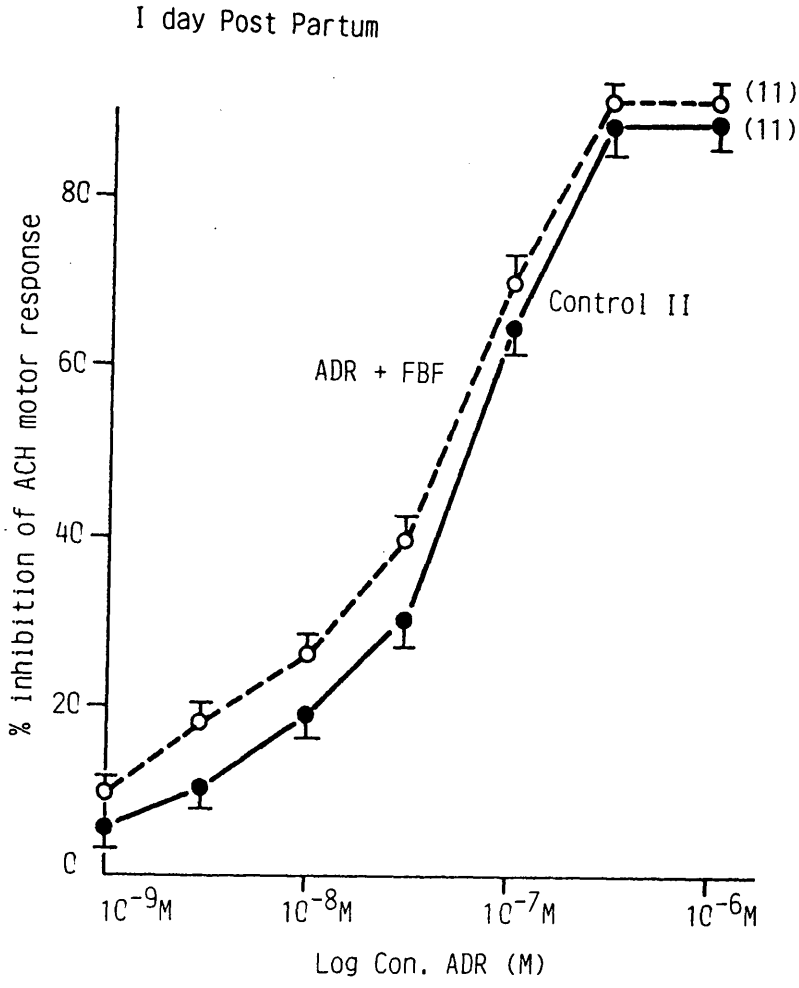


FIGURE 37: Log. concentration response curves to adrenaline in the rat isolated uterus in 1 day post partum: Control II = ADR, in presence of AZA (10^{-6} M), DMI (10^{-6} M), and NMN (10^{-6} M); in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

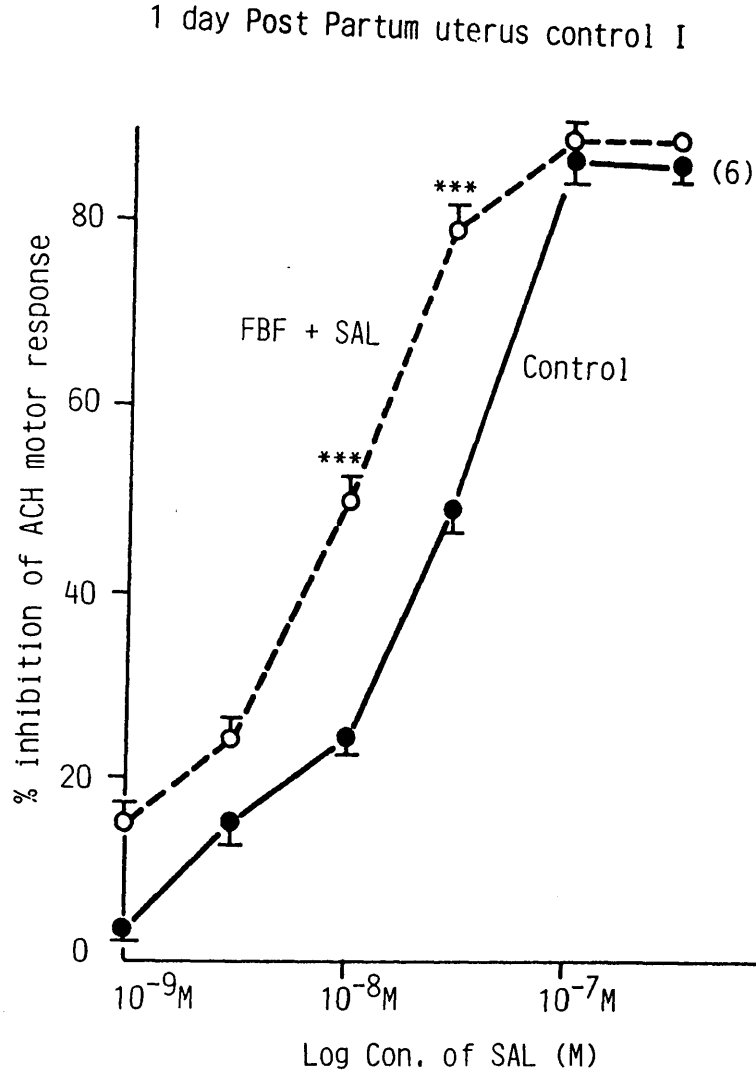


FIGURE 38: Log. concentration response curves to Salbutamol in the rat isolated uterus in 1 day post partum: Controls, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

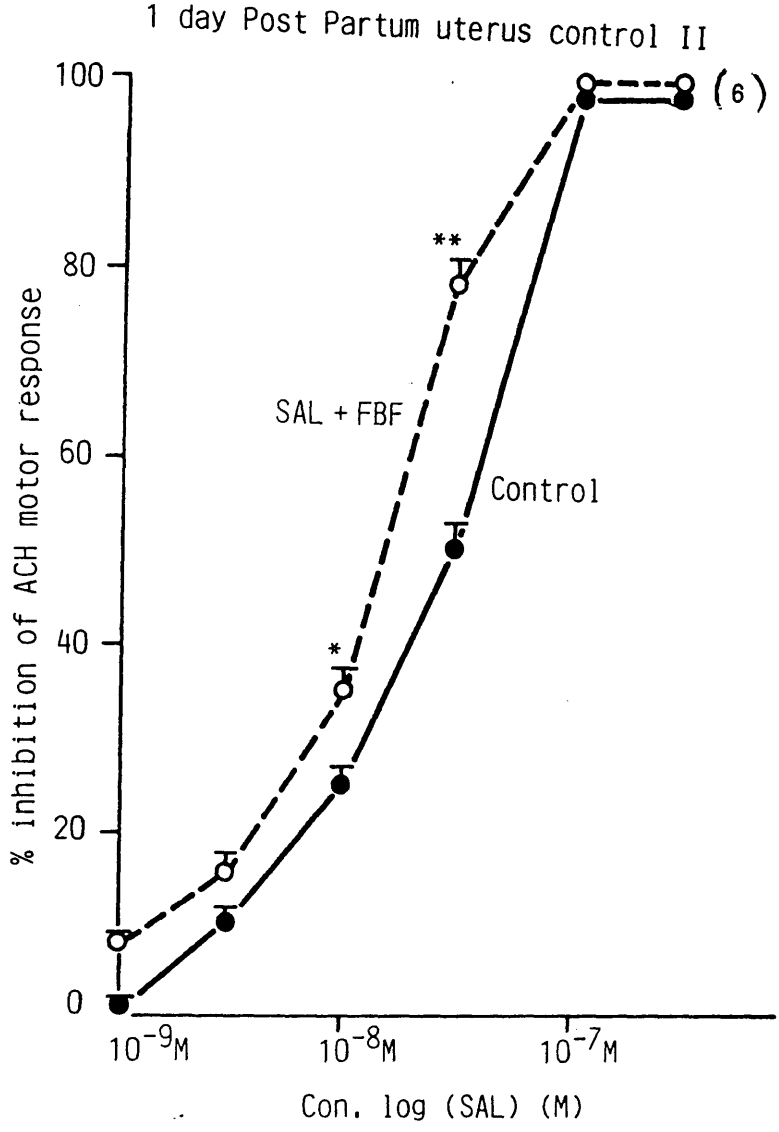


FIGURE 39: Log. concentration response curves to Salbutamol in the rat isolated uterus in 1 day post partum: Control II = SAL, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M): in presence of AZA (10^{-6}M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

Table 21:

Effect of Flurbiprofen (FBF, 10^{-5}M) on Adr and Sal potencies in uteri from one-day post partum animals (pD_2 values).

Drug	Potency (pD_2) Experiment		After FBF
Adr	Control I	7.70 ± 0.03 (6)	7.70 ± 0.05 (6)
	Control II	7.22 ± 0.21 (6)	7.30 ± 0.03 (6)
Sal	Control I	7.60 ± 0.07 (6)	8.50 ± 0.28 (6)***
	Control II	7.52 ± 0.15 (4)	7.82 ± 0.10 (4)

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

preparations in which the myometrial and endometrial layers were intact. The possibility that the endometrial layer may play a role in uterine response to adrenoceptor agonists was, therefore, investigated in a series of experiments, in which the endometrium was removed. Both control I and control II studies were performed for ADR and SAL in the absence and presence of the cyclo-oxygenase inhibitor FBF (10^{-5} M).

(i) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL IN ENDOMETRIUM FREE UTERI

Concentration-response curves and pD_2 values for the agonists in the absence and presence of endometrium are shown in Figures 40 and 41 and Table 22, respectively.

ADRENALINE:

Removal of the endometrium produced no change in the concentration response curve to ADR in control I experiments (Figure 40).

SALBUTAMOL:

In the absence of endometrium in control I experiments, there was a significant shift ($P < 0.05$) in the SAL concentration-response curve to the right at the higher concentrations (Figure 41). Compared with values obtained in whole uteri, there was no significant difference in SAL pD_2 values in the absence of endometrium (Table 22).

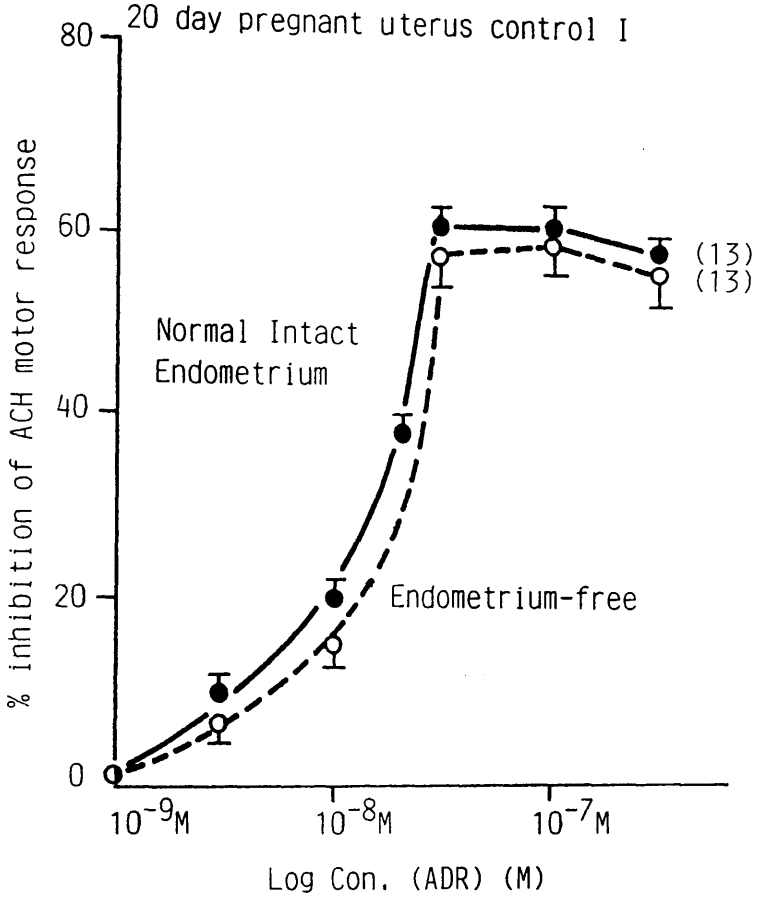


FIGURE 40: Log. concentration response curves to adrenaline in the rat isolated uterus in 20 day pregnant: Controls, with and without endometrium. Number of observations in brackets.

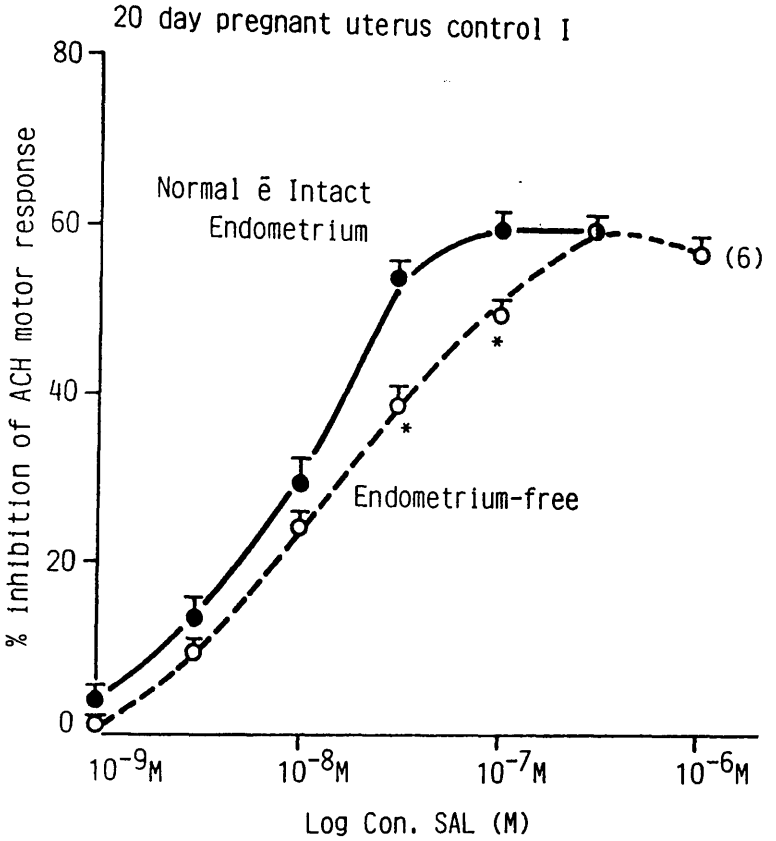


FIGURE 41: Log. concentration response curves to Salbutamol in the rat isolated uterus in 20 day pregnant: Controls, with and without endometrium. Number of observations in brackets.

