AN ABSTRACT OF THE THESIS OF

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Title: A STUDY OF SOME PHYSIOLOGICAL PROPERTIES OF OVINE AND BOVINE UTERO-OVARIAN ARTERIES

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The utero-ovarian vasculature of domestic animals has been a popular research subject, especially concerning the roles of the utero-ovarian artery and vein in the unilateral luteolytic phenomenon demonstrated in these animals. No reports in the literature, however, have been found regarding the smooth muscle physiology of these vessels.

The present study was undertaken to investigate some of the basic physiological properties of the utero-ovarian arteries from sheep and beef. Perfusion chambers equipped with platinum electrodes were utilized to examine the response characteristics of these vessels to exogenously administered pharmacologic agents and to periarterial sympathetic nerve stimulation. Also, a preliminary fluorescence histochemical study was made of a pair of bovine utero-ovarian arteries..

In sheep, an association between the presence of a corpus luteum and the physiological responses of utero-ovarian arteries was demonstrated. Arteries serving the ovaries bearing corpora lutea responded to norepinephrine, epinephrine, and serotonin with significantly greater changes in perfusion pressure than did the arteries associated with ovaries not having a luteal body. Vasoconstrictor responsiveness to periarterial sympathetic nerve stimulation agreed with these findings. In addition, baseline perfusion pressures were elevated for the arteries from the corpus luteum side of the animal. The hormonal state of the sheep at the time of sacrifice also appeared to influence the vascular smooth muscle. Utero-ovarian arteries removed during the luteal phase (Day 13) of the estrous cycle elicited greater responses to vasoactive biogenic amines than the arteries removed at the time of ovulation (Day 0), but due to small sample size and extreme intra-animal variability, it was not possible to show these differences to be significantly different.

Using utero-ovarian arteries of bovine origin, a similar relationship between the presence of a corpus luteum and the physiological properties of the smooth muscle was demonstrated. The arteries serving corpus luteum-containing ovaries responded with significantly greater vasoconstrictor responses to each of the biogenic amines administered at various doses. The data for sympathetic nerve stimulation suggested similar findings with the arterial segments from the

corpus luteum side eliciting greater changes in perfusion pressure. As in sheep, the mean initial baseline tone of the arteries from the corpus luteum-bearing ovary was significantly greater than that of its counterpart. In addition, vascular responsiveness of bovine utero-ovarian arteries was investigated during the continuous infusion of prostaglandin $F_{2\alpha}$, the propounded luteolysin in domestic animals. While prostaglandin $F_{2\alpha}$ itself did not alter perfusion pressures, responses to both periarterial sympathetic nerve stimulation and administration of norepinephrine were significantly increased.

The preliminary histochemical study of a pair of bovine utero-ovarian arteries was undertaken to ascertain if there was a visually demonstrable difference between corpus luteum-associated arteries and arteries adjacent to ovaries lacking a luteal body. Biogenic amine-containing nerve fibers were not visualized in either the vascular wall or its surrounding connective tissue. What was seen, however, is that the adventitia of the vessels is heavily populated with highly fluorescent mast cells which appear to completely ring the vessel and to contain histamine; there were no observable differences between the two arteries. The findings of this single study cannot be interpreted as unequivocal evidence that adrenergic innervation to these arteries is absent. Further examination of this vascular tissue using this microscopic procedure, as well as other techniques, is recommended.

A Study of Some Physiological Properties of Ovine and Bovine Utero-Ovarian Arteries

by

Lynda Marie Kuhl

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A STUDY OF SOME PHYSIOLOGICAL PROPERTIES OF OVINE AND BOVINE UTERO-OVARIAN ARTERIES

I. GENERAL INTRODUCTION

The utero-ovarian vasculature of domestic animals has served as a topic of continued research interest, especially with regard to the possible role of the utero-ovarian vein and artery in the unilateral luteolytic phenomenon demonstrated in these animals. It has been suggested that the utero-ovarian artery participates in a counter-current transfer of a luteolytic agent of uterine origin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), from the closely situated utero-ovarian vein. In spite of this interest in the utero-ovarian arteries, no reports in the literature have been found which discuss the basic smooth muscle physiology of these vessels. As a consequence, this study was undertaken with a twofold purpose: firstly, to define some of the basic physiological properties of ovine and bovine utero-ovarian arteries; secondly, to compare ovine and bovine utero-ovarian arterial characteristics for similarities and differences.

