

AN ABSTRACT OF THE THESIS OF

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Title: CONCERTED ACTIONS OF OVARIAN STEROIDS AND
PROSTAGLANDIN F₂α ON SMOOTH MUSCLE CONTRAC-
TILITY OF OVINE AND BOVINE UTERINE ARTERIES

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The role of estradiol-17β, progesterone and prostaglandin F₂α (PGF₂α) in altering contractility of uterine vascular smooth muscle was investigated. A segment of uterine artery (3.5 cm) supplying each uterine horn of ewes and heifers were removed at necropsy and perfused with oxygenated Krebs Henseleit Solution prior to initiating sequential drug perfusions. Arteries were subjected to electrical stimulation (ES) and changes in perfusion pressure due to changes in resistance to flow were recorded in mm Hg.

EXPERIMENT I: Uterine arteries from unilaterally-ovulating pregnant (P) and nonpregnant (NP) ewes and heifers, necropsied on days 15 and 17 post-estrus, respectively, (estrus=day 0) were subjected to three 30 min perfusions in the order: saline, PGF₂α (1 ng/ml) and saline. Overall, responsiveness to ES by arteries ipsilateral to ovaries bearing corpora lutea (CL) from both species was greater (P < .01) than that of contralateral arteries in NP animals only. Arteries from NP ewes and heifers perfused with PGF₂α exhibited greater

($P < .05$) smooth muscle contractility in response to ES than when arteries were perfused with saline. Perfusion of arteries from P animals with $\text{PGF}_2\alpha$ resulted in no change (heifers) or a decrease ($P < .05$) in contractility (ewes) to ES when compared to responses of arteries to ES during saline perfusions. Perfusion of arteries from NP ewes with conceptus brei (conceptuses of day 15 P ewes) for 30 min, caused a marked decrease ($P < .01$) in contractility of arteries to ES during a subsequent perfusion of $\text{PGF}_2\alpha$.

EXPERIMENT II: Five unilaterally-ovulating NP ewes were sacrificed on each of days 0 (estrus), 3, 6 and 10 of the estrous cycle. Regardless of stage studied, uterine arteries ipsilateral to ovaries having the greater follicular development, hereafter referred to as ipsilateral arteries, responded to ES with increased smooth muscle contractility when compared to the low consistent responses of contralateral arteries. Responses of ipsilateral arteries to ES following perfusions of saline, $\text{PGF}_2\alpha$ or norepinephrine (NE) increased from day 0 to 10 of the cycle. However, only ipsilateral arteries from day-10 ewes exhibited increased ($P < .01$) contractility to ES following perfusion of $\text{PGF}_2\alpha$. Perfusion of phentolamine reduced contractility ($P < .01$) of ipsilateral arteries to ES when compared to responses after previous perfusions of saline, $\text{PGF}_2\alpha$ or NE. Subsequent perfusion of $\text{PGF}_2\alpha$ failed to increase arterial response to ES over that exhibited following phentolamine perfusion. Perfusion of ipsilateral arteries with NE after $\text{PGF}_2\alpha$ resulted in a return of smooth muscle

contractility to ES ($P < .01$).

EXPERIMENT III: Sixteen ovariectomized ewes were assigned randomly to four groups and received the following treatments for one week: 1) none (controls); 2) estradiol-17 β (E_2 , 54 mg implant); 3) progesterone (P_4 , 15 mg twice daily); and 4) $E_2 + P_4$. Treatment of ewes with P_4 increased ($P < .01$) while treatment with E_2 decreased ($P < .01$) arterial smooth muscle contraction to ES following initial perfusions of saline, $PGF_2\alpha$ or NE compared to responses of arteries removed from control or $E_2 + P_4$ -treated ewes. Responses of arteries from control and $E_2 + P_4$ -treated ewes did not differ. Arteries from all ewes exhibited increased contractility ($P < .01$) to ES following NE when compared to responses evoked by ES following perfusions of saline or $PGF_2\alpha$. Perfusion of phentolamine depressed smooth muscle contraction ($P < .01$) of arteries of all ewes. Subsequent perfusion of $PGF_2\alpha$ failed to elicit an increase in arterial contractility to ES over that exhibited following phentolamine perfusion. Arteries from all ewes except those treated with E_2 responded to ES with increasing contraction of smooth muscle following repeated perfusions of NE after $PGF_2\alpha$.

It is proposed that progesterone and estradiol alter uterine arterial nerve concentration of NE and/or sensitivity of smooth muscle cells, and thus, in concert with the regulatory action of $PGF_2\alpha$ on the release of NE, may serve to modulate arterial smooth muscle contractility.

Concerted Actions of Ovarian Steroids and Prostaglandin
 $F_2\alpha$ on Smooth Muscle Contractility of Ovine
and Bovine Uterine Arteries

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CONCERTED ACTIONS OF OVARIAN STEROIDS AND
PROSTAGLANDIN F₂ α ON SMOOTH MUSCLE
CONTRACTILITY OF OVINE AND
BOVINE UTERINE ARTERIES

REVIEW OF LITERATURE

An adequate blood supply is essential for the function of any organ or gland. In this regard, blood flow to the uterus is particularly important since this organ serves as the site of implantation of the fertilized ovum and subsequent development of the young. Recent evidence suggests ovarian steroids may act to control blood flow through the uterine vascular bed. Alterations in uterine blood flow produced by ovarian dysfunction may have adverse effects on the reproductive performance of the female or interfere with the normal development of the young. Thus, a thorough understanding of the mechanism of action of ovarian steroids in altering uterine blood flow is of major interest.

It has long been known that during estrus, a state of persistent hyperemia develops in the uterine vascular bed. In the guinea pig, the hyperemia begins 6 to 10 hours prior to estrus and uterine blood flow begins to decline slowly following ovulation. (Markee, 1932b). In the rabbit, maximal vasodilation of the uterus occurs several hours before ovulation (Markee, 1932a) and remains high for several days. These early data led to the conclusion that the ovary, probably through

secretion of ovarian steroids, is intimately involved in the control of uterine blood flow, at least during the time of estrus. As a result of this mechanism, a high degree of vascularization of the endometrium is assured which is requisite for implantation of the fertilized ovum.

Rhythmic changes in uterine blood flow during the human menstrual cycle were observed by Prill and Gotz (1961). Uterine blood flow increased during the follicular phase and decreased during the luteal phase of the cycle with maximal flows observed near the time of ovulation. One of the first definitive studies, however, which demonstrated that ovarian secretion of progesterone and/or estrogen is involved in modulating uterine blood flow, was conducted by Greiss and Anderson (1969). These researchers observed that during the estrous cycle of the ewe, the rate of blood flow through the uterine arteries followed a consistent repetitive pattern which appeared to correspond to changes in secretion of ovarian steroids. Uterine arterial blood flow increased abruptly at or just prior to behavioral estrus followed by an abrupt decrease and then a continuous gradual decline in blood flow throughout the remainder of the estrous cycle. In the ewe, the major estrogen secreted by the ovaries during the estrous cycle is estradiol-17 β (Moore et al., 1969). The concentration of estradiol in ovarian venous blood was found to be low (40 pg/ml) about 48 hours before the onset of estrus (Scaramuzzi et al., 1970) with maximal levels of this steroid (1 ng/ml) present either

before or at the onset of estrus. Concentrations of progesterone in the utero-ovarian vein of the ewe are low (12 ng/ml) for the first two days following estrus, then increase rapidly between days 3 and 6 and remain constant (200 ng/ml) for approximately six days before returning to a low level on the day before estrus (Thorburn and Mattner, 1971). Thus, the abrupt increase in uterine arterial blood flow observed at estrus in the ewe may have resulted from a vasodilatory action of estradiol while the gradual decline in blood flow following estrus may be a result of progesterone-induced vasoconstriction. Further evidence suggesting differential effects of ovarian estrogen and progesterone secretion on altering uterine blood flow in the nonpregnant ewe was presented by Huckabee et al. (1968). These investigators observed that uterine arterial blood flow was highest when ovaries contained large follicles without corpora lutea (CL) and lowest in the presence of one or more well-developed CL and minimal follicular development.

Estrogen is known to exert a profound effect on uterine hemodynamics. Uterine hyperemia following estrogen administration has been demonstrated in the ewe (Huckabee et al., 1970), as well as the guinea pig (Markee, 1932b), rat (McKercher et al., 1973) and rabbit (Kaiser, 1947). In contrast, progesterone administration results in a decreased blood flow through the ovine uterine vascular bed (Caton et al., 1974b). Research conducted by Greiss and Anderson (1970),

however, indicates that although progesterone is capable of reducing uterine arterial blood flow in the ewe, its major effect appears to be a reduction of uterine vascular sensitivity to estrogen-induced vasodilation. A single injection or daily injections of estradiol into ovariectomized ewes increased uterine arterial blood flow markedly for only one to two days, followed, by a progressive reduction in blood flow to a constant level significantly below that achieved after the initial injections of this hormone. When superimposed on daily estradiol, progesterone decreased uterine arterial blood flow about 30 percent. Following progesterone withdrawal, uterine blood flow increased to the level observed after the initial administration of estradiol. Caton et al. (1974a) presented evidence for an antagonistic effect of estrogen and progesterone on altering uterine arterial blood flow in the ewe. Estradiol-stimulated increases in blood flow through the uterine vascular bed were decreased by a subsequent administration of progesterone. The magnitude of induced changes in uterine arterial blood flow in the above study appeared to be related to the ratio of the two ovarian steroids.

A local effect of estrogen on increasing blood flow through the uterine vascular bed of the ewe was demonstrated by Greiss and Miller (1971). Injection of estrogen into the isolated lumen of one uterine horn significantly increased blood flow through the ipsilateral uterine artery, while blood flow through the contralateral uterine

artery remained unchanged. Of special importance to this thesis was the observation by these researchers that vascular changes within a single ovine uterine horn were reflected almost exclusively by changes in blood flow through the major ipsilateral uterine artery.

The uterus of the ewe and cow is an ideal model with which to study the local effects of an ovary on ipsilateral uterine blood flow. Both the ovine and bovine uterus are bipartite and are composed of a small body which bifurcates into two prominent uterine horns, each of which receives blood by way of a separate uterine artery derived from a branch of the internal iliac artery (Tanudimadja et al., 1968; Yamauchi and Sasaki, 1969). Vascular changes within a single uterine horn are reflected almost exclusively by changes in blood flow through the ipsilateral uterine artery of the ewe, since no arterial cross-circulation appears to occur between uterine horns (Greiss and Miller, 1971). Further, it has been demonstrated that the two uterine arteries supply most of the blood to the uterus of the ewe (Greiss and Anderson, 1974). Due to the similarity in uterine anatomy between the ewe and cow, it is reasonable to assume that each uterine horn of the cow is similarly supplied with arterial blood.

It has been known for many years that most mammalian arterial beds are subject to the influence of adrenergic vasoconstrictor nerves (Bayless, 1923). This constrictor innervation is not confined to the arteriolar resistance vessels within a tissue but is distributed to the

extrinsic arteries, such as the aorta (Bevan, 1962; Paterson, 1965). The main sympathetic perivascular plexus surrounding both the ovarian and the uterine vessels are post-ganglionic fibers derived from the periaortic plexus. They become incorporated into the adventitia of these vessels at their points of origin and, therefore, bypass the hypogastric nerves. The uterine artery also receives additional post-ganglionic sympathetic fibers from the pelvic plexus (Shabanah et al., 1964). No parasympathetic (cholinergic) post-ganglionic fibers have been demonstrated to innervate uterine blood vessels (Rothe, 1971).

The neurotransmitter released at most post-ganglionic sympathetic nerve endings is norepinephrine (NE), thus, these nerves are referred to as adrenergic. In the arterial wall, adrenergic nerve terminals are confined almost exclusively to the adventitio-medial junction (periarterial adrenergic nerves), from where NE diffuses throughout the entire media during sympathetic nerve stimulation (Dolezel, 1975). Norepinephrine released from periarterial adrenergic nerves acts on the smooth muscle cell through membrane bound receptors (α or β -adrenergic receptors). Stimulation of α -adrenergic receptors by NE results in vasoconstriction while stimulation of β -adrenergic receptors results in vasodilation. Vasoconstriction, mediated by α -adrenergic receptors, predominates in blood vessels of the abdominal viscera including those of the uterus and ovaries (Innes and Nickerson, 1970).