Table 22:

Effect of endometrium removal on Adr and Sal potencies in uteri from 20-day pregnant animals (pD_2 values).

State of uterus	Potency (pD_2)	
	ADR	SAL
Normal	7.75 ± 0.19 (13)	8.00 ± 0.19 (14)
Endometrium-free	7.52 ± 0.34 (6)	7.82 ± 0.19 (6)

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

(ii) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL IN THE PRESENCE OF A CYCLO-OXYGENASE INHIBITOR

Concentration-response curves and pD_2 values for the agonists in the absence and presence of FBF are shown in Figures 42 to 45, and Table 23, respectively.

ADRENALINE:

FBF had no effect on control I or control II concentration-response curves to ADR in endometrium-free preparations (Figures 42 and 43). In control II experiments, before addition of FBF, ADR produced a complete inhibition of the Ach motor response. Compared with control values, there were no significant differences in both control I and control II pD_2 values for ADR, in the presence of FBF (Table 23).

SALBUTAMOL:

FBF had no effect on the SAL concentration response curves in control I experiments (Figure 44). In control II experiments FBF significantly increased the sensitivity to SAL at low concentration (Figure 45), but there was no significant change in the pD_2 value (Table 23). FBF did not alter the degree of inhibition produced by SAL which was already 100%.

In summary, endometrium removal caused slight changes in ADR and SAL responses in uteri from 20-day pregnant animals. With the exception of SAL in control II experiments, cyclo-oxygenase inhibition had no effect on adrenoceptor agonist responses in uteri under this

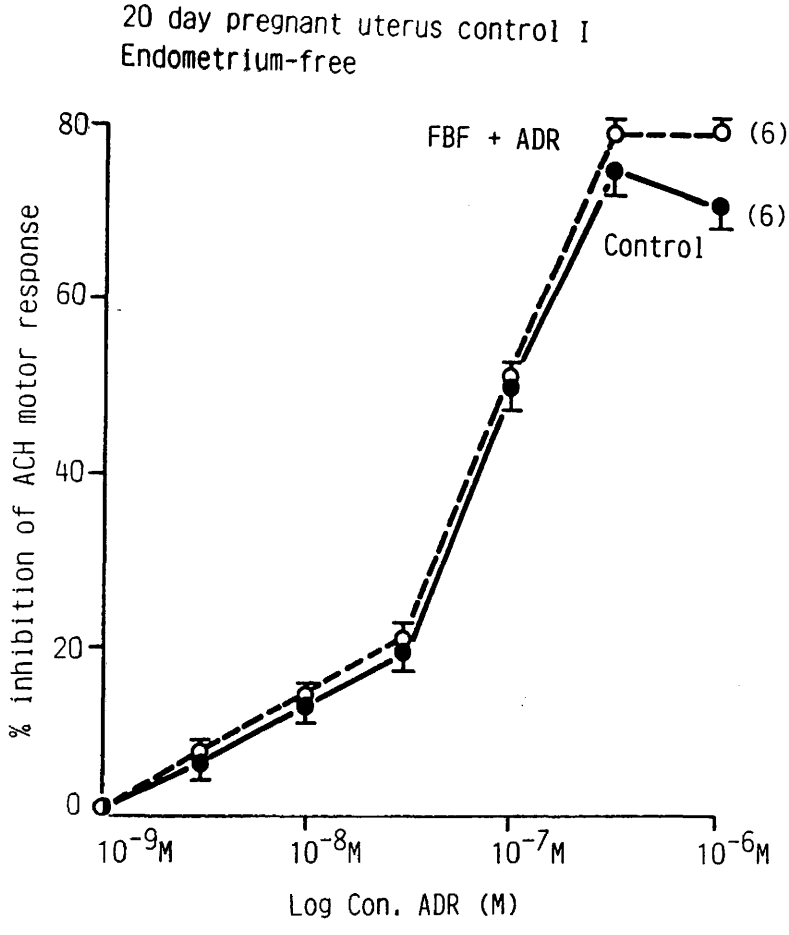


FIGURE 42: Log. concentration response curves to adrenaline in the rat isolated uterus in 20 day pregnant: Controls endometrium free, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

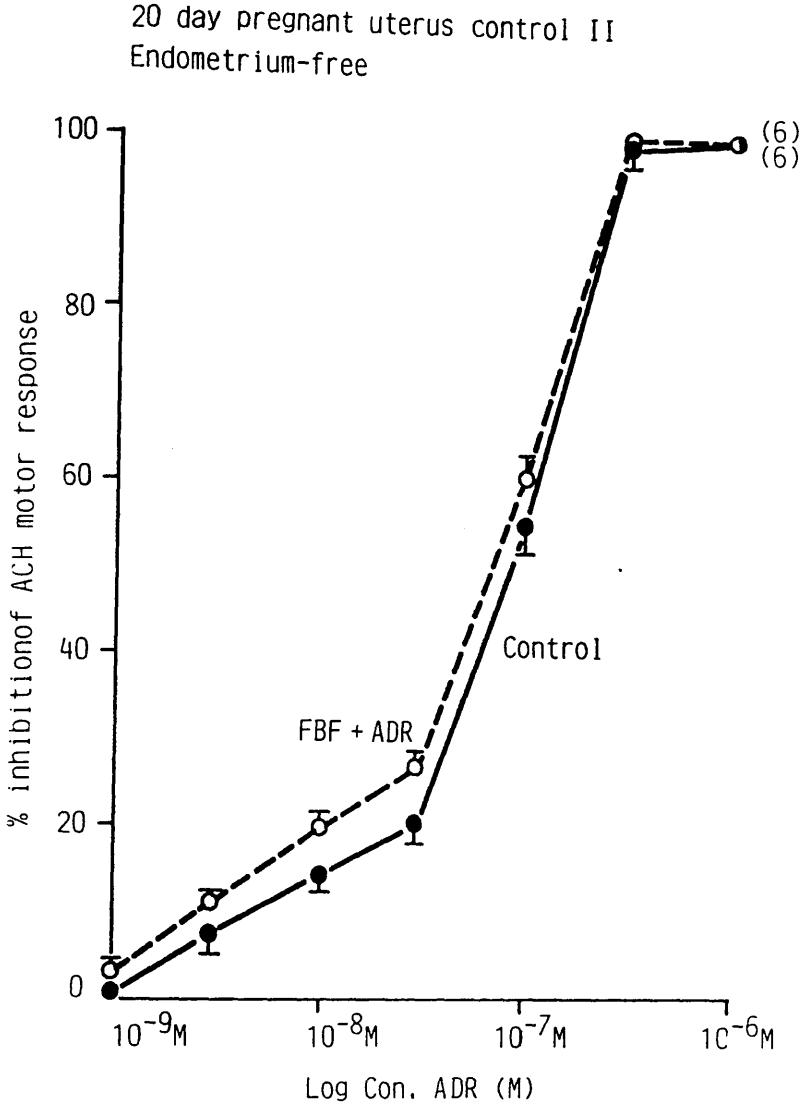


FIGURE 43: Log. concentration response curves to adrenaline in the rat isolated uterus in 20 day pregnant endometrium free, Control II = ADR, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M); in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

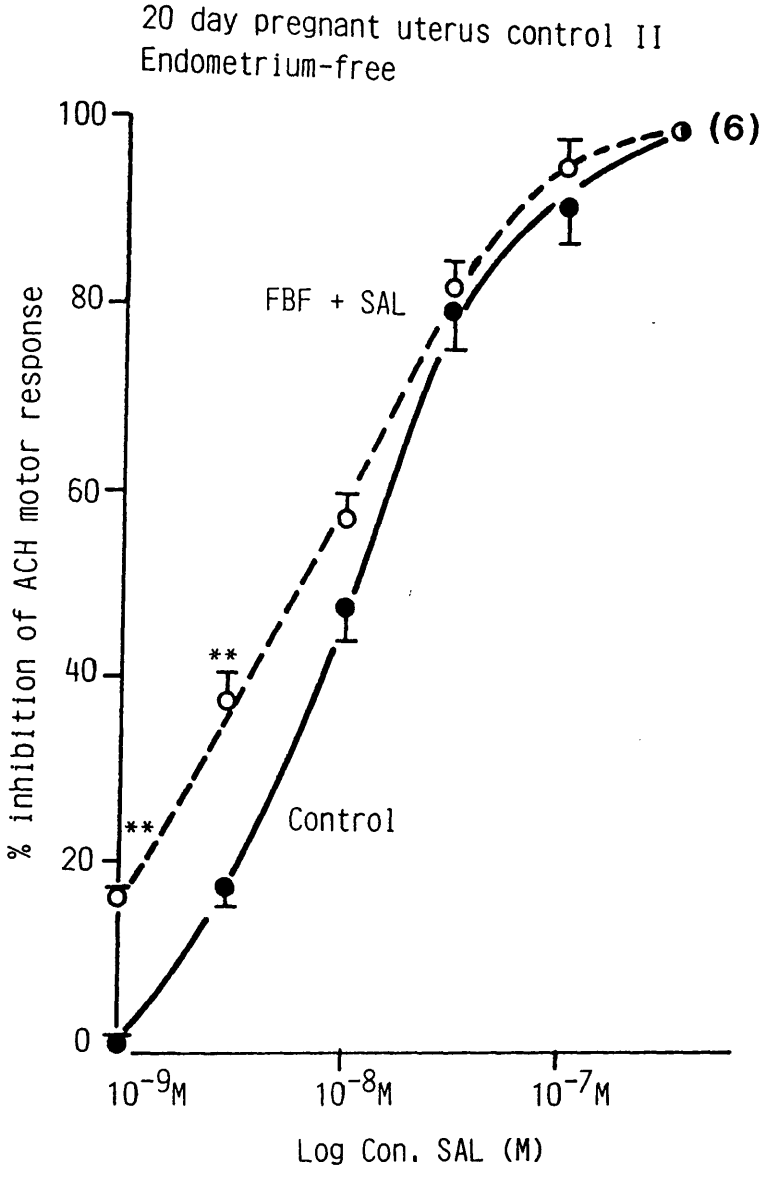


FIGURE 44: Log. concentration response curves to Salbutamol in the rat isolated uterus in 20 day pregnant endometrium free, Control II = SAL, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M): in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

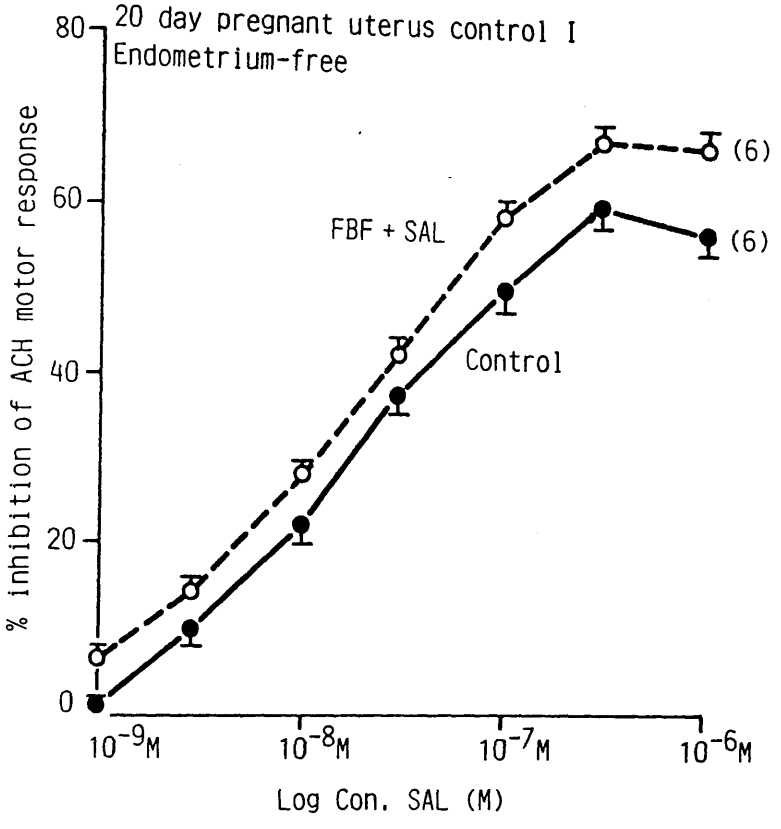


FIGURE 45: Log. concentration response curves to Salbutamol in the rat isolated uterus in 20 day pregnant: Controls, endometrium free, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

Table 23:

Effect of Flurbiprofen (FBF, 10^{-5}M) on Adr and Sal potencies in endometrium-free uteri from 20-day pregnant animals (pD_2 values).

Drug	Experiment	Potency (pD_2)	After FBF
Adr	Control I	7.10 ± 0.34 (6)	7.10 ± 0.33 (6)
	Control II	7.00 ± 0.28 (6)	7.10 ± 0.34 (6)
Sal	Control I	7.70 ± 0.19 (6)	7.75 ± 0.09 (6)
	Control II	7.92 ± 0.34 (6)	8.16 ± 0.30 (6)

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

condition.

(L) RESPONSES TO ADRENOCEPTOR AGONISTS IN ENDOMETRIUM-FREE UTERI FROM ONE-DAY POST-PARTUM ANIMALS

As with the studies on pregnant animals with endometrium-free uterine preparations, a corresponding series of experiments was performed in uteri from one-day post-partum rats. Both control I and control II experiments were carried out for ADR and SAL, in the absence and presence of the cyclo-oxygenase inhibitor, FBF (10^{-5} M).