II. CORPUS LUTEUM AND ESTROUS CYCLE INFLUENCE ON PHYSIOLOGICAL PROPERTIES OF EWE UTERO-OVARIAN ARTERIES

Introduction

The results of several investigations reviewed by Del Campo and Ginther (1) have indicated in the cow, ewe, and sow that a functional and anatomical relationship exists between the uterus and ovaries with the utero-ovarian vasculature serving as the link between these organs. Whereas most of the studies have been concerned with the possible role of the utero-ovarian vein and artery in the unilateral luteolytic phenomenon demonstrated in these animals (2 - 4), very little has been reported regarding the smooth muscle physiology of these vessels throughout the estrous cycle. Mattner and Thorburn (5) indicate there is a threefold increase in ovarian blood flow coincident with the luteal phase of the cycle in sheep. They found similar cyclic variations in blood flow for ovaries with or without corpora lutea and suggested that the tone of the ovarian vessels might be under the control of circulating progesterone levels. In contrast, other investigators have shown the estrogens to have a vasodilating effect on the uterine vessels in sheep (6 - 9).

We have found no reports regarding further investigations of possible interrelationships between the hormonal state and vascular smooth muscle properties of the reproductive system; however, it has

been demonstrated that the effects of certain vasoactive substances may be modified by varying sex steroid concentrations in the body. Lloyd (10) and Hettiaratchi and Pickford (11) have shown that progesterone can reduce the vasoconstrictor effects of vasopressin, oxytocin, and angiotensin as measured systemically in the rat, whereas Boxill and Brown (12) have reported that the pressor response to epinephrine is augmented in dogs when progesterone is present. Chronic treatment of guinea pigs with testosterone has been demonstrated to reduce or abolish contractile and pressor responses of the isolated, superfused seminal vesicles to angiotensin, barium chloride, tyramine, epinephrine, and norepinephrine (13).

The intent of this investigation was to examine the responsiveness of the utero-ovarian arteries of the ewe during two opposing hormonal stages of the estrous cycle to the biogenic amines, norepinephrine, epinephrine, serotonin and to sympathetic nerve stimulation. Estrus (Day 0) and the luteal phase (Day 13) of the estrous cycle were chosen as meeting this hormonal criterion, with estrogen being the predominant circulating ovarian steroid during the former and progesterone during the latter stage.

Materials and Methods

Crossbred ewes were examined for estrus twice daily using vasectomized rams, with the first day of detected estrus designated

as Day 0 of the cycle. Out of 15 ewes sacrificed, 12 ewes bearing a corpus luteum in only one ovary were assigned to two experimental groups representing Day 0 and Day 13 of the estrous cycle, with each group having six animals. Each ewe was sacrificed by exsanguination, and the ovarian vasculature with its accompanying ovary was excised from each side of the uterus. Note was taken of which ovary, right or left, contained the corpus luteum from the previous cycle in Day 0 animals and for the present cycle in Day 13 animals. A 3.5 cm portion of ovarian artery and its associated vein was sectioned immediately distal to the bifurcation of these vessels into ovarian and uterine components. The artery was cannulated with polyethylene tubing at both ends and the tissue mounted in a perfusion chamber, the experimental procedure being an adaptation of the isolated perfused rabbit ear artery preparation of Steinsland et al. (14). Perfusion systems were assembled in duplicate to permit simultaneous treatment and monitoring of two arterial segments. Krebs bicarbonate solution equilibrated with 5% carbon dioxide in oxygen at 37°C was delivered by two Harvard infusion pumps to both preparations with a perfusion rate of 17 ml/min and superfusion rate of 10 ml/min. Changes in perfusion pressure arising from the 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.

Periarterial sympathetic nerves were excited by field stimulation. Square wave pulses of 1 msec duration and supramaximal voltage (60-70 V) were applied through bipolar platinum electrodes located at each end of the perfusion chambers, and delivered from a Grass Model SD5 stimulator. Electrical stimulation was applied in 30 second trains separated by five minute intervals at frequencies of 10, 20, and 30 Hz.

A Harvard dual syringe infusion pump was used to administer drug solutions at a rate of 0.17 ml/min into the perfusion cannulae. The concentrations (M) of the infused drugs l-norepinephrine bitartrate, l-epinephrine bitartrate, and serotonin creatinine sulfate were calculated for the perfusion fluid.