Arteries supplying the uterus of the ewe and cow are innervated by post-ganglionic sympathetic (adrenergic) nerves from each side of the spinal cord which freely decussate to individually supply both uterine horns (Sisson and Grossman, 1953). When these uterine periarterial adrenergic nerves were subjected to electrical stimulation, profound vasoconstriction occurred in the ovine uterine vascular bed, almost to the point of entirely compromising uterine blood flow (Greiss and Gobble, 1967). Sympathetic cholinergic (vasodilator) innervation of the ovine uterine vascular bed could not be demonstrated. These data, which suggest a vasoconstrictor function of uterine periarterial adrenergic neurons, are supported by the observation that infusion of NE, at very low doses, into ovine uterine arteries decreased uterine blood flow substantially (Barton et al., 1974). Further, vasoconstriction of the uterine vascular bed induced by stimulation of periarterial adrenergic neurons in anesthetized pregnant (Greiss and Pick, 1964) and nonpregnant (Ladner et al., 1970) ewes is mediated by α -adrenergic receptors; presence of β -adrenergic receptors were not demonstrated. The sympathetic innervation of the uterine artery of the ewe appears to differ from that of the dog (Ryan et al., 1974a) and guinea pig (Bell, 1968), both of which have vasoconstrictor (adrenergic) and vasodilator (cholinergic) innervation.

Recent evidence suggests that ovarian steroids may play a role

in controlling the vasoconstrictor function of periarterial adrenergic nerves, with subsequent alterations in blood flow. Treatment of ovariectomized ewes with estradiol diminished constriction of the uterine artery to infusion of NE compared to the response of the infused uterine artery of control ewes (Barton et al., 1974). Thus, one is tempted to postulate an effect of estradiol on diminishing the activity of α -adrenergic receptors with a subsequent increase in blood flow. In addition, estradiol may reduce arterial constriction by decreasing the content of neurotransmitter in adrenergic nerves (McKercher et al., 1973). These researchers observed that the increase in uterine blood flow following an injection of estradiol into ovariectomized rats was associated with a pronounced reduction in the NE content of uterine periarterial adrenergic nerves.

Progesterone appears to augment rather than reduce the responsiveness of vascular smooth muscle to catecholamines which may serve to explain the ability of this hormone to cause reduced uterine blood flow. Progesterone increases smooth muscle contractility in canine aortic strips to NE and epinephrine (E) by inactivating catechol-O-methyl-transferase, a major enzyme in catecholamine degradation (Kalsner, 1969). These data are in agreement with results reported by Kuhl et al. (1974) who observed that arteries supplying blood to ovaries bearing CL in ewes responded to NE and

E in vitro with greater smooth muscle contractility than arteries associated with ovaries without CL. There was also a tendency for arteries removed during the luteal phase (Day 13) of the estrous cycle to elicit greater in vitro responses to vasoactive biogenic amines than arteries removed on the day of estrus (Day 0). Evidence that estradiol, in addition to progesterone, may be required for maximal constriction of vascular smooth muscle of the dog was presented by Boxill and Brown (1955) who observed that the presence of both progesterone and estradiol appeared to be required for a maximal pressor response induced by doses of epinephrine injected into the jugular vein.

Prostaglandins (PG) of the E and F series have recently been implicated as having a role in regulating uterine blood flow. Ryan et al. (1974b) observed that administration of estrogen to the female rat induces formation of uterine prostaglandins (PGE and PGF) which appear to be necessary for estrogen-induced uterine hyperemia. Prostaglandin synthesis inhibitors prevented the estrogen-stimulated increases in uterine prostaglandins and blood flow. Contractility of arterial smooth muscle is stimulated by PGFs and depressed by PGEs with a subsequent decrease and increase in regional arterial blood flow, respectively (NaKano, 1971).

Although PGE₂ and PGF₂α are synthesized by the ovine uterus (Wilson et al., 1972) only PGF₂α has been demonstrated in uterine

venous blood of ewes (Thorburn et al., 1972) and cows (Shemesh and Hansel, 1975). Like estradiol and progesterone, prostaglandin $F_2\alpha$ has been implicated in regulation of adrenergic neurotransmission in blood vessels. Results reported by Kadowitz et al. (1972) suggest that intraarterial infusion of $PGF_2\alpha$ in the dog enhances smooth muscle contraction in the cutaneous vascular bed to sympathetic nerve stimulation without changing responsiveness to NE or tyramine. These data indicate that $PGF_2\alpha$ probably facilitates the release of neurotransmitter (NE) from periarterial adrenergic nerve terminals and appears to be specific for the readily releasable pool. In addition, Kadowitz et al. (1971) demonstrated that $PGF_2\alpha$ may potentiate vasoconstriction, in part, by blockade of neuronal reuptake of norepinephrine. Both of these studies indicate that $PGF_2\alpha$ elicits its constrictor effects on vascular smooth muscle by increasing the concentration of NE at the level of the effector cell. Thus, changes in uterine blood flow during various physiological states may be due to the concerted actions of $PGF_2\alpha$ and ovarian steroids on vascular smooth muscle contractility.

Prostaglandin $F_2\alpha$ may also be involved in controlling blood flow to the ovary, thus resulting in altered ovarian function. Concentrations of $PGF_2\alpha$ are increased in ovine endometrium (Wilson et al., 1972) and uterine venous plasma of ewes (Thorburn et al., 1972), cows (Shemesh and Hansel, 1975) and guinea pigs (Blatchley et al.,

1972) during the period of luteal regression. Prostaglandin $F_2\alpha$ may be the active luteolytic agent in these species since its administration results in premature luteal regression in the ewe (Thorburn and Nicol, 1971) and cow (Rowson et al., 1972). It has been suggested that localized control of luteal function in the ewe and cow is effected by a counter current transfer of $PGF_2\alpha$ from the utero-ovarian vein to the closely apposed ovarian artery in the ovarian pedicle (McCracken et al., 1971; Delcampo and Ginther, 1973; Goding, 1974).

Infusion of $PGF_2\alpha$ into the ovarian artery of the ewe reduces blood flow through the ovary and initiates luteolysis (McCracken et al., 1970; Baird, 1974). Blood flow to the luteal ovary declines during the normal period of luteal regression in the ewe (Niswender et al., 1975). These data suggest a possible effect of $PGF_2\alpha$ in promoting vasoconstriction of ovarian arterial smooth muscle, thus reducing ovarian blood flow. Recent evidence, however, indicates that the luteolytic effect of $PGF_2\alpha$ may also involve a selective action on the vascular system of the CL. Capillary blood flow to the ovine CL declines at the time of luteal regression (Niswender et al., 1973) and in response to infusion of $PGF_2\alpha$ into the uterine vein (Thorburn and Hales, 1972). To date, however, the exact mechanism of $PGF_2\alpha$ -induced luteolysis has not been determined.

Evidence implicating uterine periarterial adrenergic nerves in the physiological regulation of uterine blood flow during ovine pregnancy

was presented by Ladner et al. (1970), who observed that the non-pregnant uterus was more sensitive to the vasoconstrictor effects of a given intra-arterial dose of NE than the pregnant uterus. These data suggest a diminished activity of α -adrenergic receptors of the uterine vasculature during ovine pregnancy leading to the observed increase in uterine blood flow during this reproductive state. This apparent affect of pregnancy in altering the function of periarterial adrenergic nerves appears to involve a local uterine component. Greiss and Anderson (1970) implanted electromagnetic flow probes around the uterine arteries supplying blood to pregnant and nonpregnant uterine horns of ewes and observed an abrupt increase in uterine blood flow only to the cornus containing the conceptus on days 17 or 18 of pregnancy. Transient increases in blood flow to the pregnant horn were also observed on days 13, 14 or 15 in some ewes. These data suggest the possibility that the conceptus exerts a local effect on uterine blood flow in the ewe.

Surface to surface contact between the blastocyst and the wall of the uterus occurs about the 10th day post-mating in the ewe (Green and Winters, 1945). These researchers observed that the attachment appears to occur by means of an adhesive, mucin like material. Assketon (1906) reported that the surface epithilium of the caruncles in the ewe is distinctly degenerated by day 17. It has been demonstrated that the embryo must be present in the uterus of the ewe by

day 13 for continued maintenance of the CL and pregnancy (Moor and Rowson, 1966). These observations indicate that a feto-maternal reaction of some type occurs by day 13. It is of interest that the transient increases in blood flow to the cornus containing the conceptus observed by Greiss and Anderson (1970) on days 13 to 15 following mating occurred on days critical for ensuring prolongation of the lifespan of the CL.

A long standing enigma in reproductive biology is that of maternal recognition of pregnancy, the mechanism by which the developing conceptus signals its presence to the mother. An essential requirement for the establishment and maintenance of pregnancy is that the ovarian cycle be arrested and the function of the CL prolonged. In the cow, ewe and pig, the recognition signal has been transmitted even before the embryonic tissue becomes intimately attached to the uterine epithelium and is, therefore, clearly distinct from implantation. Preimplantation embryos (blastocysts) of the rat (Dickmann and Dey, 1974), mouse (Dey and Dickmann, 1974), hamster (Dickmann and Gupta, 1974) and pig (Perry et al., 1973) are capable of synthesizing steroid hormones. A local effect of hormones produced by the blastocyst may be concerned with the ongoing process of implantation or with the signal whereby the developing embryo conveys its presence to the mother.

The mechanism responsible for maintenance of a CL during early pregnancy, and in particular, the way in which the embryo influences this process, is not yet clearly understood. Evidence presented by Rowson and Moor (1967) indicates that the ovine embryo

produces a "luteotropic substance" very early in development. Daily intra-uterine infusion of a homogenate of frozen and thawed 14- or 15-day sheep conceptuses into ewes prolonged the functional life of the CL. That this luteotropic substance is blood borne and reaches the ipsilateral ovary by way of the uterine venous effluent from the gravid horn has been demonstrated by Mapletoft et al. (1976). These researchers surgically separated one uterine horn in bilaterally ovulating ewes to produce a nongravid uterine horn and the uterine vein on one side was anastomosed to the corresponding vein of the opposite side. Corpora lutea regressed when the adjacent uterine vein contained blood from only the nongravid horn, whereas the CL was maintained when the adjacent uterine vein contained venous blood from a gravid horn, regardless of whether it also contained blood from a nongravid horn.

It is unlikely that the luteotropic effect of pregnancy is caused by differences in exposure of the CL to $\text{PGF}_2\alpha$. Levels of $\text{PGE}_2\alpha$ in endometrium (Wilson et al., 1972), as well as uterine venous, ovarian venous and ovarian arterial blood (Pexton et al., 1975) were found to be increased in both pregnant and nonpregnant ewes at the time of CL regression in nonpregnant ewes. It is conceivable, therefore, that the early ovine conceptus synthesizes, or stimulates uterine synthesis of a substance which prevents uterine and ovarian vasoconstriction in response to $\text{PGF}_2\alpha$. This control of uterine and/or ovarian blood flow

by the embryo may function to create optimal conditions for the continuation of pregnancy.

Although ovarian steroids are known to elicit changes in uterine blood flow of pregnant and nonpregnant animals, their mechanism of action in inducing these changes has remained obscure. Current investigations, however, strongly indicate that a relationship exists between the function of adrenergic (vasoconstrictor) nerves innervating uterine and ovarian arteries and local concentrations of progesterone and estrogen.

STATEMENT OF THE PROBLEM

As the world population continues to grow at an ever increasing rate, it is vital that food production keep pace with the demand. Although research is being conducted to maximize our ability to produce food, new areas must continually be explored. Recently, the ability of animal protein to compete with plant protein as a source of human nutrients has been questioned. If domestic livestock are to maintain their rightful place as a vital part of the agricultural industry, continual improvement in efficiency of production must be made. Efficiency of livestock production might be accomplished by a number of different approaches, one of which could be determination and subsequent elimination of factors contributing to embryo mortality.

Recently, decreased uterine blood flow has been associated with the increased incidence of embryo mortality resulting from heat stress in the ewe. In addition, physical stress has been shown to result in a significant reduction in blood flow through the uterine vascular bed of the ewe, presumably due to altered function of periarterial sympathetic vasoconstrictor nerves. An adequate blood supply is necessary for the function of any organ, and would seem to be of extreme importance to the uterus which serves as the site of implantation and subsequent embryonic development. The possible effects of reduced uterine blood flow on the fetus are unknown, but may be of

vital importance during disease states known to compromise uterine circulation such as toxemia of pregnancy.

Ovarian steroids are known to alter blood flow through the uterine vascular bed of many mammalian species and may be responsible for the normal variations in uterine blood flow during the estrous cycle and pregnancy. The way in which steroid hormones elicit changes in uterine blood flow is unknown but may involve a local effect on periarterial adrenergic neurotransmission or vascular smooth muscle sensitivity to neurotransmitter. This hypothesis is based, in part, on a recent study which demonstrated that arteries supplying blood to ovaries bearing CL in nonpregnant ewes responded to periarterial sympathetic nerve stimulation and vasoactive biogenic amines with greater smooth muscle contraction in vitro than arteries associated with ovaries without CL. These data suggest a possible local effect of an ovary on controlling vasoconstriction and thus blood flow through the ipsilateral utero-ovarian vasculature.