(i) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL

Concentration-response curves and pD_2 values for the agonists in the absence and presence of endometrium are shown in Figures 46 and 47, and Table 24, respectively.

ADRENALINE:

In the absence of endometrium, there was a shift in ADR concentration-response curves to the right and a significant decrease ($P < 0.001$) in the maximum degree of inhibition (Figure 46). Compared with values obtained in whole uteri, there was a significant decrease ($P < 0.05$) in ADR pD_2 value, in the absence of endometrium (Table 24).

SALBUTAMOL:

In the absence of endometrium, there was a significant shift ($P < 0.001$) in SAL concentration-response curves to the right (Figure 47) but the degree of inhibition was not reduced. Compared with values obtained in whole uteri,

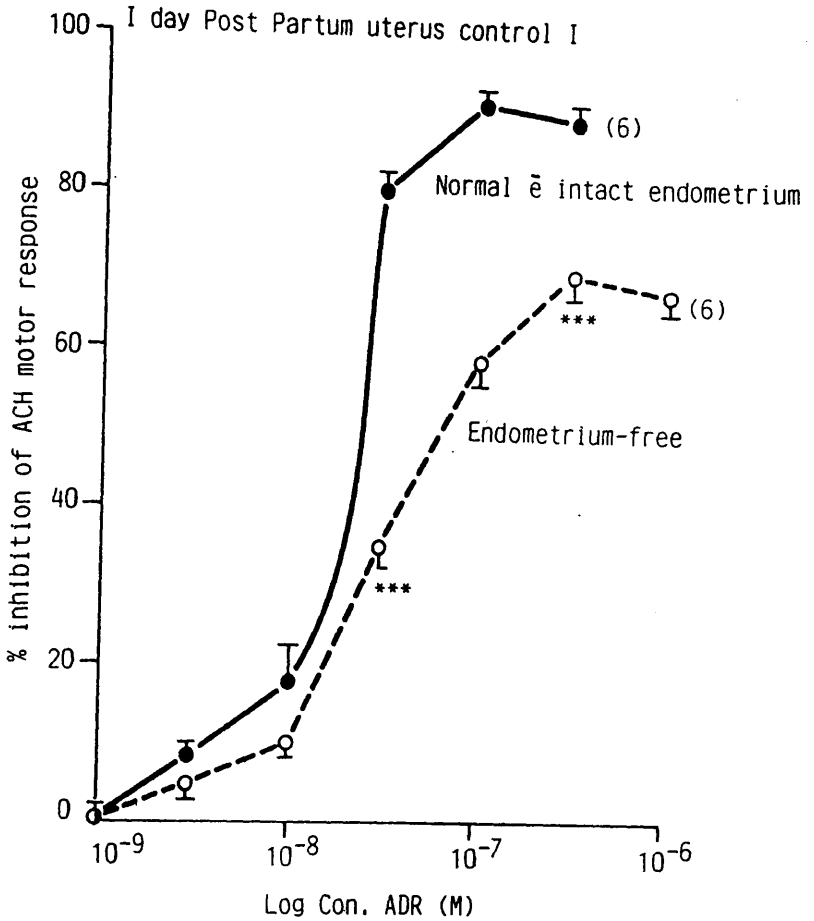


FIGURE 46: Log. concentration response curves to Adrenaline in the rat isolated uterus in 1 day post partum: Controls, with and without endometrium. Number of observations in brackets.

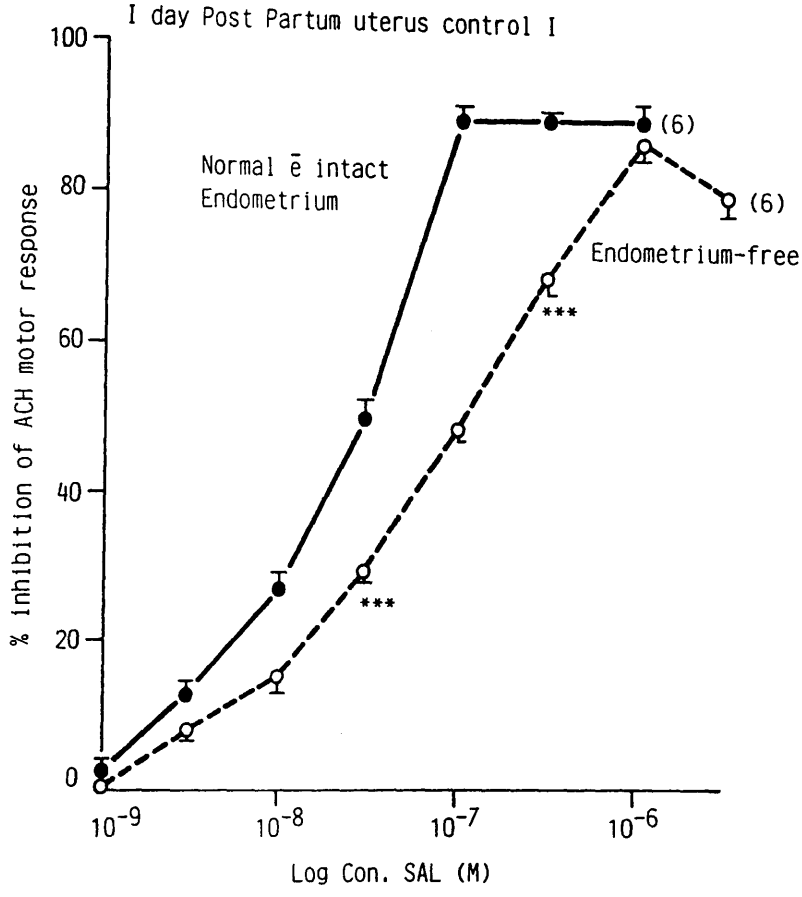


FIGURE 47: Log. concentration response curves to Salbutamol in the rat isolated uterus in 1 day post partum: Controls, with and without endometrium. Number of observations in brackets.

Table 24:

Effect of endometrium removal on Adr and Sal potencies in uteri from one-day post partum animals (pD_2 values).

State of uterus	Potency (pD_2)	
	ADR	SAL
Normal	7.70 ± 0.03 (6)	7.30 ± 0.07 (6)
Endometrium-free	7.02 ± 0.34 (6)*	7.60 ± 0.34 (6)*

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

there was a significant ($P < 0.05$) decrease in SAL pD_2 values, in the absence of endometrium (Table 24).

(ii) INHIBITORY RESPONSES TO ADRENALINE AND SALBUTAMOL IN THE PRESENCE OF A CYCLO-OXYGENASE INHIBITOR

Concentration-response curves and pD_2 values for the agonists in the absence and presence of FBF are shown in Figures 48 to 51, and Table 25, respectively.

ADRENALINE:

FBF had no effect on control I or control II concentration-response curves to ADR in endometrium-free preparations (Figures 48 and 49). Compared with control values, there were no significant differences in control I and control II pD_2 values for ADR in the presence of FBF (Table 25).

SALBUTAMOL:

FBF had no effect on control I and control II concentration-response curves to SAL in endometrium-free preparations (Figures 50 and 51). In control II experiments, SAL produced a complete inhibition of the Ach-induced contraction. Compared with control values, there were no significant differences in control I and control II pD_2 values for SAL, in the presence of FBF (Table 25).

Endometrium-removal produced a right-ward shift in ADR and SAL concentration-response curves, and decreased the maximum degree of inhibition (ADR only).

Cyclo-oxygenase inhibition had no effect on ADR and SAL responses in control I and control II experiments in

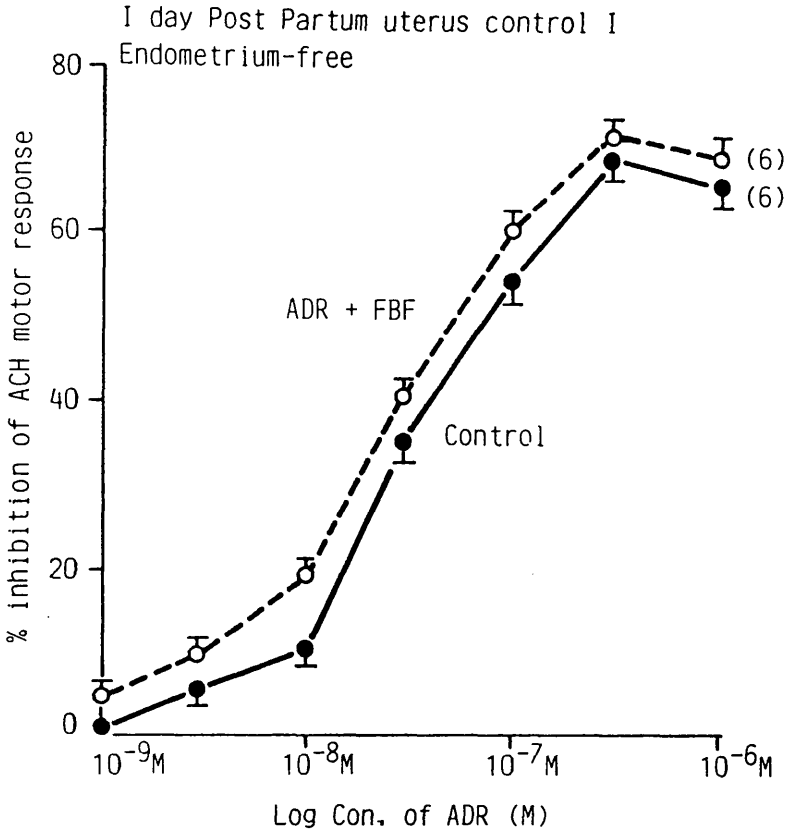


FIGURE 48: Log. concentration response curves to Adrenaline in the rat isolated uterus in 1 day post partum: Controls, endometrium free, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

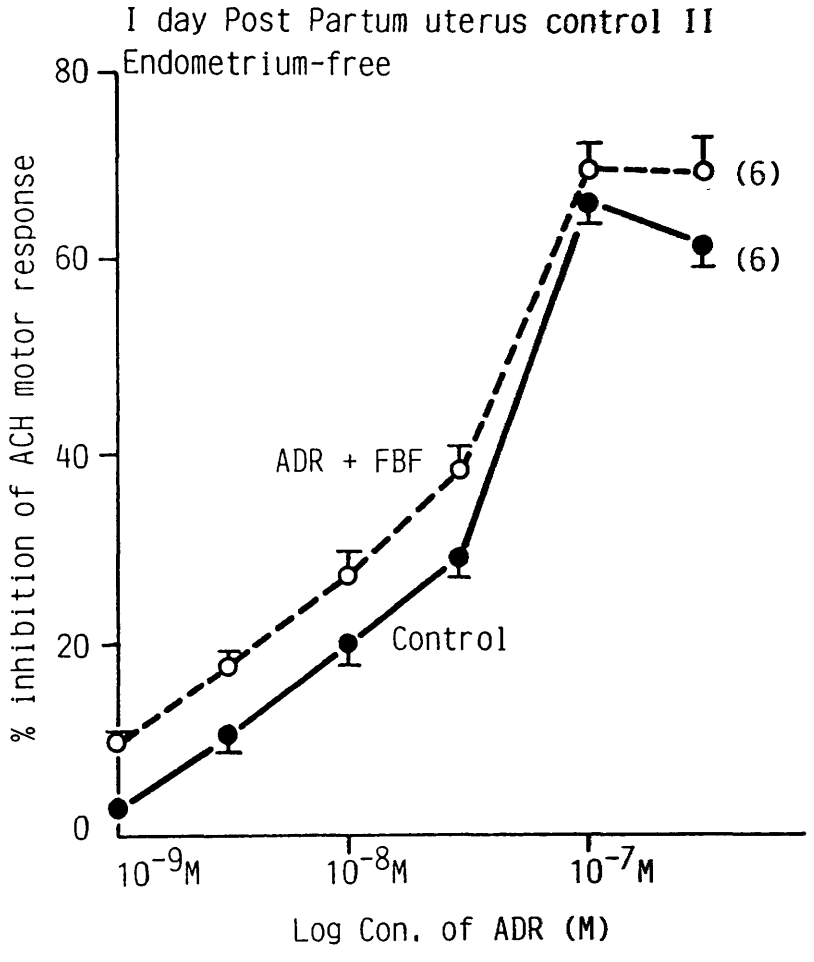


FIGURE 49: Log. concentration response curves to Adrenaline in the rat isolated uterus in 1 day post partum endometrium free, Control II = ADR, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M): in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

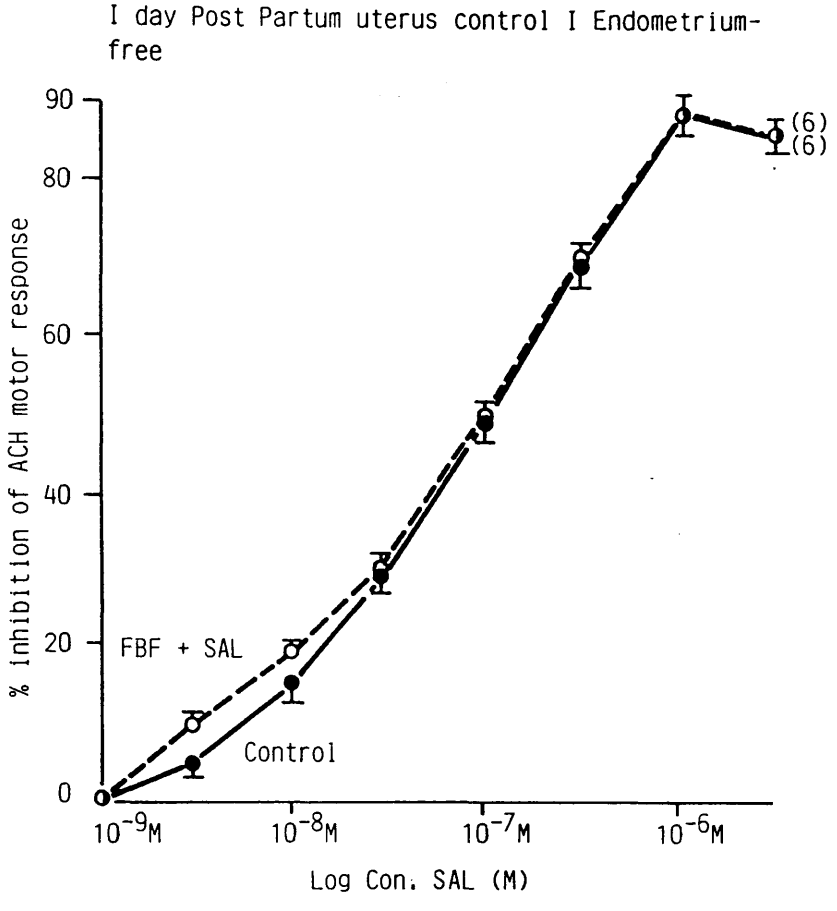


FIGURE 50: Log. concentration response curves to Salbutamol in the rat isolated uterus in 1 day post partum: Controls endometrium free, and in presence of flurbiprofen (FBF, 10^{-5} M). Number of observations in brackets.