Standard statistical techniques were used to calculate means and standard errors of the means for each set of data. Student's *t* tests for unpaired and paired data were utilized to determine the statistical significance of observed differences.

Results

Figure 1 depicts the vasoconstrictor responses elicited in sections of utero-ovarian arteries by the biogenic amines, norepinephrine, epinephrine, and serotonin. A typical dose-response relationship was demonstrated for each drug; however, transducer limitations prevented determination of the maximally effective dose for each of the

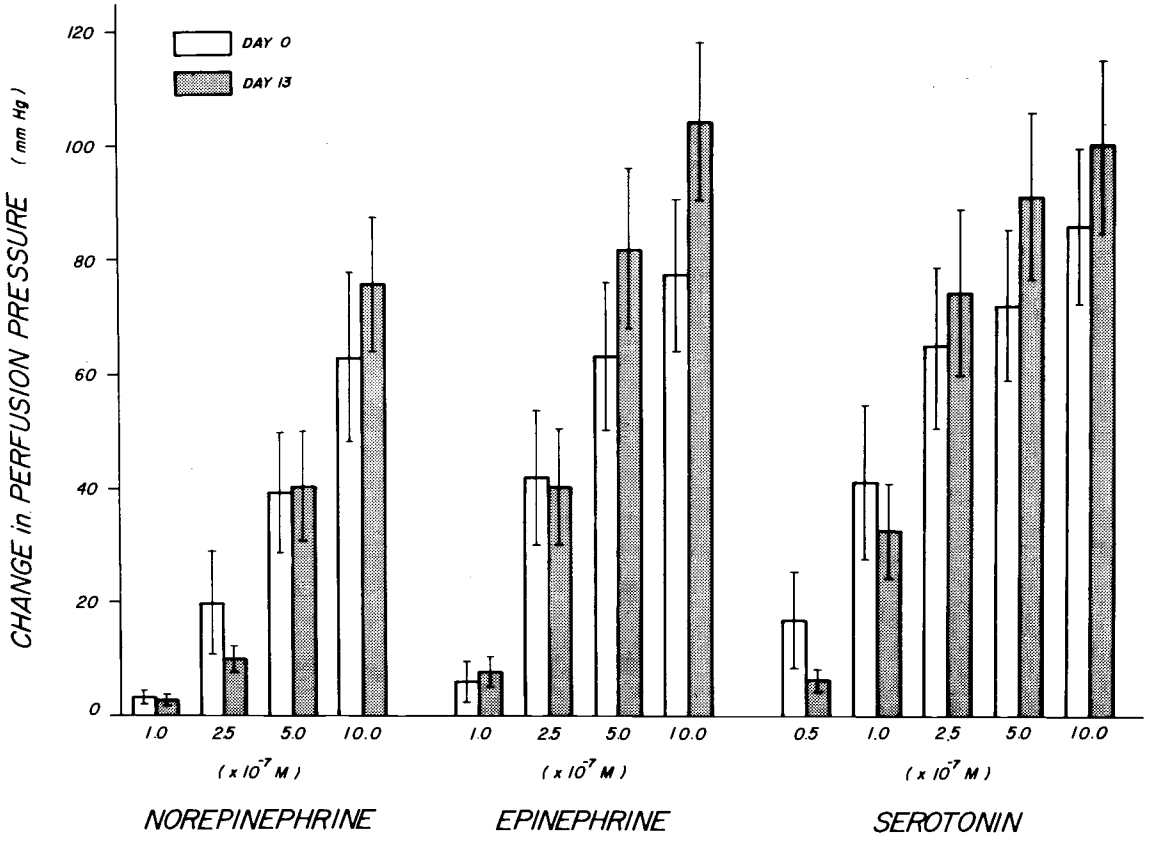

Figure 1. Vasoconstrictor responses of utero-ovarian arteries from ewes sacrificed on Day 0 (open bars) and Day 13 (shaded bars) of the estrous cycle to various doses of norepinephrine, epinephrine, and serotonin. Each bar represents the mean change in perfusion pressure (mm Hg) ± SE for 12 arteries (two samples per animal in each group).