Very little research has been conducted dealing with the physiology of uterine arterial smooth muscle. The following studies were conducted to determine the role, if any, of ovarian steroids (estradiol and progesterone) and prostaglandin $F_2\alpha$ in altering the contractile response of uterine arterial smooth muscle of pregnant and nonpregnant ewes and heifers. Increased knowledge concerning the control of uterine blood flow in these domestic species could provide more

insight into the problems associated with embryonic mortality and increase our understanding of ovarian function.

EXPERIMENT I: IN VITRO RESPONSE OF OVINE AND BOVINE
UTERINE ARTERIES TO PROSTAGLANDIN $F_2\alpha$ AND
PERIARTERIAL SYMPATHETIC
NERVE STIMULATION

Introduction

In the cow (Ginther et al., 1966) and ewe (Inskeep and Butcher, 1966), removal of the uterine horn ipsilateral but not contralateral to the ovary bearing the CL prolonged luteal lifespan. In attempting to determine the mechanism of uterine controlled luteolysis in these species, most research has been directed towards studying the possible role of the utero-ovarian vein and ovarian artery as the pathway by which the luteolysin reaches its site of action (Hansel and Echterkamp, 1972; McCracken et al., 1971; McCracken et al., 1972; DelCampo and Ginther, 1973). Concentrations of prostaglandins F are increased in uterine venous blood of ewes (Thorburn et al., 1972) and cows (Shemesh and Hansel, 1975) during the period of luteal regression. It has been proposed that $PGF_2\alpha$ may be the active luteolytic agent in these species since its administration results in premature luteal regression in the ewe (McCracken et al., 1970; Thorburn and Nicol, 1971) and cow (Rowson et al., 1972).

Few studies have been conducted on the physiology of smooth muscle of the uterine or ovarian vasculature of the cow and ewe. Kuhl et al. (1974) have demonstrated that arteries supplying blood to ovaries

bearing CL in ewes responded to norepinephrine, epinephrine or serotonin in vitro with greater increases in perfusion pressure than arteries associated with ovaries without CL. Further, there was a tendency for arteries removed during the luteal phase (day 13) of the estrous cycle to elicit greater in vitro responses to vasoactive biogenic amines than arteries removed on the day of estrus (day 0). It has also been suggested that prostaglandins may play a role in the regulation of adrenergic neurotransmission in blood vessels (Davies and Withrington, 1968; Davies et al., 1968; Hedquist, 1970).

The present experiments were undertaken to determine if uterine arterial segments removed from the side ipsilateral or contralateral to the ovary bearing the CL in pregnant and nonpregnant ewes and heifers responded differently to in vitro perfusion of $\text{PGF}_2\alpha$ and periarterial sympathetic nerve stimulation. A corollary experiment was conducted to determine whether a brei of the conceptus in uterine flushings from day 15 pregnant ewes could alter the in vitro responsiveness of uterine arterial segments of day 15 nonpregnant ewes to $\text{PGF}_2\alpha$ and nerve stimulation.

Materials and Methods

Ten Hereford heifers and 15 mature crossbred ewes, exhibiting estrous cycles of normal duration as determined by use of vasectomized males, were used in this study. All animals used were

unilaterally ovulating, which was determined at the time of necropsy. The first day of detected estrus for both ewes and heifers was designated as day 0. Five heifers and five ewes were assigned randomly for mating to intact males. At 0800 hr on day 15 post-estrus or-mating for ewes and day 17 post-estrus or-mating for heifers, jugular blood samples were taken by venipuncture and the animals sacrificed. Blood serum was frozen at -20°C until assayed for progesterone by use of radioimmunoassay techniques described by Koligian and Stormshak (1976). Verification of pregnancy was accomplished by flushing the uterus with saline to obtain the embryo and attached membranes. The conceptus in flushings was brought up to a volume of 5 ml with saline and drawn repeatedly through a fine gauge needle to form a fine mince of tissue (a brei) which was then frozen until utilized.

The uterine artery and adjacent ovary were dissected from each side of the uterus and placed into a container of oxygenated Krebs Henseleit Solution (25°C) of the following millimolar composition: NaCl, 154; KCl, 154; CaCl_2 , 110; KH_2PO_4 , 154; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 154; NaHCO_3 , 154; and glucose, 11 for transport to the laboratory. Upon arrival at the laboratory, tissue was immediately transferred to a container of continuously oxygenated Krebs Henseleit Solution (4°C) until prepared for perfusion. A 3.5 cm segment of the uterine artery supplying each uterine horn was removed immediately proximal to its

first bifurcation in the mesometrium, noting which segment was ipsilateral or contralateral to the ovary bearing the CL. The two uterine arterial segments from each animal were cannulated with polyethylene tubing and mounted in duplicate perfusion chambers (≤ 60 minutes after sacrifice of the animal) as depicted in Figure 1 to permit simultaneous treatment and monitoring. The experimental procedure used in this study was similar to that described by Kuhl *et al.* (1974) who perfused ovarian arterial segments from ewes. Krebs Henseleit Solution, equilibrated with 95% O₂-5% CO₂ at 37°C, was delivered by two Harvard infusion pumps to both preparations with an intraluminal perfusion rate of 17 ml/min for heifer arterial segments and 10 ml/min for ewe arterial segments. Intraluminal perfusion rates were determined from preliminary studies on uterine arteries from ewes and heifers and were based upon optimal responsiveness of arteries to periarterial sympathetic nerve stimulation. Extraluminal superfusion flow was 10 ml/min for arterial segments from heifers and ewes. Periarterial sympathetic nerves were excited by field stimulation. Square wave pulses of one msec duration and supramaximal voltage of 70V were applied through bipolar platinum electrodes located at each end of the perfusion chambers and delivered from a Grass Model SD5 stimulator. Changes in perfusion pressure arising from changes in resistance to flow through the arterial segments as a result of smooth muscle contraction were measured with

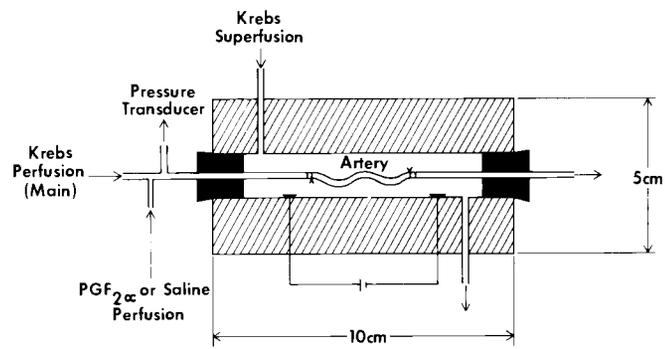


Figure 1. Schematic drawing of the perfusion apparatus (see text for details).

Statham pressure transducers and recorded in millimeters of mercury (mm Hg) by a Gould Brush 280 two channel recorder.

A 30-minute equilibration was allowed to establish a constant baseline perfusion pressure. After equilibration, arterial segments from ten heifers (five pregnant and five nonpregnant) and ten ewes (five pregnant and five nonpregnant) were subjected to three 30-minute perfusion periods with saline, $\text{PGF}_2\alpha$ and saline, respectively. The five remaining nonpregnant ewes were assigned to a corollary study in which arterial segments were subjected to four 30-minute perfusion periods with saline, conceptus brei in uterine flushings, $\text{PGF}_2\alpha$ and saline, respectively. Thirty-second electrical stimulations (10, 20 and 30 Hz, respectively) were applied at 10-minute intervals within each 30-minute perfusion period and maximal arterial responses above baseline perfusion pressure were recorded. A Harvard dual syringe infusion pump was used to administer $\text{PGF}_2\alpha$, conceptus brei in uterine flushings or saline solutions at a rate of .07 ml/min into the perfusate. The concentration of prostaglandin $\text{F}_2\alpha$ -tromethamine salt diluted by the perfusion fluid was constant at 1 ng/ml as it entered each arterial segment.

Data were analyzed statistically by use of least squares analysis of variance to determine: 1) the effect of sequential electrical stimulation during perfusion of saline or $\text{PGF}_2\alpha$ on arterial response and 2) whether in vitro responses of arteries from nonpregnant and

pregnant animals were affected by prior location of the artery within the animal (ipsilateral or contralateral to the ovary with the CL). Differences between means were tested by Least Significant Difference. Data on baseline perfusion pressures of arteries and concentrations of serum progesterone of nonpregnant and pregnant animals of each species were subjected to analysis of variance.

Data are presented in the form of histograms in which the ordinate represents mean changes in perfusion pressure of electrically stimulated arterial segments. Unless otherwise stated, each histogram represents the responses of ten arterial segments from five animals; five segments ipsilateral and five contralateral to ovaries with CL.

Results

Baseline perfusion pressures recorded at the end of equilibration are presented in Table 1. Uterine arteries ipsilateral to ovaries bearing CL from pregnant and nonpregnant ewes and heifers had greater ($P < .01$) baseline perfusion pressures than arteries removed from the contralateral side. It should be emphasized that $\text{PGF}_2\alpha$, conceptus brei in uterine flushings or saline perfusions without electrical stimulation did not alter the basal perfusion pressure of any arterial segments at the concentrations used in this study.

Uterine arteries ipsilateral to ovaries bearing CL in nonpregnant

Table 1. Baseline perfusion pressures of uterine arterial segments 30 minutes after cannulation and placement in perfusion chambers.

Species	Reproductive state	Location of artery relative to CL	No. arteries	Perfusion pressure (mm Hg) ^a
Ewe	Nonpregnant	Ipsilateral	10	39.8 ± 1.1*
		Contralateral	10	32.7 ± .9
	Pregnant	Ipsilateral	5	41.2 ± 1.3*
		Contralateral	5	33.2 ± 1.0
Heifers	Nonpregnant	Ipsilateral	5	40.8 ± 3.5*
		Contralateral	5	34.4 ± 2.8
	Pregnant	Ipsilateral	5	43.8 ± 4.9*
		Contralateral	5	34.2 ± .9

^aMean ± SE.

*Ipsilateral vs. contralateral artery, P < .05 within species.

heifers responded to sequential electrical stimulation (SES) with greater increases in smooth muscle contraction ($P < .01$) during all three perfusion periods than those removed from the contralateral side (Figure 2). When ipsilateral and contralateral arteries were perfused with $\text{PGF}_2\alpha$, perfusion pressure increased ($P < .01$) in response to SES compared to pressure changes in the same arteries during saline control perfusions before and after $\text{PGF}_2\alpha$. Changes in perfusion pressure during saline perfusions before and after $\text{PGF}_2\alpha$ for both types of arteries did not differ significantly. Arteries ipsilateral or contralateral to ovaries with CL in pregnant heifers (Figure 3), however, exhibited no differences in responsiveness to perfusion of $\text{PGF}_2\alpha$ and SES when compared to the responsiveness of these arteries during saline control perfusions before and after $\text{PGF}_2\alpha$.

The in vitro responsiveness of uterine arteries removed from nonpregnant ewes is presented in Figure 4. Similar to arterial responses of nonpregnant heifers, overall changes in smooth muscle contraction to SES of arteries ipsilateral to ovaries bearing CL were greater ($P < .01$) than those of arteries removed from the contralateral side during all three perfusion periods (saline or $\text{PGF}_2\alpha$). Only arteries contralateral to ovaries with CL displayed elevated perfusion pressures ($P < .05$) in response to SES during the $\text{PGF}_2\alpha$ perfusion when compared to the responsiveness to SES during respective saline control perfusions before and after $\text{PGF}_2\alpha$, which did not differ

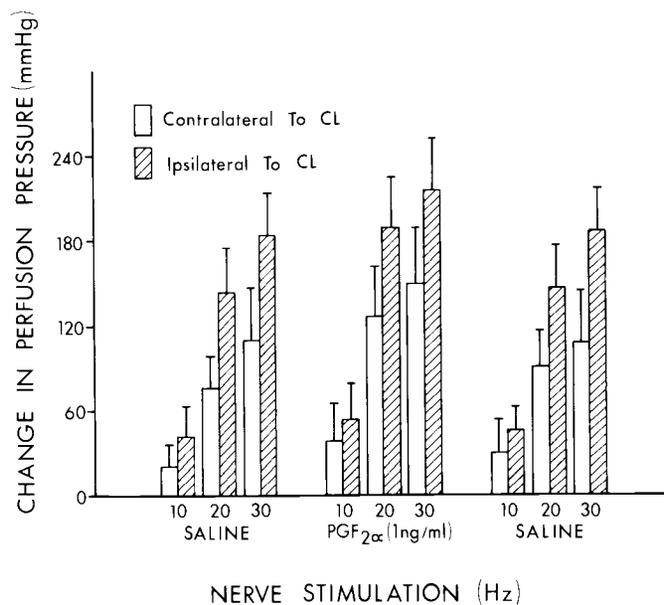


Figure 2. Effects of PGF_{2α} and sequential electrical stimulation on vasoconstriction of uterine arteries from nonpregnant heifers 17 days after detected estrus. Mean ±SE of five arteries.