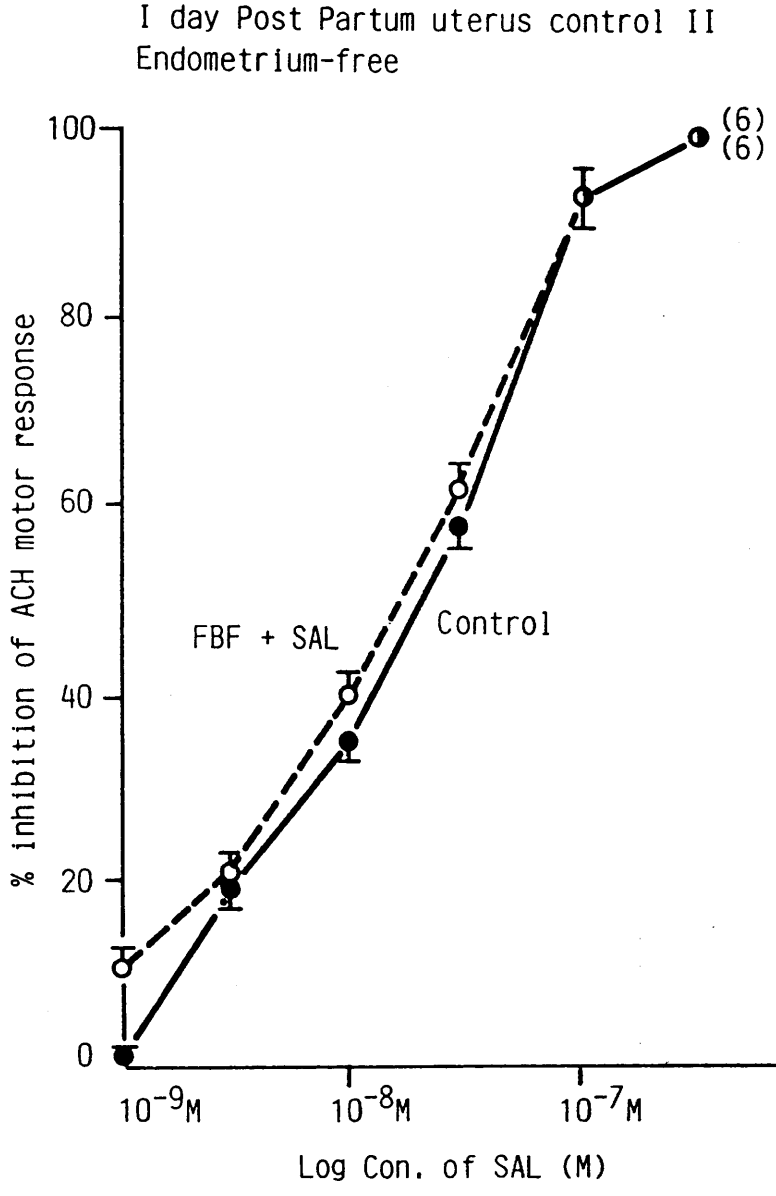


FIGURE 51: Log. concentration response curves to Salbutamol in the rat isolated uterus in 1 day post partum endometrium free, Control II = SAL, in presence of AZA (10^{-6} M), DMI (10^{-6} M) and NMN (10^{-6} M): in presence of AZA (10^{-6} M), DMI (10^{-6} M), NMN (10^{-6} M) and FBF (10^{-5} M). Number of observations in brackets.

Table 25:

Effect of Flurbiprofen (FBF, 10^{-5}M) on Adr and Sal potencies, in endometrium-free uteri from one-day post partum animals (pD_2 values).

Drug	Experiment	Potency (pD_2)	After FBF
Adr	Control I	7.52 ± 0.34 (6)	7.70 ± 0.33 (6)
	Control II	7.40 ± 0.21 (6)	7.70 ± 0.03 (6)
Sal	Control I	7.10 ± 0.34 (6)	7.10 ± 0.38 (6)
	Control II	7.70 ± 0.09 (6)	7.77 ± 0.08 (6)

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

uteri from which the endometrium had been removed.

(M) ADENOSINE 3'5' CYCLIC MONOPHOSPHATE ASSAY EXPERIMENTS IN UTERI FROM NON-PREGNANT AND PREGNANT ANIMALS

There is evidence that both the ovarian hormones and prostaglandins can alter cAMP metabolism in the rat uterus (Kishikawa, 1981; Krall et al., 1984). Since the results obtained so far indicate that adrenoceptor agonists may affect PG formation in uteri from non-pregnant and pregnant animals, the contribution of cAMP to the effects produced by a β -adrenoceptor agonist, SAL, was investigated in a series of experiments.

Basal and SAL-stimulated cAMP levels were measured in preparations from the four phases of the oestrous cycle and from 20-day pregnant animals (with and without endometrium).

(i) cAMP LEVELS IN UTERI FROM THE FOUR PHASES OF THE OESTROUS CYCLE

BASAL cAMP LEVELS

The basal cAMP levels in uteri from proestrus, oestrus, metoestrus and dioestrus are shown in Figure 52. There were no statistically significant differences in basal cAMP content in the four phases, which lay in the range 100-150 pmol/g wet weight.

EFFECT OF SAL ON cAMP LEVELS:

The effects of SAL on cAMP level in uteri from the

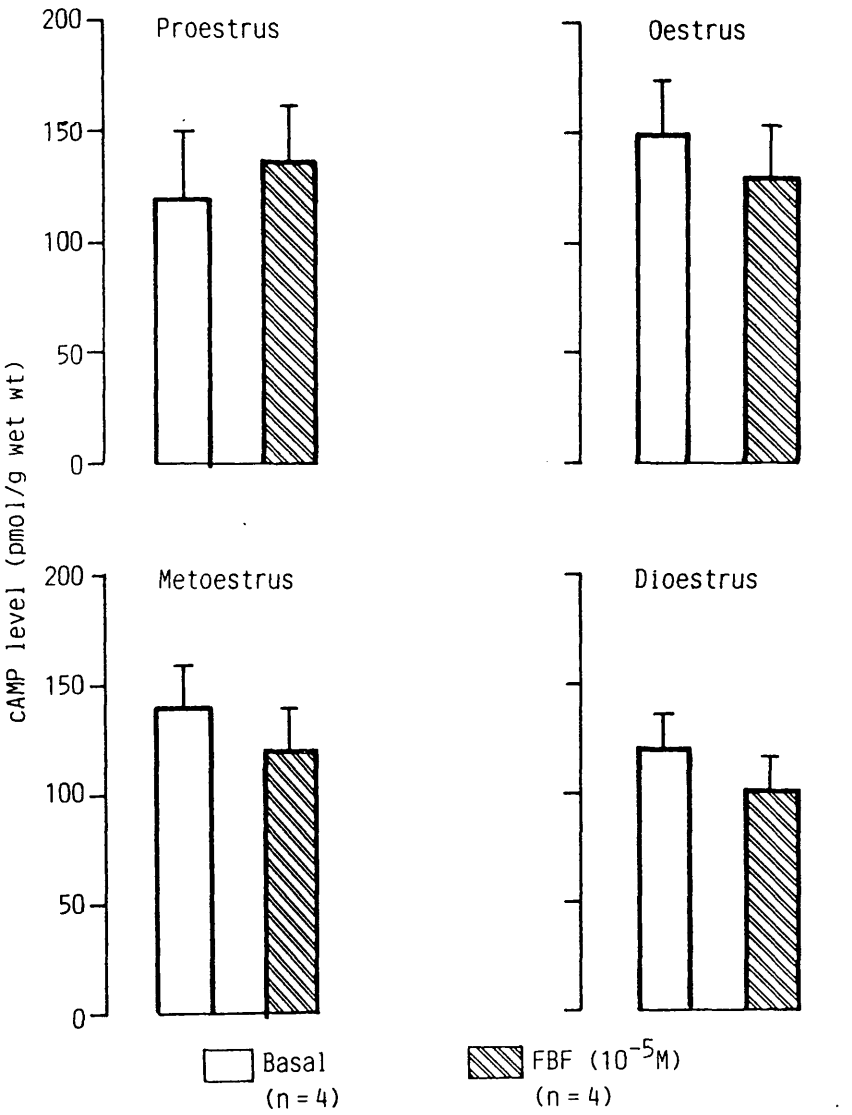


FIGURE 52: Effect of flurbiprofen (FBF) on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in proestrus, oestrus, metoestrus and dioestrus. Open columns = controls; hatched columns = in presence of FBF (10^{-5} M). Number of observations in brackets.

four phases of the oestrous cycle are shown in Figures 53 to 56. SAL (10^{-9} M - 10^{-5} M) produced a concentration-dependent increase in uterine cAMP content over the basal levels in proestrus (Figure 53), oestrus (Figure 54), metoestrus (Figure 55) and dioestrus (Figure 56).

EFFECT OF A CYCLO-OXYGENASE INHIBITION ON cAMP LEVELS

Prostaglandins have been shown to alter cAMP metabolism in the rat uterus (Krall et al., 1984). Thus the effect of cyclo-oxygenase inhibition on basal cAMP levels was examined in a series of experiments. The effects of FBF (10^{-5} M) on basal cAMP levels are shown in Figure 52. FBF did not change the basal levels of cAMP. There were no significant differences in cAMP content in the presence of FBF in any phase of the cycle.

EFFECT OF SAL ON cAMP LEVELS IN UTERI PRETREATED WITH A CYCLO-OXYGENASE INHIBITOR

In a series of experiments, the effect of SAL (10^{-9} M - 10^{-5} M) was examined in preparations pretreated with FBF (10^{-5} M). In the presence of FBF, SAL produced concentration-dependent increases in uterine cAMP content. In all phases the increases were not different from those in which prostaglandin formation had not been inhibited.

Basal cAMP levels and the increases induced by SAL were similar in all four phases of the oestrous cycle. Cyclo-oxygenase inhibition had no effect on basal cAMP levels, nor on the ability of SAL to increase tissue cAMP content.

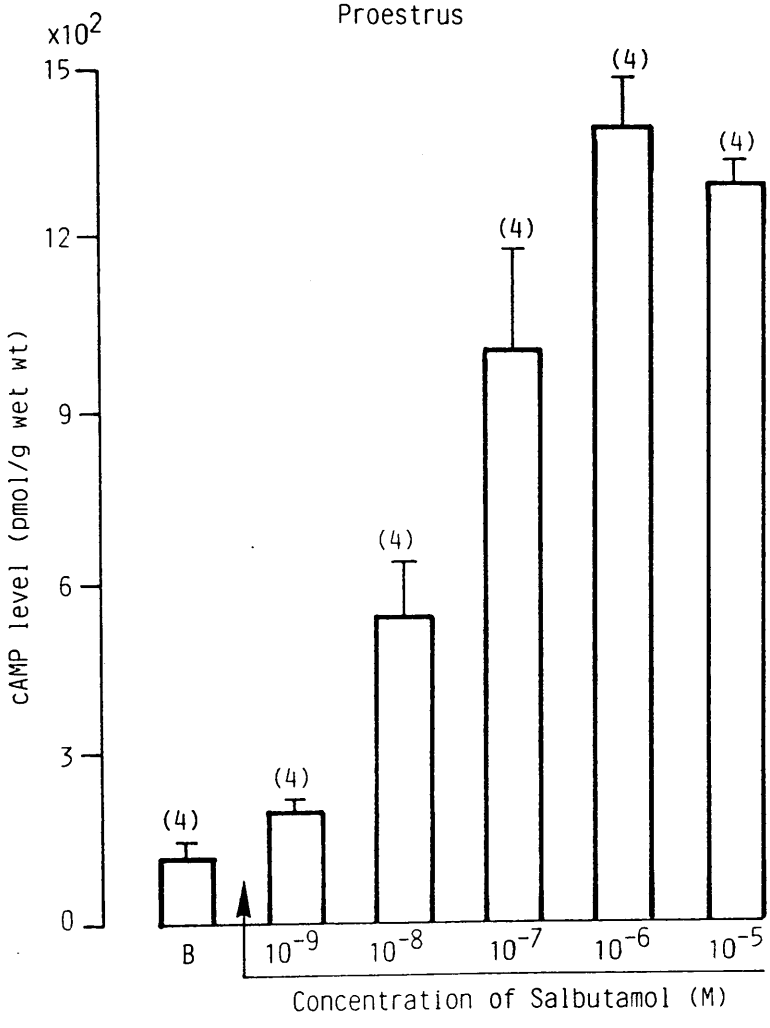


FIGURE 53: Effect of Salbutamol on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in proestrus, in the concentration range (10^{-9} M to 10^{-5} M). Number of observations in brackets.