drugs. These results suggest a tendency for arteries removed from ewes on Day 13 of the estrous cycle to be more responsive to the biogenic amines than are arterial segments from ewes sacrificed on Day 0 of their cycle. Further evaluation of these data reveal that the magnitudes of vasoconstrictor responses for arteries taken from the same animal were noticeably different for most of the ewes, suggesting that presence of the corpus luteum might be of importance. Table 1 summarizes the vascular responsiveness data with respect to corpus luteum location. For all six ewes sacrificed on Day 13 of the estrous cycle, there was a correlation between corpus luteum location and the side from which the more responsive artery was sectioned, whereas in the Day 0 group, there were two of six ewes in which the more responsive artery came from the ovary not bearing the corpus luteum of the previous cycle. Quantitative vasoconstrictor response data for the utero-ovarian arteries classified according to corpus luteum are presented in Figure 2. Changes in perfusion pressure produced by each of the three biogenic amines were significantly greater ($p < 0.05$) for the arteries removed from the side adjacent to ovaries having corpora lutea.

Analysis of baseline perfusion pressure recorded for each artery following a one-hour initial equilibration period showed this pressure to be significantly higher ($p < 0.01$) for the arteries sectioned from the luteal side. Mean equilibrated baseline pressure for Day 0

Table 1. Analysis of the presence or absence of a corpus luteum in the ovary ipsilateral to the utero-ovarian artery eliciting greater vasoconstrictor responses to administered vasoactive drugs for each ewe within each group (Day 0 and Day 13).