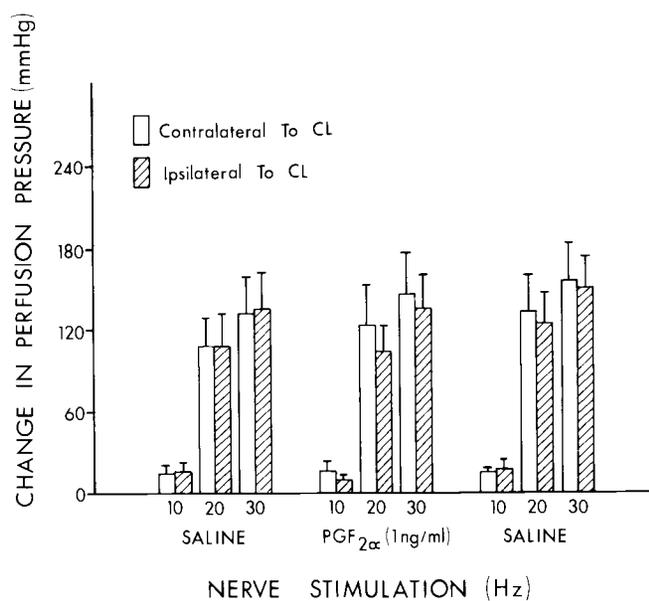


Figure 3. Effects of PGF_{2α} and sequential electrical stimulation on vasoconstriction of uterine arteries from pregnant heifers 17 days after detected estrus. Mean ±SE of five arteries.

significantly. Arteries ipsilateral to ovaries with CL responded to SES with an increase in contractility during perfusion with $\text{PGF}_2\alpha$ but the response was not significant statistically when compared to the arterial response to SES during respective saline perfusions.

During the first saline perfusion, the overall responsiveness to SES by arteries from pregnant ewes (Figure 5), removed ipsilateral to ovaries with CL, was greater ($P < .01$) than that of arteries removed from the contralateral side. During the $\text{PGF}_2\alpha$ perfusion and subsequent saline perfusion, however, no differences in responsiveness to SES between arteries removed from the ipsilateral and contralateral sides were detected. Arterial responsiveness to SES during perfusion of $\text{PGF}_2\alpha$ was depressed ($P < .01$), regardless of whether arteries were removed ipsilateral or contralateral to ovaries bearing CL when compared to changes in perfusion pressure elicited by stimulation of these same arteries during respective saline control perfusions. During the second saline perfusion, the responsiveness of arteries to SES from the luteal side only were depressed ($P < .05$) when compared to responses of the same arteries during the pre- $\text{PGF}_2\alpha$ saline control perfusion.

Uterine arteries from the remaining five nonpregnant ewes were utilized in a second study. The perfusion sequence of these arterial segments differed from that previously presented in that a 30-minute perfusion of the previously frozen conceptus brei in uterine flushings

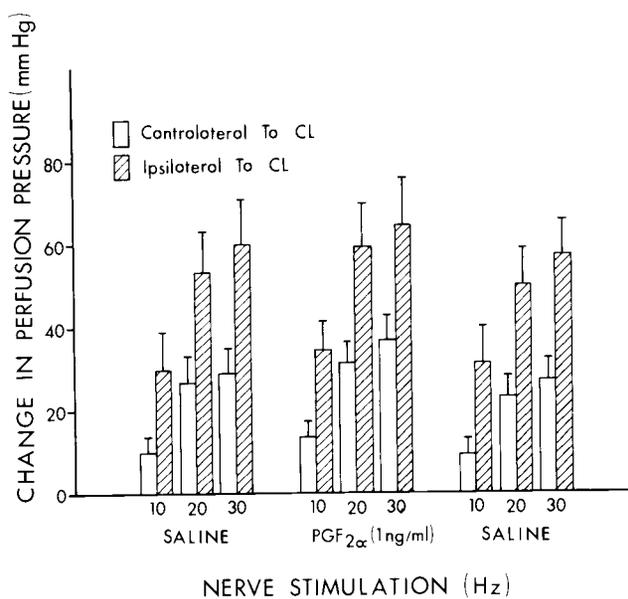


Figure 4. Effects of $\text{PGF}_2\alpha$ and sequential electrical stimulation on vasoconstriction of uterine arteries from nonpregnant ewes 15 days after detected estrus. Mean \pm SE of five arteries.

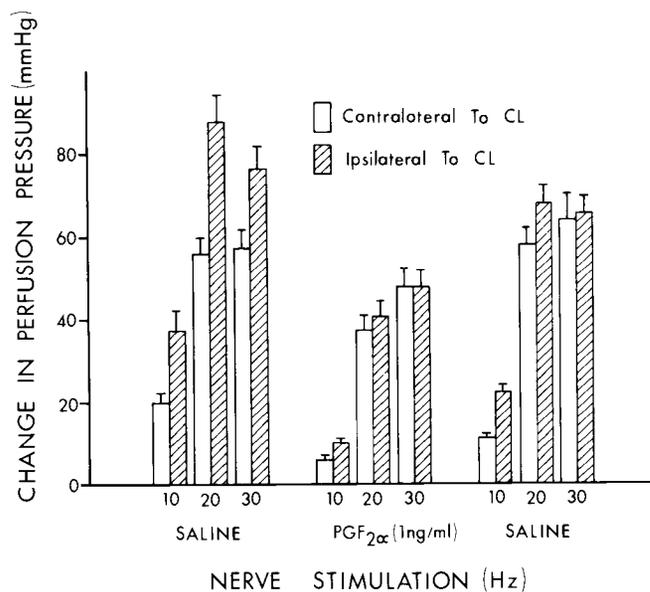


Figure 5. Effects of $\text{PGF}_2\alpha$ and sequential electrical stimulation on vasoconstriction of uterine arteries from pregnant ewes 15 days after detected estrus. Mean \pm SE of five arteries.

(one conceptus equivalent per pair of arterial segments from each animal) from day-15 pregnant ewes was administered between the first saline perfusion and perfusion of $\text{PGF}_2\alpha$. The entire perfusion sequence of these arterial segments is presented in Figure 6. Perfusion of conceptus brei in uterine flushings for 30 minutes had no effect on the responsiveness of arteries (ipsilateral or contralateral) from day-15 nonpregnant ewes to SES when compared to responses of these arteries during saline control perfusions. Similar to results of the first study, uterine arteries ipsilateral to ovaries bearing CL responded to SES with greater changes in smooth muscle contraction ($P < .01$) than arteries removed from the contralateral side during all perfusion periods (saline, conceptus brei in uterine flushings and $\text{PGF}_2\alpha$). There was a pronounced decrease ($P < .01$) in the responsiveness of arteries from both sides to SES during the $\text{PGF}_2\alpha$ perfusion when compared to responses during perfusions of saline and conceptus brei in uterine flushings. As shown previously (Figure 4), an increase in responsiveness to SES was exhibited by arteries from day-15 nonpregnant ewes during a similar $\text{PGF}_2\alpha$ perfusion when no preinfusion of conceptus brei in uterine flushings was administered, indicating an effect of this perfusate in altering arterial responsiveness to $\text{PGF}_2\alpha$.

Serum progesterone levels for all animals utilized in both studies are presented in Table 2. There was no difference in progesterone

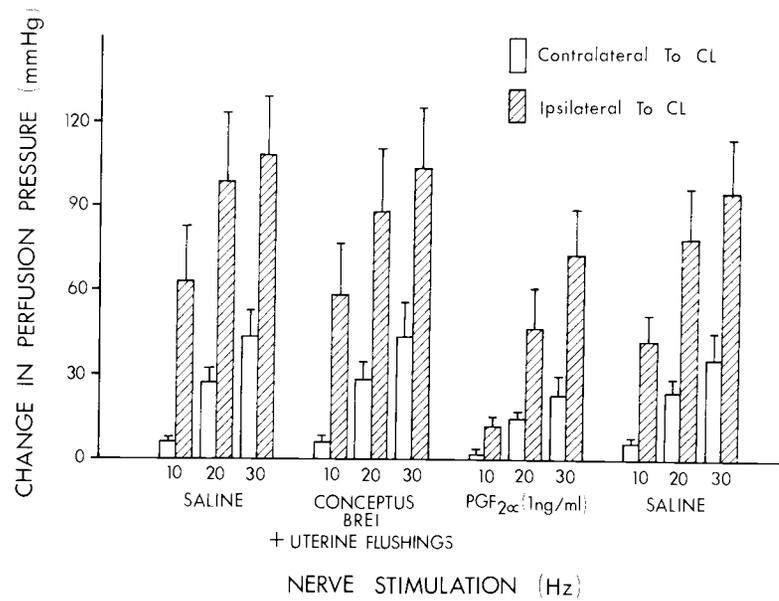


Figure 6. Effects of PGF₂α and conceptus brei in uterine flushings on vasoconstriction of uterine arteries from nonpregnant ewes 15 days after detected estrus. Mean ± SE of five arteries.

Table 2. Serum progesterone levels of ewes and heifers measured at the time of necropsy.

Species and condition	Days post-estrus	No. animals	Progesterone concentration (ng/ml) ^a
Ewes - nonpregnant	15	10	1.5 ± .3
Ewes - pregnant	15	5	2.5 ± .5*
Heifers - nonpregnant	17	5	2.1 ± 1.0
Heifers - pregnant	17	5	2.5 ± .9

^aMean ± SE.

*Pregnant vs. nonpregnant ewes, P < .05.

levels between pregnant and nonpregnant heifers 17 days post-estrus. Pregnant ewes, however, displayed elevated progesterone levels 15 days post-breeding ($P < .05$) when compared to nonpregnant animals on the same day post-estrus.

Discussion

The data presented herein indicate an association between the presence of the CL and physiological responses of vascular smooth muscle of uterine arteries, at least on the ipsilateral side in nonpregnant animals. These findings are in agreement with those of Kuhl et al. (1974) who found that the ovarian artery removed from the side ipsilateral to the ovary bearing the CL in nonpregnant ewes exhibited increased basal perfusion pressure and responsiveness to periarterial sympathetic nerve stimulation than the artery from the contralateral side. The mechanism whereby the presence of the corpus luteum is able to affect the responsiveness of vascular smooth muscle has not been determined.

Progesterone produced by the CL may, in some manner, have a local effect on vascular smooth muscle. This may account for the differences in responsiveness to SES by the uterine arteries ipsilateral and contralateral to ovaries with CL. Serum concentrations of progesterone indicate that the CL in all animals was indeed functional on the day of necropsy. Progesterone concentration in ovarian lymph of

ovaries containing CL during the luteal phase of the estrous cycle and during pregnancy of the ewe is very high; at times higher than in ovarian venous blood (Lindner et al., 1964). Further, these investigators demonstrated that rates of lymph flow from ovaries containing an active CL were much higher per unit weight of tissue than from any other organ studied in the ewe. Quantitatively, the amount of progesterone transported by the lymphatic system is quite small in relation to that transported by the blood stream, but this does not alter the fact that the local concentration of a hormone such as progesterone in the interstitial fluid of the reproductive organs may play a very significant role in regulating utero-ovarian function. In the ewe lymph flows from each uterine horn and ovary to the two pairs of internal iliac lymph nodes at the terminal bifurcation of the aorta (Meckley and Ginther, 1969). Since ovarian and uterine lymphatics from each side usually run in close apposition in the broad ligament and accompany the ipsilateral utero-ovarian vasculature (Morris and Sass, 1966) a local effect of progesterone on uterine arterial blood flow appears possible.