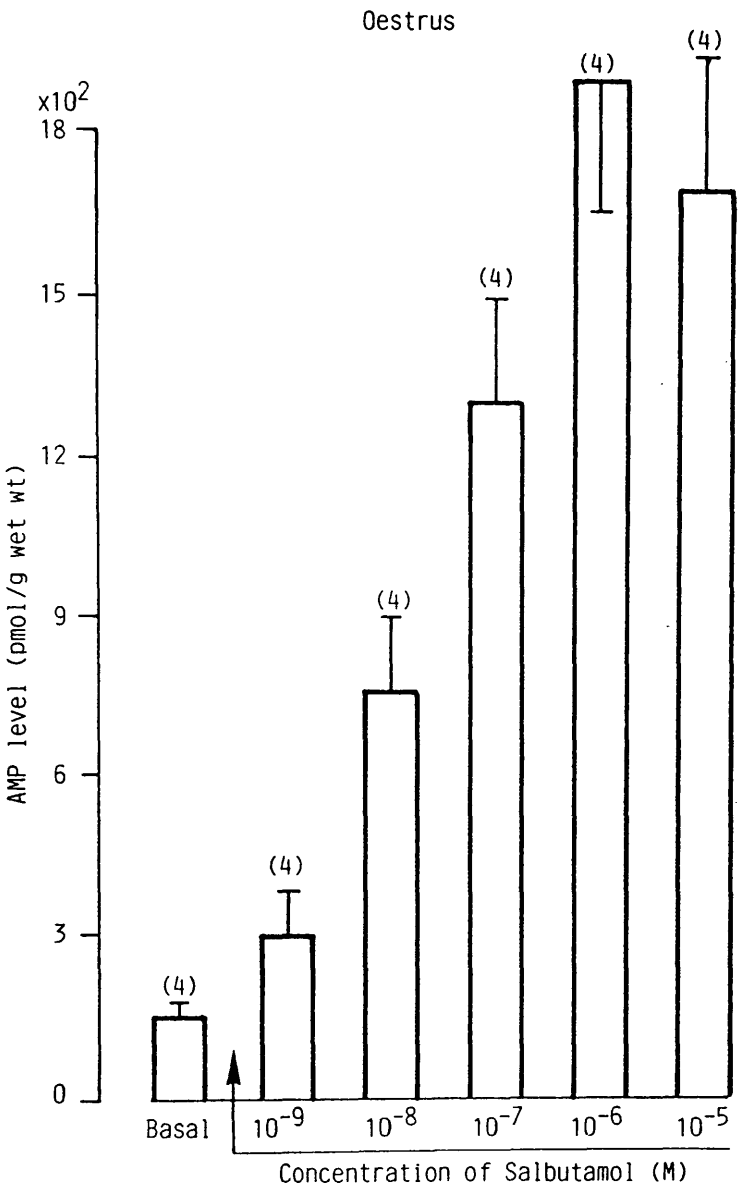


FIGURE 54: Effect of Salbutamol on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in oestrus, in the concentration range (10^{-9} M to 10^{-5} M). Number of observations in brackets.

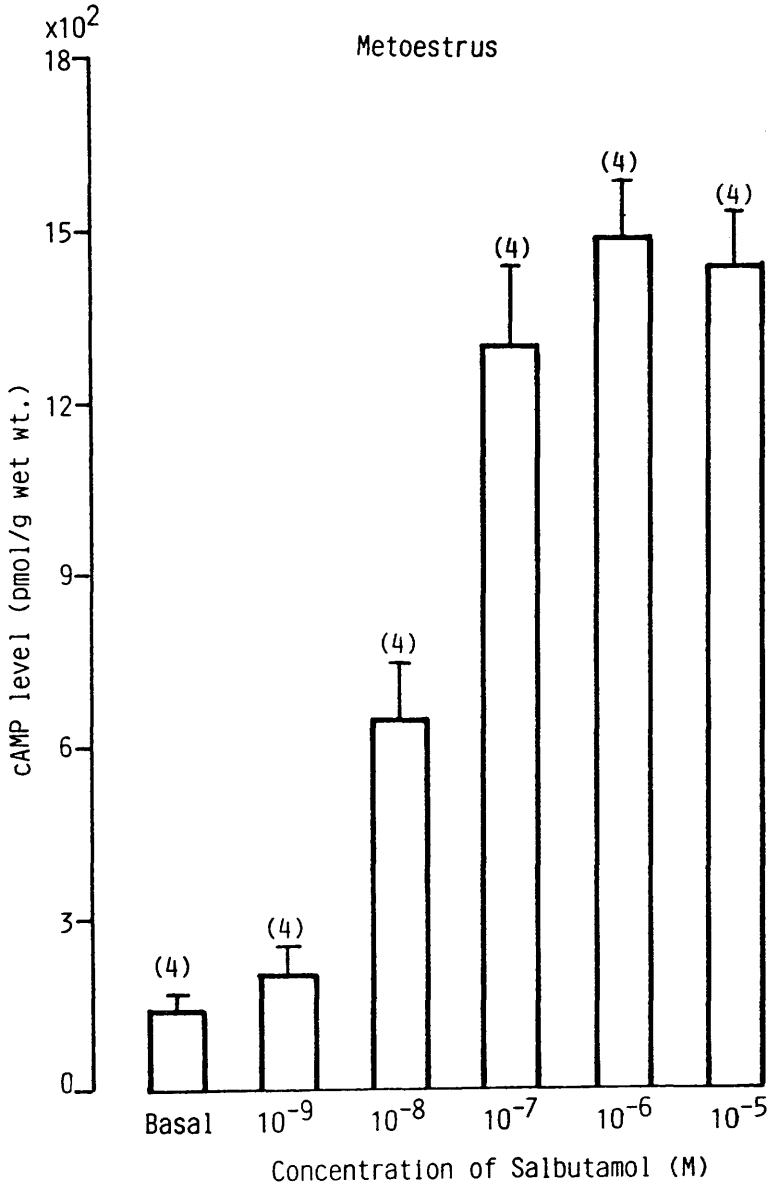


FIGURE 55: Effect of Salbutamol on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in metoestrus, in the concentration range (10^{-9} M to 10^{-5} M). Number of observations in brackets.

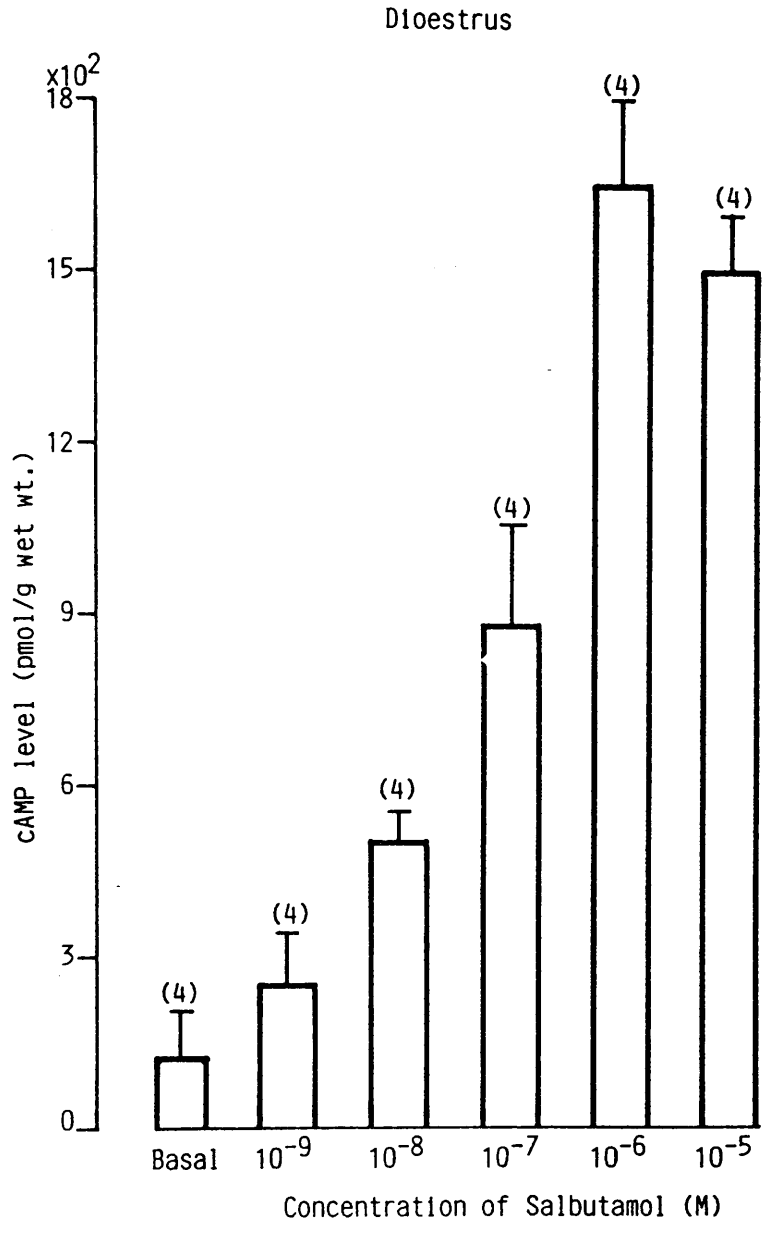


FIGURE 56: Effect of Salbutamol on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in dioestrus, in the concentration range (10^{-9} M to 10^{-5} M). Number of observations in brackets.

(ii) cAMP LEVELS IN UTERI FROM 20-DAY PREGNANT ANIMALS

BASAL cAMP LEVELS:

Basal cAMP levels were measured in uteri with and without the endometrial layer. cAMP concentrations (pmol/g wet weight) in uteri with and without endometrium are (mean ± S.E.M.) 148 ± 13 (n = 4) and 146 ± 20 (n = 4) and did not differ from those obtained in the oestrous cycle respectively. Endometrium-removal did not significantly affect basal cAMP content in the uterus.

EFFECT OF SAL ON cAMP LEVELS:

The effect of SAL on cAMP levels was measured in preparations with and without endometrium. SAL (10^{-9} M - 10^{-5} M) produced concentration-dependent increases in uterine cAMP content over the basal levels in normal and endometrium free preparations (Figure 57). The increase in cAMP levels produced by SAL in uteri from 20-day pregnant rats did not differ from that induced in uteri from the four phases of the oestrous cycle.

There was no significant difference in cAMP content between normal and endometrium-free uterine preparations.

Endometrium-removal did not alter basal tissue cAMP content neither did it affect the ability of SAL to increase uterine cAMP content.

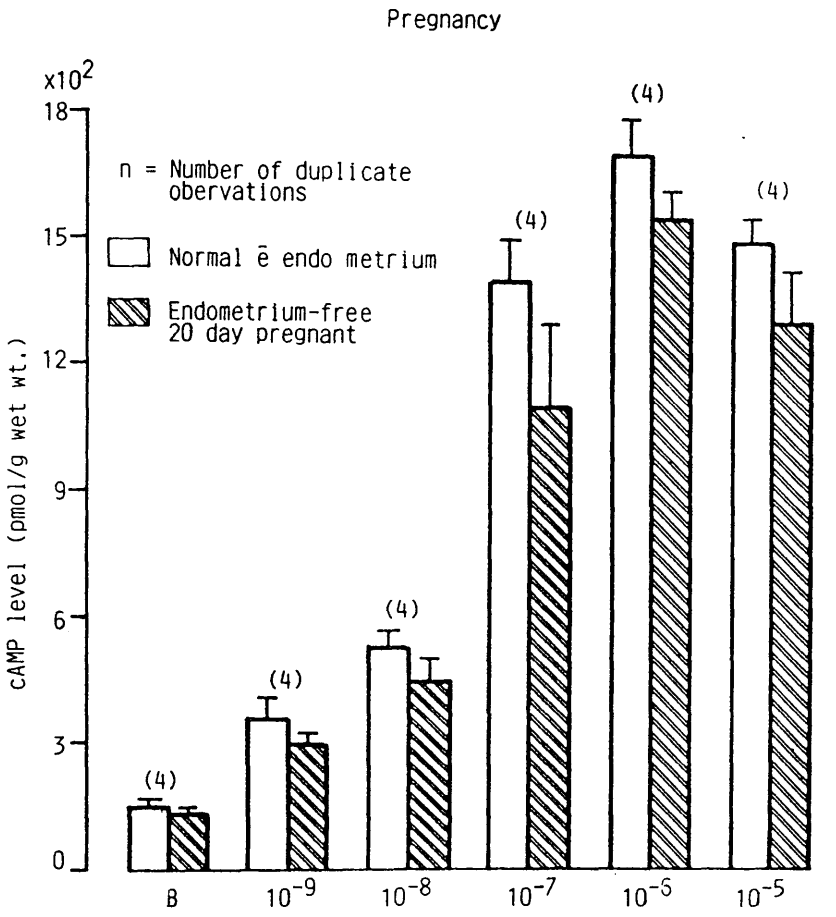


FIGURE 57: Effect of Salbutamol on basal cAMP levels (expressed as pmol/g wet weight of tissue) in the rat isolated uterus in 20 day pregnant with and without endometrium in the concentration range (10^{-9} M to 10^{-5} M). Open columns = normal and intact endometrium; hatched columns = endometrium-free. Number of duplicate observations in brackets.

CHAPTER 4

DISCUSSION

(A) RESPONSES TO ACETYLCHOLINE IN UTERI DURING THE OESTROUS CYCLE, PREGNANCY AND IN POST PARTUM ANIMALS

Uteri from rats undergoing the natural oestrous cycle and pregnancy, and in those from postpartum animals were contracted by Ach. Ach potency was similar in uteri under the different hormonal conditions indicating that a homogeneous population of muscarinic receptors which may not be influenced by the ovarian hormones exists in this preparation. During the oestrous cycle, the maximum tension developed in response to Ach varied being highest in oestrus, and lowest in dioestrus. In the oestrogen pretreated rats, Ach produced greater tension, than in naturally occurring oestrus. Tension developed in response to Ach in uteri from 20-day pregnant and one-day postpartum animals was approximately more than two-fold higher when compared with the effect observed during the oestrous cycle. The differences in tension developed to Ach may be due to the observed variation in thickness of uterine smooth muscle in non-pregnant (when compared to pregnant and postpartum states (Digges, 1980; Boyle & Digges, 1982a,b). A difference in calcium function is also possible since the weak tension in non-pregnant, pregnant and postpartum rats may be due to a limited calcium influx (Maruyama, Ochiai, Tokutome, Hachiya & Umazume, 1986; Matsuzawa, Masahashi, Kihira & Tomita, 1987).