	ABSENT CORPUS LUTEUM	PRESENT CORPUS LUTEUM
DAY 0	2	4
DAY 13	0	6

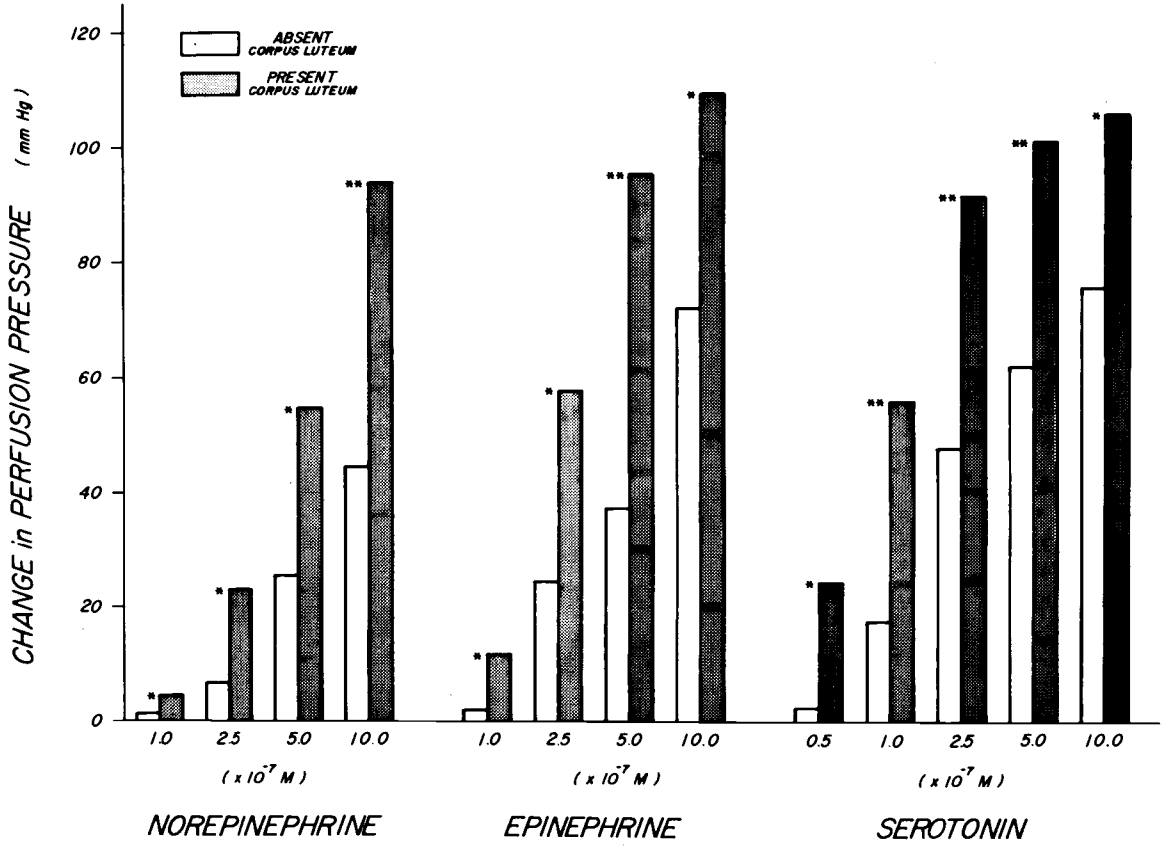

Figure 2. Vasoconstrictor responses of utero-ovarian arteries from the side adjacent to the ovary without a corpus luteum (open bars) and from the side with the ovary bearing a corpus luteum (shaded bars) to various doses of norepinephrine, epinephrine, and serotonin. Each bar represents the mean changes in perfusion pressure (mm Hg) $\pm$ SE for 12 arteries. ** $p < 0.01$; * $p < 0.05$.

and Day 13 arteries associated with the corpus luteum was 68.0 ± 2.1 mm Hg compared with that of 62.8 ± 1.7 mm Hg recorded for the arteries adjacent to ovaries not bearing corpora lutea. When compared on Day 0 and Day 13, disregarding corpus luteum location, the mean initial perfusion pressures of the arteries were 64.4 ± 2.2 mm Hg and 66.4 ± 1.9 mm Hg, respectively.

Preliminary data for sympathetic nerve stimulation with bipolar electrodes agrees with vascular responsiveness data for the biogenic amines. For the arteries tested from the Day 13 group, greater vasoconstrictor responses were elicited from the vessels removed from the corpus luteum side of the ewe. The association between luteal body and arteries responding with greater vasoconstriction was not well defined for ewes from the Day 0 group. Comparison of the magnitude of responses of arteries grouped as Day 0 and Day 13 was inconclusive.

Discussion

Contrary to the findings of Mattner and Thorburn (5), the results of this investigation strongly suggest that the corpus luteum exerts some influence on the vascular properties of, at least, the utero-ovarian artery serving the ovary which bears it. The data indicate a greater responsiveness of this vessel to biogenic amines and sympathetic nerve stimulation, and the baseline tone of this artery is greater

than that of its contralateral counterpart. These findings suggest that at some point near Day 0, the influence of the previous corpus luteum on the associated utero-ovarian artery diminishes, whereas on Day 13 of the cycle there is definite luteal influence.

The mechanism by which the corpus luteum appears to exert a local effect on the artery serving it is undefined. It is, perhaps, another example of a counter-current transfer mechanism whose existence is currently being argued for the luteolytic process in these animals (4). The luteal body may release some substance into the ovarian vein, and this substance might then be transferred to the utero-ovarian artery at some distal point via a counter-current mechanism. Anatomically, such a process is feasible.

Another finding which deserves consideration is that of the tendency for arteries removed from ewes on Day 13 of the estrous cycle to be more responsive to norepinephrine, epinephrine, and serotonin. This agrees in some respects with the findings reported by Boxill and Brown (12). While the data collected on this day did not show the differences to be statistically significant ($p > 0.05$), this might be the result of the small sample size as well as intra-animal variation associated with the corpus luteum.

Summary

An association between corpora lutea and physiological responses of the vascular smooth muscle of the utero-ovarian arteries was demonstrated. Arteries serving the ovaries bearing corpora lutea responded to norepinephrine, epinephrine, and serotonin with significantly greater changes in perfusion pressure than did the arteries associated with ovaries not having a luteal body. Vasoconstrictor responsiveness to periarterial sympathetic nerve stimulation agreed with these findings. In addition, baseline perfusion pressures were elevated for the arteries from the side adjacent to the ovary with the corpus luteum compared to values for the arteries from the other ovary. The arteries removed during the luteal phase (Day 13) of the estrous cycle elicited greater responses to vasoactive biogenic amines than the arteries removed at the time of ovulation (Day 0), but these differences were not statistically significant.

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III. ALTERATIONS OF PHYSIOLOGICAL PROPERTIES OF BOVINE UTERO-OVARIAN ARTERIES BY THE CORPUS LUTEUM AND BY ADMINISTRATION OF PROSTAGLANDIN $F_{2\alpha}$

Introduction

Numerous studies concerned with various aspects of the utero-ovarian vasculature of domestic animals have continued to appear in the literature. Several investigators have reported work directed at defining the relationship of the vascular anatomy to the unilateral luteolytic phenomenon described for these animals (1-8), whereas other groups have devoted their efforts toward elucidation of the luteolysin now thought to be prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (9-13).