Greiss and Miller (1971) reported that injection of estrogen into one uterine horn of nonpregnant ewes significantly increased blood flow through the ipsilateral uterine artery, while blood flow through the contralateral uterine artery remains unchanged. In the present study, the greater increase in responsiveness to SES by the arterial segment removed from the side ipsilateral to the ovary

bearing the CL in heifers and ewes, as compared to that of the contralateral segment, may have been due to estrogen. For this premise to be true, synthesis and release of estrogen by the ovary with the CL would have to exceed that of the contralateral ovary. In unilaterally-ovulating ewes (Dufour et al., 1972) and in heifers (Rajakoski, 1970) follicular development has been reported to be greater in the ovary bearing the CL than in the opposite ovary. England et al. (1973) also demonstrated that on day 14 following estrus in cows there was an increased ($P < .05$) content and concentration of estradiol-17 β in the largest follicle of the ovary containing the functional CL when compared to the largest follicle in the contralateral ovary. Concentrations of estrogen in the ovarian venous and peripheral plasma of the ewe (Moore et al., 1969; Scaramuzzi et al., 1970) and peripheral plasma of the heifer (Hansel and Echterkamp, 1972) are increasing on day 15 and 17 of the cycle, respectively; times at which animals were necropsied in the present experiments. Concentrations of estrogen in ovarian lymph of the ewe during the mid-luteal phase of the cycle have been reported to be approximately 300 pg/ml, a level which is many fold greater than in peripheral serum (Lindner et al., 1964). The possibility of increased estrogen production by the CL-bearing ovary resulting in increased quantities of this steroid in the ipsilateral uterine lymph vessels, which could alter uterine arterial function, warrants further investigation.

Perfusion of $\text{PGF}_2\alpha$ through uterine arteries removed ipsilateral or contralateral to ovaries with CL in nonpregnant heifers and ewes caused a greater increase in arterial smooth muscle contraction in response to SES than did the perfusion of saline prior to or after $\text{PGF}_2\alpha$. Responses of arteries (ipsilateral or contralateral) to SES during control perfusion with saline prior to or after $\text{PGF}_2\alpha$ did not differ significantly. That this vasoconstrictor effect of $\text{PGF}_2\alpha$ may be mediated by biogenic amines was indicated by Kuhl et al. (1974). These researchers demonstrated that infusion of norepinephrine, epinephrine or serotonin into ovarian arterial segments from nonpregnant ewes resulted in an increase in smooth muscle contraction. Prostaglandin $\text{F}_2\alpha$ has been implicated in regulation of adrenergic neurotransmission in blood vessels (Kadowitz et al., 1971), as well as in altering ovarian arterial blood flow (Pharriss, 1970). Thus, $\text{PGF}_2\alpha$ may act to alter the synthesis and/or release of neurotransmitters from blood vessels with ultimate alterations in blood flow to the uterus and ovaries. The increased responsiveness of the ipsilateral arterial segments to SES during perfusion of $\text{PGF}_2\alpha$, as compared to that of the contralateral segment similarly perfused, suggests that the action of $\text{PGF}_2\alpha$ is enhanced by the presence of an ovary with a CL in nonpregnant animals.

When uterine arteries from pregnant animals were perfused with $\text{PGF}_2\alpha$, no change in responsiveness (heifers) or a decrease in

responsiveness (ewes) of the arteries to SES was observed, as compared to that during respective saline control perfusions before and after $\text{PGF}_2\alpha$. Thus, uterine arteries from nonpregnant ewes and heifers respond in vitro to the same concentrations of $\text{PGF}_2\alpha$ and SES differently than arteries from pregnant animals. It is unlikely that the differences in response of arteries between nonpregnant and pregnant animals were caused by differences in in vivo exposure to endogenous $\text{PGF}_2\alpha$. Pexton et al. (1975) found no differences in PGF levels in ovarian venous, ovarian arterial and uterine venous blood of pregnant and nonpregnant ewes on day 15 of the cycle. Concentrations of prostaglandins in uterine or ovarian lymph, however, have not been determined.

The conceptus by some as yet unknown mechanism is apparently able to control uterine blood flow. Greiss and Anderson (1970) implanted electromagnetic flow probes around the uterine artery of the pregnant or nonpregnant uterine horn of the ewe and observed an abrupt increase in uterine blood flow on days 17 or 18 of pregnancy to the cornus containing the conceptus. In the present study, perfusion of a brei of the day-15 ovine conceptus in uterine flushings through uterine arteries from day-15 nonpregnant ewes altered the responsiveness of the arteries to SES during subsequent perfusion with $\text{PGF}_2\alpha$. The fact that this modified response was similar to that observed when arteries from day-15 pregnant ewes were exposed to $\text{PGF}_2\alpha$, is also suggestive

of a local effect by the conceptus in regulating uterine blood flow. It is interesting to note that daily intrauterine infusions of a similar preparation of the conceptus in uterine flushings as used in this study caused maintenance of the CL in nonpregnant ewes up to 25 days post-estrus (Rowson and Moor, 1967). It is possible that a substance produced by the conceptus or early pregnant uterus may alter vascular responsiveness to $\text{PGF}_2\alpha$ with ultimate changes in blood flow to the uterus and/or ovaries, thus serving as one mechanism by which the maintenance of pregnancy is ensured.

EXPERIMENT II: RESPONSE OF OVINE UTERINE ARTERIES
TO NERVE STIMULATION AFTER PERFUSIONS OF
PROSTAGLANDIN F₂α, NOREPINEPHRINE OR
NEUROTRANSMITTER BLOCKING DRUGS

Introduction

Ovarian hormones appear to play a major, if not dominant, role in regulating uterine blood flow in the nonpregnant ewe (Greiss and Anderson, 1969). Uterine blood flow in the ovariectomized ewe increased after treatment with estradiol (Huckabee et al., 1970) and decreased following administration of progesterone (Caton et al., 1974a). The precise mechanism(s) by which estrogen and progesterone act to alter uterine blood flow is not known but may involve an effect of the steroid on neurotransmitter synthesis and/or release. This possibility is supported in part by the observation that injection of estradiol into ovariectomized rats reduced norepinephrine content of uterine periarterial adrenergic nerves, while at the same time increasing uterine blood flow (McKercher et al., 1973).

It has been also demonstrated that ovarian hormones act locally on the uterine vasculature. Greiss and Miller (1971) found that an injection of estrogen into the lumen of one uterine horn of the ovariectomized ewe increased blood flow through the ipsilateral but not the contralateral uterine artery. In Experiment I, utilizing only ewes with a CL present in one ovary on day 15 of the estrous cycle, the

uterine artery ipsilateral to the ovary bearing the CL was found to respond to in vitro periarterial nerve stimulation with greater smooth muscle contraction than the contralateral artery. The observed effect of the presence of a CL on the response of the uterine artery in vitro is presumed to be due to an increased local concentration of progesterone (Lindner et al., 1964). However, during the estrous cycle of the ewe greater follicular development tends to occur in the ovary bearing the CL (Dufour et al., 1972). Thus, an effect of a local concentration of estrogen on the uterine artery cannot be excluded. If these premises are true, responsiveness of the uterine artery to periarterial nerve stimulation at early to late stages of the estrous cycle would be expected to reflect the changing ratio of the local concentration of progesterone to estrogen.

Prostaglandin $F_2\alpha$, which is present in high concentrations in the ovine uterus (Thorburn et al., 1972; Wilson et al., 1972), has been implicated as a regulator of neurotransmission in blood vessels (Kadowitz et al., 1971; Kadowitz et al., 1972). Perfusion of uterine arteries of nonpregnant ewes with $PGF_2\alpha$ increased smooth muscle contraction to periarterial nerve stimulation with the artery ipsilateral to the ovary bearing the CL responding to a greater extent than the contralateral artery (Experiment I). These data suggest that uterine vasoconstriction or vasodilation may be a consequence of the local concentrations of ovarian hormones and $PGF_2\alpha$ acting to alter

sensitivity of vascular smooth muscle to neurotransmitters, function of adrenergic nerves or a combination of both processes.

In the present experiments, the influence of ovarian follicular development and the presence of a CL on in vitro contraction of uterine arterial smooth muscle following perfusion of $\text{PGF}_2\alpha$ and NE was examined at four stages of the estrous cycle of the ewe. The mechanism of action of $\text{PGF}_2\alpha$ on increasing arterial smooth muscle contractility was investigated, and the role of α - and β -adrenergic and cholinergic receptors in the contractile response was determined utilizing respective neurotransmitter blocking drugs.

Materials and Methods

Twenty mature crossbred ewes with estrous cycles of normal duration, as determined by twice daily checks for estrus with vasectomized rams, were utilized in this study. First day of detected estrus was designated as day 0 of the cycle. Ewes were assigned randomly in equal numbers to be necropsied on days 0, 3, 6 or 10 after detected estrus. At 0800 hr on the day of necropsy, jugular blood samples were taken by venipuncture and the animals sacrificed. Blood serum was frozen at -20°C until assayed for progesterone by radioimmunoassay (Koligian and Stormshak, 1976).

The uterine artery and adjacent ovary were dissected free from each side of the uterus and placed into a container of oxygenated

Krebs Henseleit Solution (25°C), as described in Experiment I. After transporting to the laboratory, tissue was maintained in continuously oxygenated Krebs Henseleit Solution (4°C) until prepared for perfusion.

A 3.5 cm segment of uterine artery supplying each uterine horn was removed immediately proximal to its first bifurcation in the mesometrium, noting which segment was ipsilateral and which segment was contralateral to the ovary containing the greatest follicular development. The position of the corpus luteum was also noted. Both ends of the two arterial segments were cannulated with polyethylene tubing while immersed in continuously oxygenated Krebs Henseleit Solution (25°C) and then mounted in duplicate perfusion chambers (≤ 60 minutes after sacrifice of the animal), as described in Experiment I. Krebs Henseleit Solution, equilibrated with 95% O_2 -5% CO_2 at 37°C , was delivered by two Harvard infusion pumps to both preparations with intraluminal and extraluminal perfusion rates of 10 ml/min.

A 30-minute equilibration was allowed to establish constant baseline perfusion pressures. After equilibration, drugs were perfused for 10-minute periods into the perfusate (Krebs Henseleit, 10 ml/min) of both arterial segments simultaneously at a rate of .07 ml/min using a Harvard dual syringe infusion pump. The order of drug perfusions and the final concentration of each drug in the perfusion fluid were as follows: 1) saline (vehicle); 2) prostaglandin

$F_2\alpha$ -tromethamine salt ($PGF_2\alpha$, 1 ng/ml); 3) saline; 4) L-norepinephrine (NE, 500 ng/ml); 5) phentolamine HCL (100 ng/ml); 6) $PGF_2\alpha$ (1 ng/ml); 7) NE (500 ng/ml); 8) NE (500 ng/ml); 9) propranolol HCL (100 ng/ml); 10) NE (500 ng/ml); 11) atropine sulfate (100 ng/ml) and 12) NE (500 ng/ml). Following each perfusion period periarterial sympathetic nerves were excited by field stimulation. Square wave pulses of 1 msec and 70 V were applied in 30-second trains with a frequency of 30 Hz through bipolar platinum electrodes located at each end of the perfusion chambers and delivered from a Grass Model SD5 stimulator. Changes in perfusion pressure arising from changes in resistance to flow through the arterial segments were measured with Statham pressure transducers and recorded in millimeters of mercury (mm Hg) by a Gould Brush 280 two channel recorder. Increases in perfusion pressure were considered to be a result of arterial smooth muscle contraction.

For purposes of microscopic measurement of arterial lumen diameter and wall thickness, a small portion of uterine artery adjacent to each ipsilateral and contralateral arterial segment was prepared for histological fixation with Bouin's solution and stained with Mallory's triple stain.

After examination of each ovary to determine the number and size of all follicles > 3 mm in diameter, the ovary was blotted dry and weighed to the nearest .1 gram. After weighing, CL were enucleated

from the ovarian stroma, weighed, and the remainder of the ovary was minced and blotted dry. The difference between the initial ovarian weight and the weight of the minced ovarian tissue plus the weight of the CL was used as an estimate of the follicular fluid weight.

Data were analyzed statistically by use of analysis of variance. Differences between means were tested by Least Significant Difference.

Results

At each stage of the cycle studied, one ovary of each ewe always contained the largest follicle and greatest follicular fluid weight ($P < .05$, respectively, Table 1). Uterine arteries adjacent to ovaries containing the greatest follicular development, hereafter referred to as ipsilateral arteries, had greater ($P < .05$) baseline perfusion pressures than arteries from the contralateral side only on day 10 after detected estrus (33.6 ± 1.9 and 27.8 ± 3.7 mm Hg, respectively, Table 1). All five ewes necropsied on day 10 also had a CL present in the ovary with the greatest follicular development. The ovary containing the largest follicle and greatest follicular fluid weight also contained a corpus albicans or a CL, respectively, in four of five ewes necropsied on each of days 0 and 3 and a CL in three of five ewes necropsied on day 6. Thus, the ovary containing the greatest follicular development contained a functional or regressed CL in 16 of the 20 ewes utilized.