(B) RESPONSES TO ADRENOCEPTOR AGONISTS IN THE OESTROUS CYCLE AND AFTER OESTROGEN PRETREATMENT

NA, ADR, and SAL produced inhibitory responses in proestrus, oestrus, metoestrus and dioestrus. Antagonism of the Ach-induced contraction by the adrenoceptor agonists is physiological, and is mediated via β-adrenoceptor stimulation (Krall et al., 1978; Boyle & Digges, 1982a; Acritopoulou-Fourcroy et al., 1985; Ohia & Boyle, 1987). The β-adrenoceptor population in the rat uterus is predominantly of the β_2-subtype (Levy & Apperley, 1978; Johanssen, Andersson & Wikberg, 1980; Ohia & Boyle, 1987), but β_1- and β_2-adrenoceptors have been shown to co-exist in oestrogen dominated uteri (Johanssen et al., 1980; Nahorski, 1981). The potencies displayed by NA, ADR and SAL in the present study are similar to those reported by other workers (Boyle & Digges, 1982a; Ohia, 1986; Ohia & Boyle, 1987). Thus supporting the above classification of β-adrenoceptors in the rat uterus during the oestrous cycle.

For each adrenoceptor agonist both the degree of inhibition and potency varied throughout the oestrous cycle. The variation could have been partially due to stimulation 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 blocked by the α-adrenoceptor antagonist, AZA. Similar AZA-sensitive motor responses have also been demonstrated in other studies (Boyle & Digges, 1982a; Boyle

& Ohia, 1984). During proestrus and oestrus, there is an increase in α -adrenoceptor activity (Krall et al., 1978; Acritopoulou-Fourcroy et al., 1985; Acritopoulou-Fourcroy & Marcias-Collado, 1988). Thus, it would seem 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 degree of inhibition produced by NA was enhanced in proestrus, oestrus and dioestrus. Enhancement of NA inhibitory effects in the rat uterus by α -adrenoceptor antagonists has also been shown by other workers (Brooks et al., 1965; Acritopoulou-Fourcroy et al., 1985).

AZA also produced paradoxical shifts in ADR concentration-response curves to the right (oestrus and dioestrus), at the lower concentrations. Non-specific excitatory effects of AZA (Qayum & Yusuf, 1977) and other α-adrenoceptor antagonists such as phentolamine and tolazoline (Tothill, 1967; Paton, 1968) have also been described. It thus appears that the rightward shifts caused by AZA may be explained in terms of a non-specific stimulant action (Ohia, 1986).

Despite the non-specific effect produced by AZA, it can be concluded that there are α -excitatory receptors in the rat uterus which can be activated by NA and ADR. α -adrenoceptors appear to contribute to the variations seen with NA and ADR but not SAL. It seems, therefore,

that other factors may be involved in the observed differences in uterine response to adrenoceptor agonists during the oestrous cycle.

The concentration of adrenoceptor agonists at receptor sites is regulated by uptake processes into neuronal and extraneuronal tissue (Gillespie, 1973; Iversen, 1973; La Bella, 1985). The possible role of these removal mechanisms in the variation in uterine response to adrenoceptor agonists was investigated in the presence of a neuronal uptake inhibitor (DMI), and an extraneuronal uptake inhibitor (NMN). DMI and NMN shifted the concentration-response curves elicited by all three agonists to the left and enhanced their maximum degree of inhibition. The increased sensitivity induced by blockade of agonist sites of loss has been described as deviation supersensitivity (Fleming, 1975; Guimaraes & Trendelenburg, 1985). Although DMI and NMN shifted adrenoceptor agonists concentration response curves to the left, corresponding increases in their pD_2 values were not achieved in all cases. One possible explanation could be that in addition to the observed leftward shifts, there was an increase in the maximum degrees of inhibition elicited by the agonists. Both changes in agonists response may serve to conceal alterations in their pD_2 values induced by uptake inhibition.

The increased maximum response to agonists observed in the presence of the uptake inhibitors has also been demonstrated in the rat vasa deferentia (Kenakin, 1980,

1984). Since the rat uterus has a sparse adrenergic innervation (Silva, 1966), extraneuronal uptake may be more important than neuronal uptake in the disposal of catecholamines. In support of this view, Boyle & Digges (1982a) showed that inhibition of the extraneuronal process alone produced effects similar to blocking both neuronal and extraneuronal uptake processes. However, in the rat atria and vasa deferentia, Morton (1987) could not find any relationship between the density of adrenergic innervation and the degree of extraneuronal uptake confirming the earlier observations of Gillespie (1973).

In the present study and that by Ohia (1986), inhibition of the uptake processes produced greater effects (in terms of shifts in agonists concentration-response curves and increases in the maximum degree of inhibition) than blockade of α -excitatory adrenoceptors. Removal mechanisms may, therefore, play a more important role than α-adrenoceptor activity in the observed variation in uterine response during the oestrous cycle.

A series of experiments was performed in the presence of AZA, DMI and NMN in order to ensure that an effect on β-adrenoceptors uncomplicated by α -adrenoceptor activity was being observed. In the presence of the combined antagonists, the maximum degrees of inhibition produced by NA, ADR and SAL were increased in all phases of the oestrous cycle. Except for ADR, agonists concentration-response curves were also shifted to the left. The effects

produced by the combined antagonists were similar to those achieved in the presence of uptake inhibitors alone, again supporting the view that agonists removal mechanisms are more important than α -adrenoceptor activity. Since the variation in uterine response to adrenoceptor agonists persisted even in the presence of AZA, DMI and NMN, it seems that α -adrenoceptor activity and the removal mechanism may only account in part for the observed differences.

There is evidence that ovariectomy enhanced uterine inhibitory response to catecholamines suggesting a role for the ovarian hormones in the observed variation in their action during the oestrous cycle (Ohia, 1986; Ohia & Boyle, 1987). In the present study, the role of ovarian hormones was further investigated using a different model. Animals were pretreated with doses of oestradiol in order to induce oestrus and the effects of SAL re-examined alone and in the presence of AZA, DMI and NMN in order to compare the effects of the removal mechanisms in uteri from oestrogen pretreated animals with those in natural oestrus. When compared to effects observed in natural oestrus, uteri from oestrogen pretreated animals were more sensitive to the inhibitory action of SAL. Interestingly, the combined antagonists had no effect on the SAL response in uteri from oestrogen pretreated animals. One possible explanation of the enhanced response to SAL alone could be that high levels of oestrogen inhibit the extraneuronal uptake of SAL leading to an enhancement of its action. Indeed, SAL may

be a substrate for the removal mechanisms in the rat uterus since its effect during the oestrous cycle was also potentiated by DMI and NMN (Ohia, 1986; present study).

The ovarian steroid used in this study has been shown to be a potent inhibitor of the extraneuronal uptake (Iversen & Salt, 1970; Salt, 1972). The lack of effect by the combined antagonists may, therefore, be due to the fact that uptake inhibition produced by oestradiol is maximal.

The finding that oestrogen pretreatment altered uterine response to SAL supports the view that the ovarian hormones may play a role in the observed variation in response to adrenoceptor agonists. Ovarian hormonal effects on adrenoceptor agonists responses did not appear to involve a direct action on adrenoceptor (Ohia & Boyle, 1987). It seems more likely that the ovarian hormones affected some intracellular processes such as intramural prostaglandin production (Ham et al., 1975; Sterin-Speziale et al., 1980; Wilson, 1983; Gimeno & Gimeno, 1984) or calcium utilization (Batra, 1986; Ishii, Kano & Ando, 1986), which in turn would account for the variation in uterine response to the adrenoceptor agonists.

(C) EFFECT OF ADRENOCEPTOR AGONISTS ON INTRAMURAL PROSTAGLANDIN PRODUCTION

Evidence from biochemical studies reveal that catecholamines can stimulate cyclo-oxygenase in tissue homogenates and isolated cells (Egan et al., 1978; Baumann et al., 1979). There is evidence that adrenoceptor agonists may interact with the cyclo-oxygenase pathway leading to prostaglandin production in rat uteri in the different hormonal conditions of the oestrous cycle, and in the absence of the hormones following ovariectomy (Boyle & Ohia, 1985a, b; Ohia, 1986; Chaud et al., 1986). In the present study, the possible effects of adrenoceptor agonists on uterine prostaglandin production has been further investigated. The effects of the cyclo-oxygenase inhibitor, FBF, were examined on SAL responses in uteri from the four phases of the oestrous cycle. Both Control I and Control II studies were performed as described previously. FBF shifted SAL Control I and Control II concentration-response curves to the left and enhanced the maximum degree of inhibition in proestrus, oestrus (Control I only), metoestrus and dioestrus. After FBF SAL produced a complete inhibition of the Ach motor response in all phases. There was a corresponding increase in SAL potency, but it did not reach significance in all experiments. This failure may be due to the fact that in addition to the observed leftward shifts in SAL concentration-response curves, there was an increase in the maximum degree of inhibition. As observed earlier with the uptake

inhibitors, changes in potency may be occurring but are concealed by the increased intensity of SAL response in the presence of FBF. Similar observations were made by Ohia (1986) for the effects of FBF on SAL response during the oestrous cycle.

Since FBF enhanced SAL responses during the oestrous cycle and uteri from oestrogen pretreated animals are more sensitive to the inhibitory effects of this amine, it was considered of interest to examine the effect of cyclo-oxygenase inhibition on the responses produced by β -adrenoceptor agonists, SAL, ISO and ISOX. FBF enhanced uterine inhibitory responses to SAL and ISO, but had a lesser effect on ISOX response. Thus cyclo-oxygenase inhibition also enhanced β -adrenoceptor agonists inhibitory responses in uteri from oestrogen pretreated animals.

Arachidonic acid is located predominantly in the 2-position of phospholipids and its release occurs by hydrolysis which is controlled by phospholipase A_2. The fact that intracellular levels of arachidonic acid are extremely low suggests that phospholipase A_2 activation represents a rate limiting step in the production of eicosanoids (Chang, Musser & McGregor, 1987). A series of experiments was performed in order to investigate the effect of phospholipase A_2 inhibition by Quinacrine on SAL response in uteri from oestrogen pretreated animals. Quinacrine enhanced uterine inhibitory response to SAL

which would suggest that products of the cyclo-oxygenase and lipooxygenase pathways may be involved in the β - adrenoceptor agonist effect. However, Ohia (1986) showed that BW 755C (a dual inhibitor of cyclo-oxygenase and lipooxygenase activities) produced effects similar to those elicited by FBF indicating that products of the lipooxygenase pathway i.e. leukotrienes are not involved in adrenoceptor agonist responses.

The blockade produced by cyclo-oxygenase inhibitors such as indomethacin and aspirin has been shown to be partly reversed by arachidonic acid in the guinea-pig (Takei & Moritoki, 1987) and human (Quaas, Goppinger & Zahradnik, 1987) uterus. Thus the ability of arachidonic acid to reverse the effects elicited by FBF was tested in a series of experiments. FBF enhanced SAL inhibitory response in uteri from oestrogen pretreated animals, but this effect was unaffected by arachidonic acid. It seems, therefore, that in the rat uterus, effects produced by the cyclo-oxygenase inhibitor, FBF, are irreversible.

The possible involvement of prostaglandins in the inhibitory response produced by adrenoceptor agonist was investigated in uteri from late pregnancy. Both Control I and Control II experiments were performed for ADR and SAL in the absence and presence of FBF. In 20-day pregnant rats the maximum response to ADR and SAL in Control I never exceeded 60% inhibition of the Ach induced contraction, while in Control II ADR reached 80% and SAL 90% inhibition.

Thus in the Control I ADR was more effective in the

pregnant rat than in the non-pregnant rat where inhibition never exceeded 45% of the Ach induced contraction although the degree of inhibition varied throughout the oestrous cycle. For SAL, the differences between the pregnant and non-pregnant animals were less marked, and SAL produced 65% inhibition in oestrus and 100% inhibition in metoestrus. The pD_2 values for ADR and SAL did not differ between the two groups of animals.

Since adrenoceptor agonists responses were altered by FBF treatment in uteri from non-pregnant and pregnant rats, a series of experiments was carried out to determine if the same relationship existed in preparations from one-day post-partum animals. Both Control I and Control II studies were performed for ADR and SAL in the absence and presence of FBF.

In one day post partum rats the maximum response to ADR in Control I never exceeded 95%, while in Control II around 90% inhibition of the Ach induced contraction, that produced by SAL in Control I reached 95% and in Control II 100%. Thus in the post partum rat, both ADR and SAL are more effective inhibiting Ach induced contractions than in either 20 day pregnant or non-pregnant rats.

FBF shifted agonists Control I and Control II concentration-response curves to the left and enhanced their maximum degree of inhibition. There were corresponding increases in agonists potencies which did not reach significance in all experiments. Thus, intramurally

generated prostaglandins may also be involved in uterine response to adrenoceptor agonists in uteri from 20-day pregnant animals. FBF also shifted SAL concentration-response curves to the left, but had no effect on ADR response. in uteri from 1 day post-partum animals

Generally, effects elicited by FBF in uteri from post partum animals were less than those observed in non-pregnant and pregnant ones. It is unclear why cyclo-oxygenase inhibition had little or no effects on ADR response in uteri from post partum animals. One possible explanation for the reduced effects produced by cyclo-oxygenase inhibition may be that uteri under this condition have a lower basal prostaglandin activity than in those from pregnant animals (Harney, Sneddon & Williams, 1974). However, the finding that SAL responses were enhanced by the cyclo-oxygenase inhibitor, FBF, would tend to support the view that endogenous prostaglandins are involved in adrenoceptor agonists effects in post partum uteri.