The physiological properties of the smooth muscle of utero-ovarian vessels has received attention only just recently. In Section II, evidence has been presented that in sheep there exists a relationship between the corpus luteum location and the magnitude of vasoconstrictor responses elicited by utero-ovarian arteries in response to periarterial sympathetic nerve stimulation and to administration of vasoactive biogenic amines.

The reproductive systems of cattle and sheep are reported to be anatomically and functionally quite similar (1-2, 5, 14). A comparative investigation was undertaken, therefore, to define some of the physiological properties of bovine utero-ovarian arteries. In addition, since $PGF_{2\alpha}$ has been reported by several workers to affect

vascular smooth muscle (15-20), and because it is apparently a normal physiological substance associated with this vascular system, this study also included an examination of vascular responses to sympathetic nerve stimulation and to administered norepinephrine in the presence of $PGF_{2\alpha}$.

Materials and Methods

Pairs of utero-ovarian arteries and their adjacent ovaries were obtained at a slaughterhouse from heifers of mixed breeds ranging in age from one to three years. Based on weight and visible characteristics of the corpus luteum (21-22), arteries were used for this study only from animals killed during the luteal phase of their estrous cycle. Pairs of arteries from six animals comprised the group for study of corpus luteum influence on arterial response characteristics, in which case note was made of which ovary contained the corpus luteum. Two other groups of vessels, each containing arteries from six heifers, were also obtained for the experiments on the effects of $PGF_{2\alpha}$ on the arterial smooth muscle responsiveness.

The comparative portion of this study was performed using the same experimental protocol as described for sheep in Section II.

The effects of $PGF_{2\alpha}$ on the responsiveness of bovine utero-ovarian arteries to periarterial sympathetic nerve stimulation and to exogenous administration of norepinephrine were studied using a

modification of the experimental set-up previously reported (Section II). Arterial segments were assembled in perfusion chamber preparations, and allowed to equilibrate for one hour; control values of responses to the stimuli were then obtained. For the test group used to examine alterations in responses to periarterial sympathetic nerve stimulation, electrical pulses were applied in 30 second trains separated by 10 minute intervals at frequencies of 10, 20, and 30 Hz, and changes in perfusion pressures were recorded. For the arteries designated to test effects of $PGF_{2\alpha}$ on norepinephrine administration, changes in vascular tone were measured in response to four different doses of the agent, to obtain control responses. Next, for both groups, a continuous infusion of one of two selected doses of $PGF_{2\alpha}$ was begun, the concentrations being 0.10 ng/ml and 0.25 ng/ml in the perfusate. After a 10 minute exposure to the prostaglandin, values for vascular responses to the respective stimuli were again obtained, where the infusion of the $PGF_{2\alpha}$ was continued throughout the remainder of the experiment.

Prostaglandin $F_{2\alpha}$ as the tromethamine salt, supplied by Dr. John Pike of the Upjohn Company, Kalamazoo, Michigan, was prepared daily as a stock solution (0.1 mg/ml base) in tris-(hydroxymethyl)-aminomethane (THAM) solution buffered to a pH of 9.5. Necessary dilutions were made with THAM buffer just prior to initiation of each of the infusions. The other drugs used in this study included

l-norepinephrine bitartrate, l-epinephrine bitartrate, and serotonin creatinine sulfate.

Standard statistical techniques were used to calculate means for each set of data. Student's *t* test for paired data was utilized to determine the statistical significance of observed differences.

Results

Each of the biogenic amines, norepinephrine, epinephrine, and serotonin, as well as periarterial sympathetic nerve stimulation, elicit vasoconstrictor responses in bovine utero-ovarian arterial segments. For each pair of arteries used in the comparative portion of this study, the arterial segment removed adjacent to the corpus luteum-containing ovary responded with greater increases in perfusion pressure to both periarterial sympathetic nerve stimulation and to the administered pharmacological agents.