Table 1. Uterine arterial baseline perfusion pressures and associated ovarian follicular characteristics.

Day of cycle	Position of artery	No. arteries	Arterial baseline perfusion pressure (mm Hg) ^a	Largest follicle (mm, dia.) ^a	Follicular fluid wt. (gms) ^a
0	Ipsilateral	5	31.8 ± 5.4	6.6 ± .2*	.54 ± .05*
	Contralateral	5	30.6 ± 4.7	5.0 ± .6	.41 ± .03
3	Ipsilateral	5	37.6 ± 2.0	5.2 ± .4*	.37 ± .04*
	Contralateral	5	41.6 ± 9.7	3.4 ± .9	.27 ± .04
6	Ipsilateral	5	36.4 ± 3.9	8.0 ± 2.1*	.81 ± .26*
	Contralateral	5	31.0 ± 3.7	2.4 ± 1.1	.34 ± .12
10	Ipsilateral	5	33.6 ± 1.9*	5.2 ± .5*	.44 ± .06*
	Contralateral	5	27.8 ± 3.7	3.4 ± .9	.28 ± .06

^aMean ± SE.

*Ipsilateral vs. Contralateral, P < .05.

Regardless of stage of the cycle, ipsilateral uterine arteries responded to electrical stimulation (ES) with elevated perfusion pressures ($P < .01$) when compared to responses of arteries from the contralateral side during all drug perfusions, except perfusions 5 (phentolamine) and 6 ($\text{PGF}_2\alpha$), during which basal responses were elicited by both arterial segments. Contralateral arteries responded to ES with increased smooth muscle contraction during the first perfusion period (saline). However, response of these arteries to ES decreased to a consistent low level following subsequent drug perfusions, as depicted in Figures 1, 2 and 3 (bottom panels). Ipsilateral uterine arteries from day-10 ewes (Figure 1, top panel) responded to ES with increased ($P < .01$) contractility following perfusions of saline, $\text{PGF}_2\alpha$ or NE (perfusion periods 1 to 4) when compared to responses to these perfusions by ipsilateral arteries from the other three groups of ewes (day 0, 3 and 6). Further, only ipsilateral arteries from day-10 ewes exhibited elevated ($P < .01$) perfusion pressures in response to ES following perfusion of $\text{PGF}_2\alpha$ (perfusion 2) when compared to responses following respective saline perfusions before and after $\text{PGF}_2\alpha$. Ipsilateral arteries from ewes necropsied 3 and 6 days after detected estrus (Figure 2, top panel), responded similarly to all drug perfusions and ES. Ipsilateral uterine arteries from ewes on days 3 and 6 responded with elevated contractility ($P < .01$) to ES following perfusions of saline, $\text{PGF}_2\alpha$ or NE (perfusion periods 1 to 4)

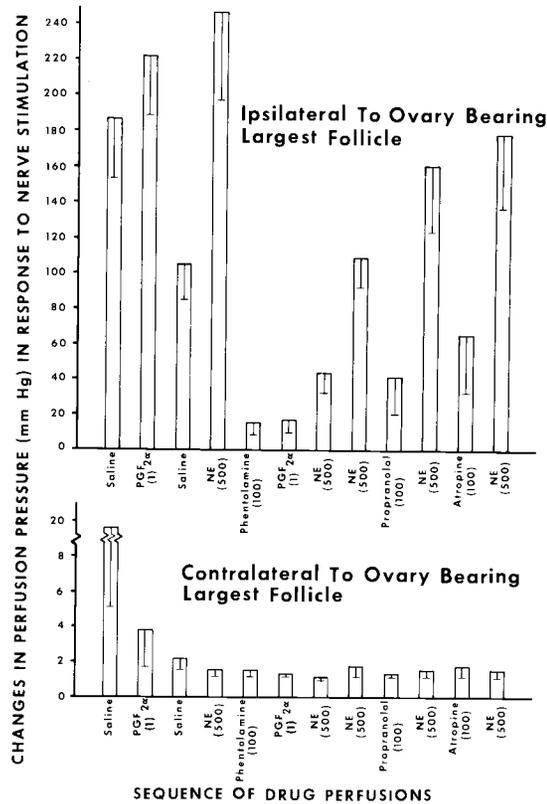


Figure 1. Effect of electrical stimulation on vasoconstriction of ipsilateral (top) and contralateral (bottom) uterine arterial segments from ewes 10 days after estrus following sequential 10 min drug perfusions. Each bar represents the mean and standard error of five arterial segments. Numbers in parenthesis are concentrations of drugs (ng/ml) in perfusion fluid.

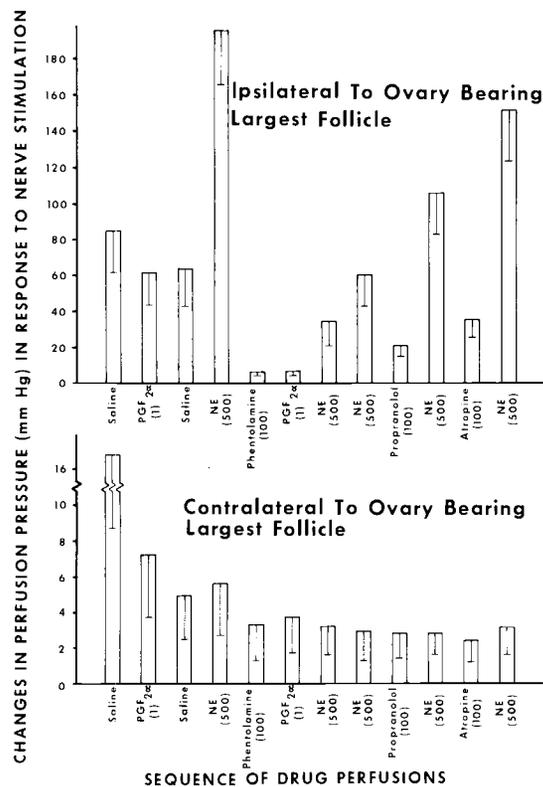


Figure 2. Effect of electrical stimulation on vasoconstriction of ipsilateral (top) and contralateral (bottom) uterine arterial segments from ewes three and six days after estrus following sequential 10 min drug perfusions. Each bar represents the mean and standard error of ten arterial segments. Numbers in parenthesis are concentrations of drugs (ng/ml) in perfusion fluid.

when compared to responses of similar arteries from estrous ewes (Figure 3, top panel).

Phentolamine, a competitive inhibitor of NE for α -adrenergic receptor sites, reduced smooth muscle contractility ($P < .01$) of ipsilateral uterine arteries to a basal level when subjected to ES. Subsequent perfusion of $\text{PGF}_2\alpha$ failed to elicit any increase in arterial responsiveness to ES over that exhibited during perfusion of phentolamine. Perfusion of ipsilateral arteries with NE after $\text{PGF}_2\alpha$ resulted in a return of contractility to ES ($P < .01$) when compared to responses evoked following perfusions of phentolamine or $\text{PGF}_2\alpha$ (perfusion periods 5 and 6, respectively). This gradual return in contractility to NE occurred in spite of intervening perfusions of propranolol and atropine (in that order). Propranolol produces selective blockage of β -adrenergic-receptor sites to the effects of stimulation and sympathomimetic agents. Atropine is a selective competitive antagonist of acetylcholine's muscarinic effects on vascular smooth muscle.

Serum progesterone levels for all animals utilized in the study are presented in Table 2. There was no significant difference in progesterone levels between ewes necropsied on days 0 and 3, although all ewes sacrificed 3 days after detected estrus had slightly elevated serum progesterone levels. By day 6, however, progesterone levels had increased ~ 3 -fold and by day 10 a 5-fold increase in this hormone

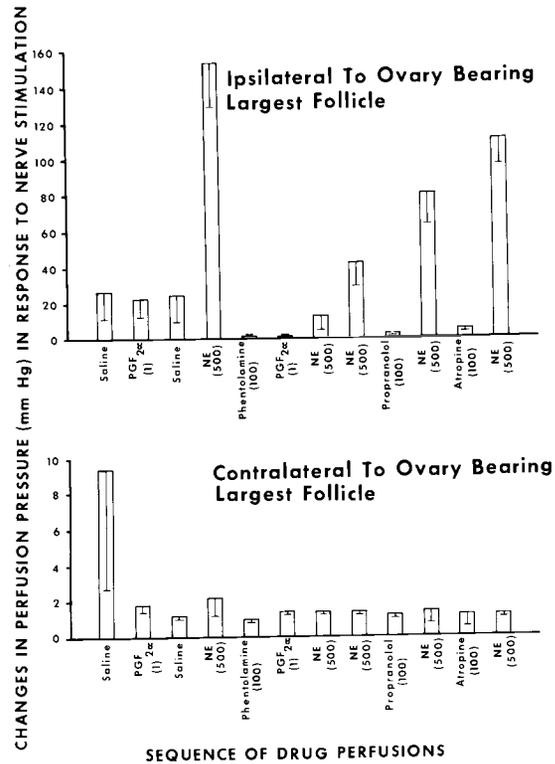


Figure 3. Effect of electrical stimulation on vasoconstriction of ipsilateral (top) and contralateral (bottom) uterine arterial segments from ewes in estrus following sequential 10 min drug perfusions. Each bar represents the mean and standard error of five arterial segments. Numbers in parenthesis are concentrations of drugs (ng/ml) in perfusion fluid.

Table 2. Serum progesterone levels of ewes at the time of necropsy.

Day of cycle	No. animals	Progesterone concentration (ng/ml)
0	5	.30 ± .01 ^a
3	5	.53 ± .04 ^a
6	5	1.09 ± .20 ^b
10	5	1.57 ± .13 ^c

a, b, c Means ± SE with different superscripts are significantly different, $P < .01$.

was observed.

Discussion

In the present study, baseline perfusion pressure was significantly increased only in uterine arteries ipsilateral to ovaries of ewes with the greatest follicular development on day-10 of the cycle. However, in these ewes the ovary with the greatest follicular development also contained a corpus luteum. Thus, the observed increase in baseline perfusion pressure may have been due to the presence of the corpus luteum exerting an influence through an increased local concentration of progesterone. Indeed, when baseline perfusion pressures of uterine arteries removed adjacent to ovaries containing CL were compared to those of arteries removed from the nonluteal side for all ewes in this study, regardless of follicular development, presence of the CL was found to have a marked influence on arterial response ($P < .01$, $38.0 \pm .9$ and $29.6 \pm .7$ mm Hg, respectively). These data on the effect of the presence of a CL on baseline perfusion pressure are consistent with those of the previous study (Experiment I) on the responses of uterine arteries removed from ewes on day 15 of the estrous cycle. The effect of the CL on increasing baseline perfusion pressure of arterial segments in the present study appears to be due to increased vascular tone since uterine arterial lumen diameter and thickness of the tunica media did not differ significantly between

ipsilateral and contralateral arteries among days of the cycle studied (overall means, 440 ± 20 and 300 ± 10 μ vs. 420 ± 20 and 300 ± 10 μ , respectively).

Results of the present experiment suggest that local concentrations of ovarian hormones may modulate the responsiveness of uterine arteries to electrical stimulation following perfusion of $\text{PGF}_2\alpha$ or NE. Ipsilateral uterine arteries exhibited increased in vitro responsiveness to ES following drug perfusion (saline, $\text{PGF}_2\alpha$ or NE) as compared to the response of the contralateral arteries. Due to the large percentage of ewes in which the same ovary contained a CL and the greater follicular development, the precise role of each hormone (estrogen or progesterone) could not be delineated in this study. The mechanism whereby ipsilateral uterine arteries are exposed to increased levels of estradiol and progesterone has been suggested by Lindner et al. (1964). These researchers demonstrated that rates of lymph flow from ovaries of ewes containing a functional CL were 50-fold higher than from ovaries with no CL. Quantitatively, levels of progesterone and estradiol were found to be many fold higher in the ovarian lymphatics than in peripheral serum. Since ovarian and uterine lymphatics run in close apposition in the broad ligament and accompany the ipsilateral utero-ovarian vasculature (Morris and Sass, 1966) a local effect of progesterone and/or estradiol from the ovary bearing the CL and largest follicle on ipsilateral uterine arterial

blood flow appears possible. Additional support for a local effect of ovarian hormones has been demonstrated by an observed association between ovarian follicular and luteal characteristics and uterine blood flow (Huckabee et al., 1968). The lowest uterine blood flow was observed when at least one ovary contained a large protruding CL with minimum follicular development, and the highest flows were obtained when one or both ovaries contained large follicles but no corpus luteum. Caton et al. (1974a) found that when progesterone was injected into ewes pretreated with estrogen and exhibiting elevated uterine blood flow rates, the rate of uterine blood flow declined. These investigators concluded that the effects of progesterone and estrogen on uterine blood flow were antagonistic in that the magnitude of blood flow changes appeared to be related to the ratio of the two ovarian hormones.