A series of experiments was performed in order to assess the contribution of the endometrium in the interaction between adrenoceptor agonist and the cyclo-oxygenase pathway. Uteri from rats in late pregnancy and post partum were employed for this study because it is easier to remove the endometrium from these preparations as compared to those from non-pregnant animals. Both Control I and Control II studies were carried out for ADR and SAL in the absence and presence of FBF. Endometrium removal caused a reduction in uterine response to ADR and SAL, with

the effects greater in one-day post partum animals than in 20-day pregnant animals. The mechanism responsible for the reduction in adrenoceptor agonist response in endometrium-free uteri is unclear. FBF had no significant effect on ADR and SAL responses in endometrium-free preparations from 20-day pregnant and one-day post partum animals. It thus seems that the endometrium could play a role in the interaction between adrenoceptor agonists and the cyclo-oxygenase pathway. The present finding appears consistent with previous reports from biochemical studies that the endometrium could be a major source of prostaglandins in the rat uterus (Campos et al., 1980; Brown & Poyser, 1985).

In summary, cyclo-oxygenase inhibition enhanced uterine inhibitory responses to adrenoceptor agonists in uteri from animals in the four phases of the oestrous cycle and after oestrogen pre-treatment. Inhibitory responses elicited by the adrenoceptor agonists in uteri from late pregnancy and in those from post partum animals were also enhanced by the cyclo-oxygenase inhibitor. The effect produced by the cyclo-oxygenase inhibitor was, however, not reversed in the presence of exogenous arachidonic acid. Cyclo-oxygenase inhibition had no effect on adrenoceptor agonists responses in endometrium-free uteri from late pregnancy and in those from post partum animals. Inhibition of phospholipase A_2 activity by Quinacrine also enhanced adrenoceptor agonists responses. The above results indicate that adrenoceptor agonists may interact

with the cyclo-oxygenase pathway leading to prostaglandin production in uteri from non-pregnant, pregnant and post partum animals. Endogenous prostaglandins thus formed as a result of adrenoceptor agonists effects would then oppose the β -adrenoceptor mediated inhibition. The endometrium seems to play a major role in the adrenoceptor agonist effect on intramural prostaglandin generation. The finding that phospholipase A_2 inhibition also altered uterine response to adrenoceptor agonist would tend to suggest that the availability of arachidonic acid for conversion to prostaglandins by cyclo-oxygenase may represent an important step in the process.

In support of results from the present study, involvement of prostaglandins in adrenoceptor agonists responses have also been shown in guinea-pig (Takei & Moritoki, 1987), sheep (Lye et al., 1987) and pregnant human (Quaas & Zahradnik, 1985; Wikland, Lindblom & Wigvist, 1985; Quaas et al., 1987) uterus. The participation of prostaglandins in adrenoceptor agonists responses has been demonstrated in a vascular (Rubanyi & Vanhoutte, 1985) and non-vascular (Trachte, 1987) smooth muscle. The mechanism by which prostaglandin biosynthesis could be stimulated by adrenoceptor agonists is not well understood. There is evidence that the agonists may directly activate cyclo-oxygenase activity by acting as phenolic co-factors for the cyclo-oxygenase of arachidonic acid (Egan et al., 1978; Baumann et al., 1979). It may also be possible that stimulation of cyclo-oxygenase could

be an indirect event produced by activation of adrenoceptors. α -adrenoceptors have been implicated in uterine prostaglandin production in humans (Wikland et al., 1985; Quaas et al., 1987), while β -adrenoceptors may mediate the process in sheep (Lye et al., 1987). In contrast, both α - and β -adrenoceptors may be involved in rat uterine prostaglandin production in the four phases of the oestrous cycle (Ohia, 1986) and in ovariectomized animals (Ohia, 1986; Chaud et al., 1986). The link between adrenoceptor activation and increased prostaglandin activity is unclear, but recent evidence suggests that GTP binding (G) proteins may be involved in the process (Reimer & Roberts, 1986; Burgoyne, Cheek & O'Sullivan, 1987; Axelrod, Burch & Jelsema, 1988). Axelrod et al (1988), proposed that upon activation of phospholipase A_2 by a receptor-coupled G protein, arachidonic acid and its numerous biologically active metabolites are generated. It is feasible, therefore, that adrenoceptor agonists effects on prostaglandin production in the rat uterus could be achieved in two ways: (a) by a direct effect of the agonists on cyclo-oxygenase and/or (b) by an indirect action on cyclo-oxygenase activity via an effect on adrenoceptor-coupled G protein leading to activation of phospholipase A_2.

(D) ROLE OF cAMP

An increase in cAMP accumulation has been shown to accompany β -adrenoceptor activation in the rat uterus (Meisheri, Diamond & McNeill, 1981; Marshall & Fain, 1985; Dokac, D'Albis, Janmot & Harbon, 1986; Tanfur & Harbon, 1987). There is evidence that both oestrogen (Sandborn et al, 1973; Flandroy & Galand, 1978; Kishikawa, 1981) and prostaglandins (Vesin & Harbon, 1974; Vesin, Dokhac & Harbon, 1978, 1979; Dokhac et al., 1986; Tanfin & Harbon, 1987) can alter cAMP production in the rat uterus. It is, therefore, possible that β -adrenoceptor agonists, ovarian hormones and prostaglandins may interact at the level of cAMP formation. Changes in cAMP metabolism induced by these factors may partially account for the differences in uterine response to adrenoceptor agonists during the oestrous cycle.

The possible role of cAMP metabolism in the variation in uterine response to adrenoceptor agonists was investigated in a series of experiments. Basal and SAL-stimulated increases in uterine cAMP content were measured in proestrus, oestrus, metoestrus and dioestrus. Basal cAMP concentrations were similar in uteri from all four phases. SAL elicited a concentration-dependent increase in cAMP levels which were similar in all phases. The above results are consistent with the report by Ohia (1986, 1988) and suggest that the variation in uterine response to adrenoceptor agonists may not be due to effects of endogenous oestrogen on cAMP, and the differential action

of the agonists on cAMP production during the oestrous cycle.

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. The basal levels of cAMP did not differ significantly in the four stages of the oestrous cycle. FBF had no effect on basal cAMP content in the four phases of the oestrous cycle indicating that in physiological concentrations, prostaglandins may not affect uterine cAMP production. A similar effect has been reported by Tanfin & Harbon (1987) who found that basal cAMP levels in the pregnant rat uterus were unaltered by aspirin, a cyclo-oxygenase inhibitor.

Based on the hypothesis that exogenous prostaglandin E_2 may antagonize uterine inhibitory response to β - adrenoceptor agonists by a desensitizing action on adenylate cyclase (Krall et al., 1984), a series of experiments was performed to investigate the role of endogenous prostaglandins on SAL-induced cAMP accumulation. FBF had no effect on SAL-stimulated cAMP levels in uteri from the four phases of the oestrous cycle. It thus seems that the site of interaction between the adrenoceptor agonists and the endogenous prostaglandins generated by them may not be at the level of cAMP formation. Tanfin and Harbon (1987) also found that aspirin did not affect ISO-induced increases in uterine cAMP content.

The results presented above so far show that endometrium removal caused a reduction in adrenoceptor agonists responses in uteri from late pregnancy and post partum. The contribution of the endometrium to SAL-induced increases in uterine cAMP content was, therefore examined in a series of experiments. Basal and SAL-stimulated cAMP levels were measured in uteri from 20-day pregnant animals in the presence and absence of endometrium. Basal cAMP levels were similar in both normal and endometrium-free preparations. SAL produced a concentration-dependent increase in uterine cAMP content which was similar in both normal and endometrium-free preparations. Thus, it would appear that absence of the endometrium does not affect the ability of SAL to increase uterine cAMP content. An effect on tissue cAMP metabolism may, therefore, not account for the observed reduction in uterine response to adrenoceptor agonists in endometrium-free preparation.

In conclusion, presence of a heterogeneous population of adrenoceptors, avid removal mechanisms into neuronal and extraneuronal sites, and the ovarian hormones contributed to the observed variation in uterine response to adrenoceptor agonists during the oestrous cycle. Agonist effects on intramurally generated prostaglandins could also be involved in the observed varition and may indeed, play a crucial role since it affected agonists response in uteri from non-pregnant, pregnant and post partum animals. Intracellular cAMP was the mediator of uterine response to adrenoceptor agonists in preparations from non-pregnant and

pregnant animals. However, changes in tissue cAMP metabolism did not appear to be of major importance in the variation in uterine response to adrenoceptor agonists during the natural oestrous cycle. The present study thus provides further pharmacological evidence for the involvement of several factors in rat uterine response to adrenoceptor agonists.

REFERENCES

REFERENCES

ABDEL-AZIZ, A. & BAKERY, 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.

ABE, Y. (1970)
The hormonal control and the effects of drugs and ions on the electrical and mechanical activity of the uterus. In: Smooth Muscle: An Assessment of Current Knowledge. pp. 396-417. London: Arnold.

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

ACRITOPOULOU-FOURCROY, S. & MARCAIS-COLLADO, H. (1988)
Involvement of α-adrenoceptors in myometrial responses in the pro-oestral rat.
Br. J. Pharmacol. 93, 185-191.

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. Pharmacolyn Ther. 139, 38-41.

AIKEN, J.W. (1972)
Aspirin and indomethacin prolong parturition in rats: evidence that prostaglandins contribute to expulsion of foetus.
Nature 240, 21-25.

AXELROD, J., BURCH, R.M. & JELSEMA, C.C. (1988)
Receptor-mediated activation of phospholipase A via GTP-binding proteins: arachidonic acid and its metabolites as second messengers.
Trends Neurol. Sci. 11, 117-123.

BAIRA, S. (1986)
Effect of estrogen and progesterone treatment on calcium uptake by the myometrium and smooth muscle of the lower urinary tract.
Eur. J. Pharmacol. 127, 37-42.

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

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

BOYLE, F.C. & DIGGES, K.G. (1982a)
Responses to catecholamines of the rat isolated uterus throughout the natural oestrous cycle.
Naunyn-Schimiedeberg'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.

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, London, 389P.

BOYLE, F.C. & OHIA, S.E. (1985a)
Changes in catecholamine inhibitory effects in the rat isolated uterus induced by ovariectomy and cyclo-oxygenase inhibition.
Br. J. Pharmacol. 86, 648.

BOYLE, F.C. & OHIA, S.E. (1985b)
Specificity of enhancement of salbutamol responses by cyclooxygenase inhibition in the rat isolated uterus.
Br. J. Pharmacol. 84, 42.

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

BRODY, S. & WIQVIST, N. (1961)
Ovarian hormones and uterine growth: Effects of estradiol, progesterone and relaxin on cell growth and cell division in the rat uterus.
Endocrinology 68, 971-977.

BORDA, E.S., PERCELO, H. & GIMENO, M.F. (1983)
Alpha adrenergic stimulation modified prostaglandins release in vas def erens.
Prostaglandins 26, 701.

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

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 -synthesis by noradrenaline in primary cell cultures from rabbit splenic pulpa is mediated by atypical alpha-adrenoceptors.
Naunyn-schmiedeberg's Arch. Pharmacol. 316, 1-7.

BULBRING, E., CASTEELS, R. & KORIYAMA, H. (1968)
Membrane potential and ion content in cat and guinea pig myometrium and the response to adrenaline and noradrenaline.
Br. J. Pharmacol. 34, 388-407.

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

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. & TOMITA, T. (1987)
Catecholamine action on smooth muscle. (Pharmacological review).
Pharmacol. Rev. 39, 49-96.

BURGOYNE, D.R., CHEER, R., O'SULLIVAN, T. & ANTHONY, J. (1987)
Receptor-activation of phospholipase A in cellular signalling.
Trends Biochem. Sci. 12, 332-333.

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

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

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

CAMPOS, G.A., LIGGINS, G.C. & SEAMARK, R.F. (1980)
Differential production of PGF and 6-keto-PGF1 by the rat endometrium and myometrium in response to oxytocin, catecholamines and calcium ionophore.
Prostaglandins 20, 297-310.

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

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 oestrogen 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.

CHANG, J., McGREGOR, J.H., MUSSER & HERB, (1987)
Phospholipase A function and pharmacological regulation.
Biochem. Pharmacol. 36, No.15, 2429-2436.

CHAUD, M.A., FRANCHI, A.M., GONZALEZ, E.T., GIMENO, M.F. & GIMENO, A.L. (1986)
Norepinephrine alters PGE2/PGE1 output ratio in isolated uterus from ovariectomized rats.
Prostaglandins, Leukotrienes & Medicine 22, 201-210.

CHOW, E.G.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.

COLE, W.C., GARFIELD, R.E. & KIRKALDY, J.S. (1985)
Gap junctions and direct intercellular communication between rat uterine smooth muscle cells.
Am. J. Physiol. 18, C20-C31.

DALE, H.H. (1906)
On some physiological actions of ergot.
J. Physiol. 34, 163-206.

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

DELICONSTANTINOS, G. & FOFIOU, S. (1986)
Effect of prostaglandins E2 and F2 on membrane calcium binding, C^{2+}/Mg^{2+}-ATPase activity and membrane fluidity in rat myometrial plasma membranes.
J. Endocrinol. 110, 395-404.

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

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

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. Pharmacol.Exp. Ther. 168, 21-30.

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.

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.
Br. J. Pharmac. 67, 466p.

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

DOKHAC, L., D'ALBIS, A., JANMOT, A.C. & HARBON, S. (1986)
Myosin light chain phosphorylation in intact rat uterine smooth muscle. Role of calcium and cyclic AMP.
J. Muscle Res. & Cell Motil. 7, 259-268.

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

DUSLING, G.J. & NOLAN, R.D. (1981)
Stimulation of prostacyclin release from the epicardium of anaesthetized dogs.
Br. J. Pharmacol. 74, 553-562.

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. & SJOBERG, N. (1974)
Ovarian influence on the content of norepinephrine transmitter in guinea-pig and rat uterus.
Endocrinology 94, 1475-1479.

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 (3), 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 oestrogen 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., CHASE, D., KORENMAN, S.G. & KRALL, J.F. (1983)
Beta-adrenergic catecholamine-dependent properties of rat myometrium primary cultures.
Am. J. Physiol. 245, C84-C90.