Contralateral arteries responded to the first saline perfusion and ES with increased contractility, but responsiveness to ES decreased to a low but consistent value following the remaining drug perfusions. The fact that these arteries initially responded to ES with increased contractility indicates a possible initial release of NE from periarterial adrenergic nerves with subsequent depletion and/or failure to release additional neurotransmitter. Evidence in support of a reduced NE content of contralateral uterine arteries was presented by McKercher et al. (1973). They demonstrated that

administration of estradiol into ovariectomized rats reduced the levels of NE in uterine periarterial adrenergic nerves. Since, in the present study, contralateral uterine arteries presumably would have only received local exposure to follicular estrogens through the ovarian lymph, depletion of NE from periarterial adrenergic nerves appears possible.

The increased responsiveness of ipsilateral uterine arteries to ES following perfusion of NE in this study may be a function of progesterone. An effect of endogenous progesterone on increasing ovarian arterial responsiveness was demonstrated by Kuhl et al. (1974). These researchers observed that arteries supplying blood to ovaries bearing CL in nonpregnant ewes responded to norepinephrine, epinephrine or serotonin in vitro with greater increases in perfusion pressure than arteries associated with ovaries without corpora lutea. There was also a tendency for arteries removed during the luteal phase (Day 13) of the estrous cycle to elicit greater in vitro responses to vasoactive biogenic amines than arteries removed on the day of estrus (Day 0). Kalsner (1969) demonstrated that progesterone elevated vascular smooth muscle contractility by inhibiting the action of catechol-O-methyl transferase on catecholamine degradation. These data are in agreement with the hypothesis that the increased smooth muscle contractility exhibited by ipsilateral arteries may be a function of progesterone stimulated increases in

NE content of periarterial sympathetic nerves. Further evidence that progesterone is involved in the potentiation of smooth muscle contractility of ipsilateral arteries is provided by the overall significant correlation ($r = .72$; $P < .01$) found to exist between arterial responsiveness to ES following initial perfusion of saline and serum progesterone levels.

A period of progesterone and/or estrogen exposure appears to be necessary before uterine arteries respond with elevated contractility to ES following perfusion of $\text{PGF}_2\alpha$. Ipsilateral arteries from day-10 ewes responded to ES with greater increases in smooth muscle contraction following perfusion of $\text{PGF}_2\alpha$ than after perfusions of saline prior to or after $\text{PGF}_2\alpha$. The concentration of $\text{PGF}_2\alpha$ perfused into the uterine arterial segments in this study is comparable to levels in the peripheral blood of the ewe (Coudert et al., 1972). It has been demonstrated (Nakano, 1971) that PGF 's increase systemic arterial and venous pressure with a corresponding decrease in regional arterial blood flow. Prostaglandin $\text{F}_2\alpha$ is known to potentiate vasoconstriction by enhancement of sympathetic neurotransmission. Results reported by Kadowitz et al. (1972) suggest that since intra-arterial infusion of $\text{PGF}_2\alpha$ enhances the response to nerve stimulation without changing responsiveness to NE or tyramine, it probably facilitates the release of neurotransmitter from sympathetic nerve terminals. This effect appears to be specific for the pool of transmitter released

by nerve stimulation. In the present study, $\text{PGF}_2\alpha$ was unable to enhance arterial smooth muscle contraction to ES following perfusion of phentolamine suggesting that the action of $\text{PGF}_2\alpha$ requires functional α -adrenergic receptors. These data indirectly support the aforementioned possibility that $\text{PGF}_2\alpha$ may be involved in regulating the release of NE by periarth sympathetic nerves.

In conclusion, it is tentatively proposed that progesterone and/or estrogen alters uterine arterial nerve concentration of NE and, thus, in concert with the regulatory action of $\text{PGF}_2\alpha$ on the release of NE, may serve to modulate the magnitude of arterial smooth muscle contraction with subsequent alterations in blood flow.

EXPERIMENT III: ROLE OF ESTRADIOL-17 β AND
PROGESTERONE IN ALTERING OVINE UTERINE
ARTERIAL CONTRACTILITY TO PERIARTERIAL
SYMPATHETIC NERVE STIMULATION

Introduction

Vasoconstriction of uterine blood vessels is generally attributed to the response of vascular smooth muscle to NE liberated from innervating adrenergic nerves (Chusid, 1973). It has been demonstrated that ovarian steroids can regulate uterine blood flow in the ewe (Greiss and Anderson, 1969). Treatment of ovariectomized ewes with progesterone caused vasoconstriction (Caton et al., 1974a), while administration of estradiol promoted vasodilation (Huckabee et al., 1970) in the uterine vascular bed. The precise mechanism of action of ovarian steroids in regulating uterine blood flow is not known. McKercher et al. (1973) observed that administration of estradiol to ovariectomized rats reduced the level of NE in uterine periarterial adrenergic nerves while increasing uterine blood flow. On the other hand, treatment of ovariectomized ewes with estradiol reduced uterine arterial vasoconstriction in response to an intraarterial dose of NE (Barton et al., 1974) suggesting a reduced sensitivity of the smooth muscle cell to the catecholamine. Progesterone, in contrast to estradiol, appears to augment the vasoconstrictor effects of stimulated uterine periarterial adrenergic nerves. Uterine arteries removed

ipsilateral to ovaries bearing CL in unilaterally ovulating nonpregnant ewes, responded with greater smooth muscle contractility to nerve stimulation (Experiment I) and perfusion of NE (Experiment II) than uterine arteries from the nonluteal side. These data suggest that exposure of the ovine uterine vascular bed to increasing concentrations of progesterone and/or estrogen may affect the function of uterine periarterial adrenergic nerves, the sensitivity of the smooth muscle cell to adrenergic neurotransmitter, or both.

Prostaglandin $F_2\alpha$, which is produced in high concentrations in the uterus of the ewe (Thorburn et al., 1972; Wilson et al., 1972) is a potent vasoconstrictor. It has been suggested that $PGF_2\alpha$ promotes vasoconstriction by facilitating the release of NE from sympathetic nerve terminals (Kadowitz et al., 1972). Results of Experiments I and II indicate that exposure of uterine arteries to progesterone potentiates the vasoconstrictor effects of $PGF_2\alpha$ on vascular smooth muscle in response to nerve stimulation. Thus, changes in uterine blood flow during various physiological states may be due to the concerted actions of $PGF_2\alpha$ and ovarian steroids on vascular smooth muscle contractility.

The present experiment was undertaken to determine the effect of treatment of ovariectomized ewes with estradiol-17 β and/or progesterone on in vitro smooth muscle responses of uterine arteries to perfusion of NE, $PGF_2\alpha$ and periarterial nerve stimulation. The

role of α - and β -adrenergic receptors in mediating the effects of steroids and $\text{PGF}_2\alpha$ on arterial smooth muscle contraction was also studied using neurotransmitter blocking drugs.

Materials and Methods

Sixteen mature crossbred ewes, having been ovariectomized for at least 60 days, were utilized in this study. Animals were assigned randomly in equal numbers to four groups and were treated as follows: 1) control; 2) estradiol-17 β ; 3) progesterone and 4) estradiol-17 β and progesterone. Estradiol-17 β (54 ± 3 mg) was placed into a silastic capsule (ID x length; 3.35 x 15 mm, respectively) and implanted subcutaneously at 0900 hr on day 0, where it remained throughout the experimental period. Twice daily (0900 and 2100 hr) intramuscular injections of 15 mg progesterone dissolved in one ml of sesame oil were initiated 48 hr after implantation and continued until 12 hr prior to necropsy at 0900 on day 7. Control and P_4 -treated ewes were implanted with an empty capsule and control and E_2 -treated ewes also received an injection of vehicle. Jugular blood was taken by venipuncture from each ewe at 24 hr intervals (0900) from day 0 until the day of necropsy. Serum was frozen at -20°C until assayed for progesterone (Koligian and Stormshak, 1976) and estradiol-17 β utilizing radioimmunoassay (RIA) techniques. Estradiol was assayed utilizing the RIA procedure described by Wu and Lundy (1971), with several

modifications which allowed a more sensitive determination of estradiol in ovine serum (K. B. Koligian, personal communication). The inter-assay precision and the accuracy of the estradiol RIA were determined by adding incremental amounts of estradiol (2.5, 5, 10, 15, 25 and 75 pg/ml, N=4) to a serum pool obtained from anestrous ewes ($1.6 \pm .1$ pg/ml, N=4). After RIA and the subtraction of the pooled serum, the following values were derived: $3.0 \pm .3$, $4.6 \pm .2$, 8.7 ± 1.2 , 14.4 ± 1.2 , 26.2 ± 2.4 and $71.4 \pm .6$ pg/ml, respectively. Sensitivity was determined to be approximately 2 pg/ml. Solvent blanks from independent assays (N=4) averaged $.8 \pm .1$ pg/tube.

The uterine artery and associated mesometrium was excised from each side of the uterus and placed immediately into a container of oxygenated Krebs Henseleit Solution (25°C). At the laboratory, tissue was transferred to continuously oxygenated Krebs Henseleit Solution (4°C) until utilized. A 3.5 cm segment of uterine artery supplying each uterine horn was removed immediately proximal to its first bifurcation in the mesometrium. Segments were cannulated at both ends with polyethylene tubing and mounted in duplicate perfusion chambers (≤ 60 minutes after sacrifice of the animal) as described in Experiment I.

Krebs Henseleit Solution, equilibrated with 95% O_2 -5% CO_2 at 37°C was delivered by two Harvard infusion pumps to both preparations with intraluminal and extraluminal perfusion rates of 10 ml/min.

Consecutive ten minute drug perfusions were begun after a 30-minute equilibration period during which constant baseline perfusion pressures were established. Drugs were perfused into the perfusate (Krebs Hanseleit Solution, 10 ml/min) at a rate of .07 ml/min using a Harvard dual syringe infusion pump. The order of drug perfusions and the final concentration of each drug in the perfusion fluid were as follows: 1) saline (vehicle); 2) prostaglandin $F_2\alpha$ -tromethamine salt (PGF $_2\alpha$, 1 ng/ml); 3) saline; 4) L-norepinephrine (NE, 500 ng/ml); 5) phentolamine HCl (100 ng/ml); 6) PGF $_2\alpha$ (1 ng/ml); 7) NE (500 ng/ml); 8) NE (500 ng/ml); 9) propranolol HCl (100 ng/ml); and 10) NE (500 ng/ml). Following each perfusion period periarterial sympathetic nerves were excited by field stimulation. Square wave pulses of 1 msec duration and 70 V were applied in 30-second trains with a frequency of 30 Hz through bipolar platinum electrodes located at each end of the perfusion chambers. Changes in perfusion pressure due to changes in resistance to flow through the arterial segments were measured with Statham pressure transducers and recorded in millimeters of mercury (mm Hg) by a Gould Brush 280 two channel recorder. Increases in perfusion pressure were considered to be a result of arterial smooth muscle contraction.

Responses of both arterial segments from each ewe were averaged for purposes of statistical analyses. Data on the effect of treatment on arterial contractility (mm Hg) and concentrations of

estradiol-17 β (pg/ml) and progesterone (ng/ml) in systemic blood were analyzed statistically by use of split-plot analysis of variance. Differences between means were tested by Least Significant Difference.

Results

Serum concentrations of estradiol-17 β in all ewes receiving estradiol were significantly greater ($P < .05$) than levels of this steroid in control or progesterone-treated ewes (overall means, 6.5 ± 1.5 vs. $3.2 \pm .2$ pg/ml, respectively). Ovariectomized ewes receiving injections of progesterone had increased ($P < .05$) serum concentrations of this hormone compared to that of all ewes receiving injections of oil vehicle (overall means, 7.90 ± 2.00 vs. $.35 \pm .04$ ng/ml, respectively). There was no effect of treatment on baseline perfusion pressures (31.1 ± 1.0 mm Hg) of uterine arterial segments. Treatment of ewes with progesterone increased ($P < .01$), while treatment with estradiol decreased ($P < .01$) smooth muscle contraction of arterial segments to ES following perfusions of saline, PGF₂ α or NE (perfusions 1 to 4) compared to responses of arteries removed from controls or ewes treated with estradiol and progesterone (Figure 1). Response of arteries from control and estradiol + progesterone-treated ewes did not differ after perfusion of any drug or the vehicle. Arterial segments from all ewes exhibited increased contractility ($P < .01$) to

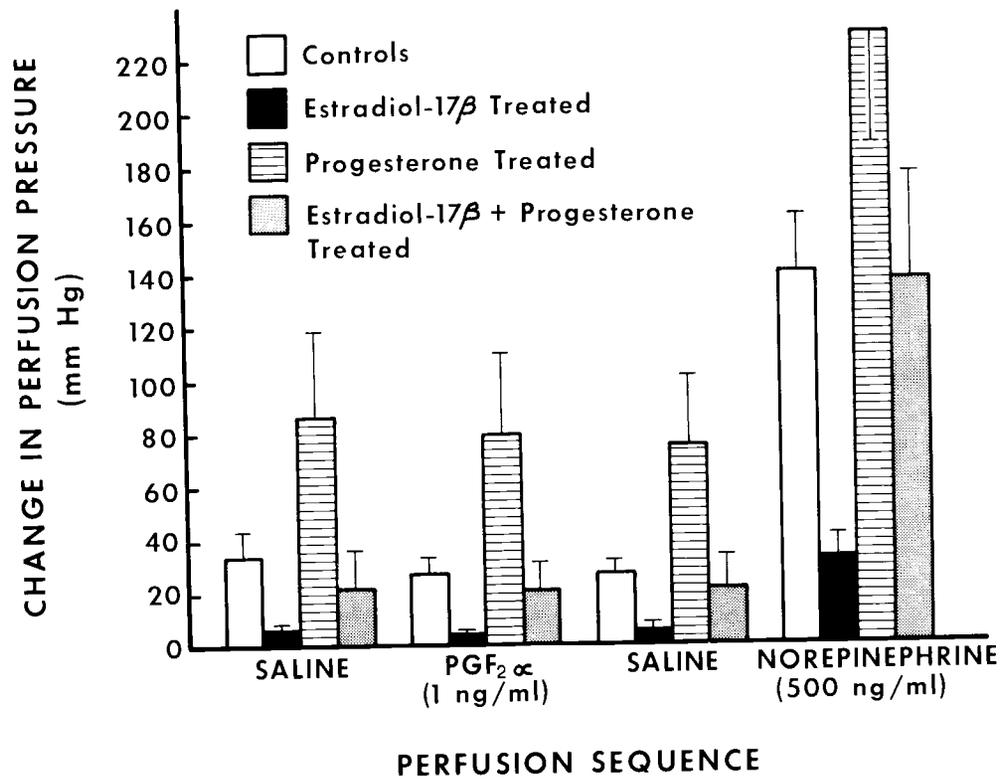


Figure 1. Effects of electrical stimulation (30 Hz) following sequential 10 min perfusions (perfusions 1 to 4) on vasoconstriction of uterine arterial segments from control or steroid-treated ovariectomized ewes. Each bar represents the mean and standard error of arterial segments from four ewes.

ES following initial perfusion of NE (perfusion 4) when compared to respective responses to ES following perfusions of saline, $\text{PGF}_2\alpha$ and saline (perfusions 1 to 3). Perfusion of $\text{PGF}_2\alpha$ failed to effect the response of smooth muscle to ES as compared to responses evoked following the perfusion of saline before and after $\text{PGF}_2\alpha$.

Responsiveness of uterine arterial segments to ES following perfusion of phentolamine, $\text{PGF}_2\alpha$, NE, NE, propranolol and NE (perfusions 5-10) are presented in Figure 2. Phentolamine (competitive blocker of α -adrenergic receptors) reduced responsiveness ($P < .01$) of all arterial segments to a consistent low level. Subsequent perfusion of $\text{PGF}_2\alpha$ (perfusion 6) failed to elicit any increase in smooth muscle contractility of arterial segments to ES over that exhibited during perfusion of phentolamine. Repeated perfusions of NE after $\text{PGF}_2\alpha$ resulted in a return of responsiveness to ES of arterial segments from all ewes, except those of estradiol-treated ewes. This gradual return in responsiveness to NE occurred in spite of an intervening perfusion of propranolol which produces selective blockage of β -adrenergic-receptor sites to the effects of stimulation and sympathomimetic agents.

Discussion

Uterine arterial segments from progesterone-treated ovariectomized ewes exhibited increased smooth muscle contractility to

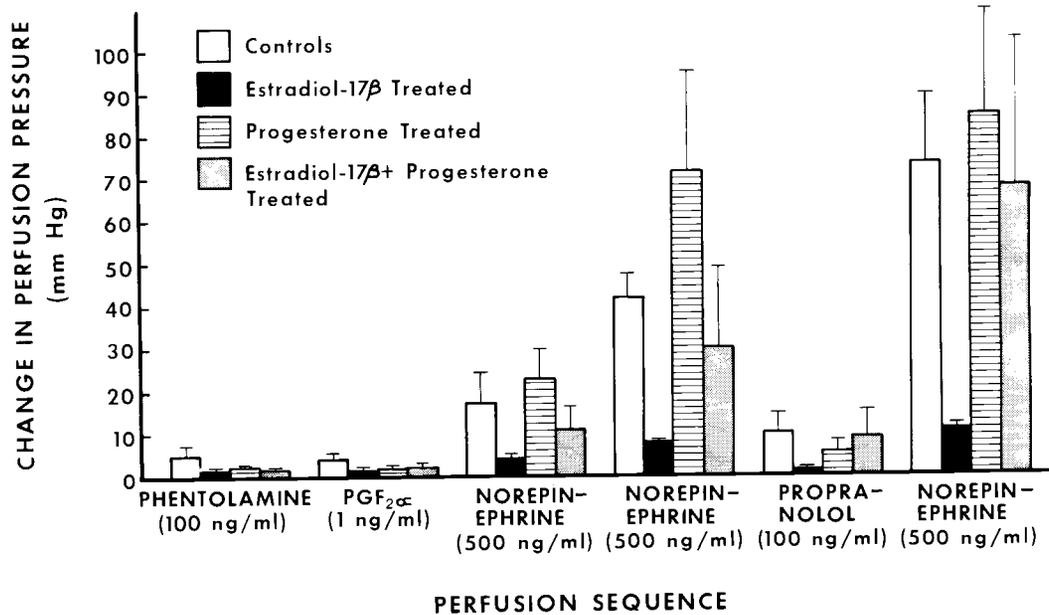


Figure 2. Effects of electrical stimulation (30 Hz) following sequential 10 min perfusions (perfusions 5 to 10) on vasoconstriction of uterine arterial segments from control or steroid-treated ovariectomized ewes. Each bar represents the mean and standard error of arterial segments from four ewes.

ES following perfusions of saline, $\text{PGF}_2\alpha$ and NE (perfusions 1 to 4) when compared to responses of arteries from non-treated control ewes. These data are in agreement with results of Experiment I which utilized only ewes with a CL present in one ovary on day 15 of the estrous cycle. This experiment demonstrated that uterine arteries ipsilateral to ovaries bearing CL responded to in vitro periarterial nerve stimulation with greater smooth muscle contraction than the contralateral arteries. Further, as progesterone levels increase throughout the luteal phase of the ovine estrous cycle, contractility of uterine arterial segments ipsilateral to ovaries containing CL increase in a parallel fashion (Experiment II). The observed effect of the presence of a CL on the response of the ipsilateral uterine artery in vitro was presumed to be due to an increased local exposure of the artery to progesterone in the ovarian lymph (Lindner et al., 1964). The ability of progesterone to enhance vasoconstriction may involve increased exposure of the smooth muscle cells to norepinephrine. Progesterone increased smooth muscle contractility of aortic strips to NE by inhibiting the action of catechol-0-methyl transferase, thus reducing the rate of catecholamine degradation (Kalsner, 1969). However, the possibility of the enhanced in vitro response of uterine arteries from progesterone-treated ewes being due to the ability of this steroid to increase levels of NE in periarterial adrenergic nerves or alter sensitivity of the smooth muscle cell to neurotransmitter

cannot be excluded.

Only those uterine arterial segments from ovariectomized ewes treated with estradiol alone exhibited depressed contractility to ES following perfusions of saline, $\text{PGF}_2\alpha$ and saline, in that order, as compared to arterial responses of control ewes. Reduced responsiveness of uterine arteries from estradiol-treated ewes to ES may be due, at least in part, to an estradiol-induced depletion of norepinephrine from adrenergic nerves. McKercher et al. (1973) reported that injection of estradiol into ovariectomized rats reduced the NE content of uterine periarterial adrenergic nerves with a resultant increase in uterine blood flow. Exposure of uterine arterial smooth muscle to estradiol may also result in a diminished activity of α -adrenergic receptors which have been shown to mediate the effects of NE on vasoconstriction in the ovine uterine vascular bed (Greiss and Pick, 1964). This possibility is supported by data from the present experiment in which treatment of ewes with estradiol reduced arterial contractility to ES following perfusion of NE (perfusion 4) when compared to responses of control arteries to this neurotransmitter. These data are similar to those of Barton et al. (1974) who demonstrated that treatment of ovariectomized ewes with estradiol reduced vasoconstriction of the uterine artery to an intra-arterial dose of norepinephrine.

The fact that uterine arteries removed from ewes receiving estradiol + progesterone responded to perfusions and stimulations

with responses similar to those from controls demonstrate an apparent antagonism between the two ovarian steroids. Evidence for an antagonistic effect of estrogen and progesterone on altering uterine blood flow has been presented by Caton et al. (1974a). These researchers demonstrated that estradiol-stimulated increases in uterine blood flow in ewes were decreased by administration of progesterone. The magnitude of induced changes in uterine blood flow in the above study appeared to be related to the ratio of the two ovarian steroids. Blood levels of estradiol and progesterone in the present study were maintained in a physiological range (Obst et al., 1971; Thorburn et al., 1969). It thus appears that the differing effects of these steroids on altering uterine arterial contractility, may play an important role in controlling blood flow to the uterus during the ovine estrous cycle and pregnancy.

Uterine arterial segments from all ewes, regardless of treatment, failed to respond to ES with increased smooth muscle contractility following perfusion of $\text{PGF}_2\alpha$ (perfusion 2) when compared to responses evoked following saline perfusions before and after prostaglandin $\text{F}_2\alpha$. Previous research (Experiment II) demonstrated that uterine arterial segments ipsilateral to ovaries bearing CL in unilaterally ovulating ewes acquired sensitivity to the vasoconstrictor effects of $\text{PGF}_2\alpha$ between 6 and 10 days after estrus. Although the length of exposure of ovariectomized ewes to progesterone was

similar in this study, local exposure of uterine arteries to this steroid would have been considerably reduced. This conclusion is based on the fact that ovarian lymphatics, which accompany the ipsilateral utero-ovarian vasculature in the ewe (Morris and Sass, 1966), contain lymph concentrations of progesterone which are as high or higher than those found in ovarian venous blood (Lindner et al., 1964). Further evidence supporting a reduced local exposure of uterine arteries to progesterone in this study, is indicated by the failure of arterial segments from progesterone-treated ewes to exhibit elevated baseline perfusion pressures. Results of the two previous studies (Experiments I and II) demonstrated a local effect of the CL on increasing baseline perfusion pressures of ipsilateral uterine arterial segments from unilaterally ovulating ewes. These data, however, do not preclude the possibility that some factor in addition to progesterone and estradiol, secreted by the luteal ovary, may function to regulate ipsilateral arterial smooth muscle contractility in response to prostaglandin $F_2\alpha$.

Blockade of α -adrenergic receptors with phentolamine reduced uterine arterial contractility to a consistent low level, regardless of which treatment the ewe had received. These data indicate that periarterial α -adrenergic receptors play an active role in modulating steroidal-induced changes in uterine arterial contractility in vitro. Perfusion of $PGF_2\alpha$ after phentolamine did not increase arterial

contractility to ES when compared to arterial responsiveness exhibited during phentolamine perfusion. Thus $\text{PGF}_2\alpha$, as well as ovarian steroids, appear to require the presence of functional α -adrenergic receptors to elicit their effects.

Repeated perfusions of uterine arteries with NE after phentolamine, except those from ewes receiving estradiol only, exhibited an almost linear return in contractility to ES as phentolamine was displaced from α -adrenergic receptors. This observation supports the premise that estradiol may function to reduce the activity of α -adrenergic receptors. The exact mechanism whereby estradiol reduces responsiveness of arteries to exogenous NE, however, needs further elucidation. Perfusion of propranolol had no apparent effect on the return of arterial contractility to NE after phentolamine perfusion. Thus, functional β -adrenergic receptors do not appear to be required for NE stimulated increases in in vitro arterial contractility.

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