FORTIER, M. & KRALL, J.F. (1983)
Adenylate cyclase activity of circular and longitudinal muscle layers of rat myometrium.
Biochem. Pharmacol. 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 estrous cycle.
Prostaglandins 22 (4), 637-649.

GARFIELD, R.E., KANNAN, M.S. & DANIEL, E.E. (1980)
Gap junction formation in the myometrium control of oestrogens, 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.

GILMORE, N., VANE, J.R. & WYLLIE, J.H. (1968)
Prostaglandins released by the spleen.
Nature 218, 1135-1140.

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

GOTO, M. & Csapo, A. (1959)
The effect of the ovarian steroids on the membrane

potential of uterine muscle.
J. Gen. Physiol. 43, 455-466.

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 oestrous 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., GIRILLO, V.J., ZANETTI, M.E. & KHEHL, F.A. (1975)
Estrogen-directed synthesis of specific prostaglandins in uterus.
Proc. Nat. Acad. Sci. USA 72, 1420-1424.

HARBON, S. & CLAUSER, H. (1971)
Cyclic adenosine 3',5'-monophosphate cAMP 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. Endocrinol. 60, 343-351.

HEDQVIST, P. (1977)
Basic mechanisms of prostaglandin action on autonomic neurotransmission.
Ann. Rev. Pharmacol. Toxicol. 17, 259-279.

HERVONEN, A., KANVERA, L. & LIETZEN, R. (1973)
Histochemistry demonstrable catecholamines and cholinesterases in the rat uterus during oestrus cycle, pregnancy and after oestrogen treatment.
Acta Physiol. Scand. 87, 283-288.

HILLIER, K. & TEMPLETON, W.W. (1982)
Stimulation of prostaglandin synthesis in rat cerebral cortex via a beta-adrenoceptor.
Gen. Pharmacol. 13, 21-25.

HORTON, E.W. & POYSER, N.L. (1976)
Uterine luteolytic hormone: A physiological role for prostaglandin F2 .
Physiol. Rev. 56, 595-651.

ISAII, K., KANO, T. & ANDO, J. (1986)
Calcium channel, Ca mobilization and mechanical reactivity of estrogen and progesterone-treated rat uterus.
Japan. J. Pharmacol. 41, 47-54.

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

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

JENSEN, K.B. & VENNEROID, 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.M., & WIKBERG, J.E.S. (1980)
Comparison of betaβ_1 - and betaβ_2 -receptor stimulation in oestrogen or progesterone dominated rat uterus.
Acta Pharmacol. et toxicol. 47, 252-258.

KAWARABAYASHI, T. & OSA, T. (1976)
Comparative investigations of alpha- and beta-effects on longitudinal and circular muscles of the pregnant rat myometrium.
Japan 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. Pharmacol. 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.
Pharmacol. Rev. 36 (3), 165-222.

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

KOGO, H., YAMADA, K. and 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 and beta-adrenergic catecholamines in the regulation of uterine smooth muscle motility and adenylate cyclase in the rat.
J. Endocrinol. 102, 329-336.

KRALL, J.F. & KORENMAN, S.G. (1979)
Regulation of uterine smooth muscle cell beta-adrenergic catecholamine-sensitive adenylate cyclase by Mg and guanyl nucleotide.
Biochem. Pharmacol. 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.
Biochem. Biophys. Acta 448, 578-588.

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. (1973)
Beta-adrenergic effects on rat myometrium: Mechanisms of membrane hyperpolarization.
Am. J. Physiol. 225, 1339-1345.

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 isoprenaline.
Life Sci. 6m 2241-2249.

LEVINE, L. & MOSKOWITZ, M.A. (1979)
Alpha- and beta-adrenergic stimulation of arachidonic acid metabolism in cells in culture.
Proc. Natn. Acad. Sci. USA 76, 6632-6636.

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

LYE, S.J., Christopher, P. & Casper, R.F. (1987)
Effects of beta-adrenergic agonist infusions on myometrial activity and plasma prostaglandin levels in the non-pregnant sheep.
Can. J. Physiol. Pharmacol. 65, 2297-2301.

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

MALATHY, P.V., CHENG, H.C. & DEY, S.K. (1986)
Production of leukotrienes and prostaglandins in the rat uterus during peri-implantation period.
Prostaglandins 32, 605-614.

MALIK, K.U. (1978)
Prostaglandins - modulation of adrenergic nervous system.
Fed. Proc. 37, 203-207.

MALTIER, J.P. & LEGRAND, C. (1985)
Characterization of alpha-adrenoceptors of preparturient rats.
Eur. J. Pharmacol. 117, 1-13.

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

MARSHALL, J.M. (1967)
Comparative aspects of the pharmacology of smooth muscle.
Fedn. Proc. 26, 1104-1110.

MARSHALL, J.M. (1968)
Relation between the ionic environment and the action of drugs on the myometrium.
Fed. 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.
Am. Rev. Pharmacol. 13, 19-32.

MARSHALL, J.M. (1977)
Modulation of smooth muscle activity by catecholamines.
Fed. 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.

MARUYAMA, M., OCHIAI, K., TOKUTOME, S., HACHIYA, S. & UMAZUME, Y. (1986)
Effects of Ca and calmodulin on contraction of chemically skinned muscle fibers from pregnant rat uteri.
Japan. J. Physiol. 36, 1179-1191.

MATSUZAWA, KATSUJI, MASAHASHI, TETSUO, KIHIRA, M. & TOMITA, T. (1987)
Contracture caused by sodium removal in the pregnant rat myometrium.
Japan. J. Physiol. 37, 19-31.

MEISHERI, K.D., McNEILL, J.H. & MARHSALL, J.M. (1979)
Effect of isoproterenol on the isolated pregnant rat myometrium.
Eur. J. Pharmacol. 60, 1-6.

MEISHERI, K.D. & McNEILL, J.H. (1979a)
Mg^{2+} - Ca^{2+} - Na^{+} -interaction 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. 239, 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.

MOLDERINGS, G.J. & SCHUMANN, H-J. (1987)
Influence of cyclo-oxygenase inhibitors and of lithium on the positive inotropic effect of mediated by alphaα_1-adrenoceptors in the guinea-pig left atrium.
Naunyn-Schmiedeberg's Arch. Pharmacol. 336, 403-408.

MORRISON, J.F.B., NIMMO, A.J. & WHITAKER, E.M. (1987)
The distribution of beta-adrenoceptors in the rat uterus.
J. Physiol. 386, 72P.

MORTON, A.J. (1987)
An investigation of the role of adrenergic innervation in the regulation of the extraneuronal uptake of [^{3}H]-isoprenaline into rat vasa deferentia and atria.
Br. J. Pharmacol. 91, 333-346.

NAFTALIN, N.J., PHEAR, W.P.C. & GOLDBERG, A.H. (1973)
Isometric contractions of isolated rat uterine muscle: Relation to oestrous 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. by J.W. Amble. pp.71-77.
Elsevier/North Holland Biomedical Press, Amsterdam.

NEEDLEMAN, P., DOUGLAS, J.R., STAECKLEIN, P.B. & JOHNSON, E.M. (1974)
Release of renal prostaglandin by catecholamines: Relationship to renal endocrine function.
J. Pharmacol. Exp. Ther. 188, 453-460.

NISHIKORI, K. & MAENO, H. (1979)
Close relationship between adenosine 3',5' monophosphate-dependent endogenosins phosphorylation of a specific protein and stimulation of calcium uptake in rat uterine microsomes.
J. Biol. Chem. 254, 6099-6106.

NOVY, J. & LIGGINS, G.C. (1980)
Role of prostaglandins and thromboxanes in the physiological control of the uterus and in parturition.
Seminars in Perinatalology 4, 45-66.

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

OHIA, S.E. (1986)
Adrenoceptor mediated inhibition in the rat uterus.
Ph.D. Thesis, Department of Pharmacology, University of Glasgow.

OHIA, S.E. & BOYLE, F.C. (1987)
Ovariectomy alters rat uterine inhibitory response to catecholamines.
Med. Sci. Res. 15, 859-861.

OHIA, S.E. & BOYLE, F.C. (1988)
Involvement of prostaglandins in the inhibitory response of adrenoceptor agonists in the rat isolated uterus.
Gen. Pharmacol. 19, No.6, 807-813.

OHIA, S.E. & BOYLE, F.C. (1988)
Role of cyclic AMP in rat uterine inhibitory response to salbutamol during the natural oestrous cycle.
Archives internationales de pharmacodynamie et de Therapie, 293, 245-256.

OJEDA, S.R., NEGRO-VILAV, A., MECANN, S.M. (1982)
Evidence for involvement of alpha-adrenergic receptors in norepinephrine-induced prostaglandin E2 and leuteinization hormone-releasing hormone release from the median eminence.
Endocrinology 110, 409-412.

OSA, T. & WATANABE, M. (1978)
Effects of catecholamines on the circular muscle of rat

myometria at term during pregnancy.
Jap. J. Physiol. 28, 647-658.

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

POLACER, I. & DANIEL, E.E. (1971)
Effect of alpha and beta-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 Sciences 21, 1617-1620.

QMINI, C., FOLCO, G., SAUTEBIN, L., NAVA, G., MANDELLI, V., FERRUCCIO, S. (1981)
Beta-adrenoceptor activation induces prostaglandin E2 generation in guinea-pig trachea.
Eur. J. Pharmacol. 72, 227-231.

QUAAS, L. & ZAHRADNIK, H.P. (1985)
The effects of alpha- and beta-adrenergic stimulation on contractility and prostaglandin (prostaglandins E2 and F2 and 6-keto-prostaglandin F1) production of pregnant human myometrial strips.
Am. J. Obstet. Gynecol. 152, 852-856.

QUAAS, L., GOPPINGER, A. & ZAHRADNIK, H.P. (1987)
The effect of acetyl salicyclic acid and indomethacin on the catecholamine and oxytocin-induced contractility and prostaglandin (6-keto-PGF1 , PGF2) production of human pregnant myometrial strips.
Prostaglandins 34, 257-269.

REINER, O. & MARSHALL, J.M. (1976)
Action of prostaglandin, PGF2 , 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 beta-adrenoceptor in rat uterus. In: Recent Advances in the Pharmacology of Adrenoceptors. Ed. E. Szabadi, Bradshaw, C.M. & Bevan, P. pp.335-336. Amsterdam: Elsevier.

RIEMER, K.R. & ROBERTS, M.J. (1986)
Activation of uterine smooth muscle contraction: Implications for eicosanoid action and interactions. Seminars in Perinatology 10, 276-287.

RITTA, M. & CARDINALI, D.P. (1982)
Involvement of alpha-adrenoceptors in norepinephrine induced prostaglandin E2 release by rat pineal gland in vitro.
Neuroscience Letters 31, 307-311.

RUBANYI, G. & VAN HOUTTE, P.M. (1985)
Inhibitors of prostaglandin synthesis augment beta-adrenergic responsiveness in ca ine coronary arteries.
Circ. Res. 56, 117-125.

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

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

SAMUELSSON, B. & WENNHALM, A. (1971)
Increased nerve stimulation induced release of noradrenaline from the rabbit heart after inhibition of prostaglandin synthesis.
Acta Physiol. Scand. 83, 163-168.

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.

SHAFFER, J.E. & MALIK, R. (1982)
Enhancement of prostaglandin output during activation of veta-1-adrenoceptors in the isolated rabbit heart.
J. Pharmacol. Exp. Ther. 233, 729-735.

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.

SJOBERG, 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.
Pharmacol. Rev. 27, 207-286.

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.

STILES, G.L., CARON, M.G. & LEFKOWITZ, R.J. (1984)
Beta-adrenergic receptors; biochemical mechanisms of physiological regulation.
Physiol. Rev. 64, 661-743.

SZURSZEWSKI, J.H. & BULBRING, E. (1973)
The stimulant action of acetylcholine and catecholamines on the uterus.
Phil. Trans. Roy. Soc. Lond. B. 265, 867, 149-156.

TAKEI, M. & MORITOKI, H. (1987)
Potentiation by sotalol of the contractions induced by epinephrine and ATP on guinea-pig uterus. Abstract of the 10th IUPHAR Congress, Sydney, Australia.

TANFIN, Z. & HARBON, S. (1987)
Heterologous regulations of cAMP responses in pregnant rat myometrium. Evolution from a stimulatory to an inhibitory prostaglandin E2 and prostacyclic effect.
Molecular Pharmacology 32, 249-257.

TAWFIK, O.W., HUET, Y.M., MALATHY, P.V., JOHNSON, D.C. & DEY, S.K. (1987)
Release of prostaglandins and leukotrienes from the rat uterus is an early estrogenic response.
Prostaglandins 34, 805-815.

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.

TRACHTE, G.J. (1987)
Adrenergic receptors mediating prostaglandin production in the rabbit vas deferens.
Prostaglandins 33, 25-35.

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.

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

VAN ORDEN, D.E., GOODALE, D.B., BAKER, H.A., BARLEY, D.B. & BHATNAGER, 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. Pharmacol. 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. Pharmacol. Exp. Ther. 235, 178-185.

WEIS, M.T. & MALIK, K.U. (1986)
Regulation by calcium of arachidonic acid metabolism in the isolated perfused rabbit heart.
Circ. Res. 59, 694-703.

WENNMALM, A. & BRUNDIN, T. (1978)
Prostaglandin-mediated inhibition of noradrenaline release: IV. Prostaglandin synthesis is stimulated by myocardial adrenoceptors differing from the alpha- and beta-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.

WILLIAMS, K.I., SNEDDON, J.M. & HARNEY, D.J. (1974)
Prostaglandin production by the pregnant rat uterus *in vitro* and its relevance to parturition.
Pol. J. Pharmacol. Pharm. 26, 207-215.

WILSON, L. (1983)
Effects of estradiol and progesterone on uterine prostaglandin levels in the pregnant rat.
Prostaglandins 26 (1), 47-54.

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

YOHAI, D. & DANON, A. (1987)
Effect of adrenergic agonists on eicosanoid output from isolated rabbit choroid plexus and iris-ciliary body.
Prostaglandins, Leukotrienes & Medicine 28, 227-235.

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