Figure 3 illustrates the mean vasoconstrictor responses elicited to periarterial sympathetic nerve stimulation delivered at three different frequencies. It can be seen that there is a positive correlation between increases in vascular tone and the rate of electrical impulse delivery. The actual experimental data suggests that for each of the levels of stimulation, the arteries which had served the corpus luteum-containing ovary responded with significantly greater increases in smooth muscle tone ($p < 0.05$) than did the arteries which had been

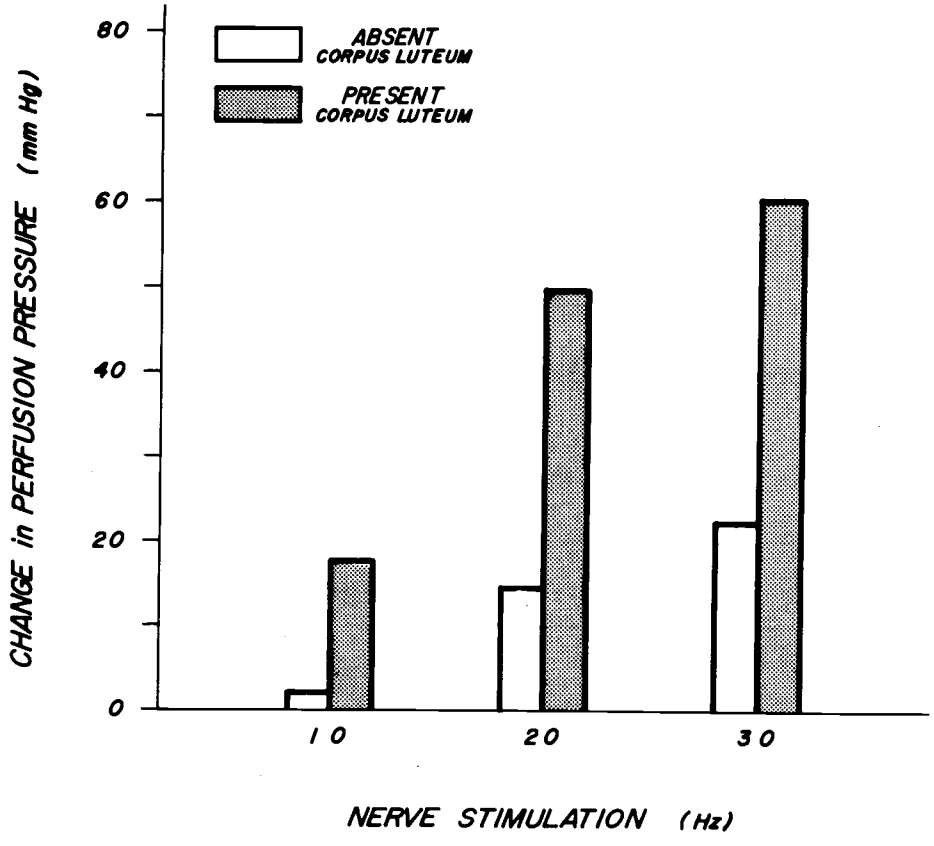

Figure 3. Vasoconstrictor responses of utero-ovarian arteries from the side adjacent to the ovary without a corpus luteum (open bars) and from the side with the ovary bearing a corpus luteum (shaded bars) to various frequencies of periarterial sympathetic nerve stimulation. Each bar represents the mean change in perfusion pressure (mm Hg) for six arteries. Differences in responsiveness between sides are significant ($p < 0.05$).

adjacent to ovaries lacking a luteal body.

The data for changes in perfusion pressure in response to administration of several doses of each of the biogenic amines are illustrated in Figure 4. For each of the drugs, the bovine utero-ovarian arterial segments demonstrated a typical dose-response relationship. The mean changes in perfusion pressure for each of the doses of norepinephrine, epinephrine, and serotonin were significantly greater ($p < 0.05$) for the arteries from the corpus luteum side of the uterus.

In addition to the above findings for vasoconstrictor responsiveness, analysis of baseline perfusion pressure recorded for each artery following a one hour initial equilibration period showed this pressure to be significantly higher ($p < 0.05$) for the segments from the luteal side. Mean equilibrated baseline pressure for the arteries delivering blood to the corpus luteum-containing ovary was 76.2 mm Hg compared with that of 67.3 mm Hg for the arterial segments associated with non-corpus luteum-containing ovaries.

The effect of $PGF_{2\alpha}$ on vascular responses to periarterial sympathetic nerve stimulation and to administration of norepinephrine was one of enhancement in both cases. Neither of the two doses of $PGF_{2\alpha}$ used, however, affected the vascular tone itself.

Figure 5 illustrates the effects of $PGF_{2\alpha}$ on sympathetic nerve stimulation. It can be seen that the vasoconstrictor responses

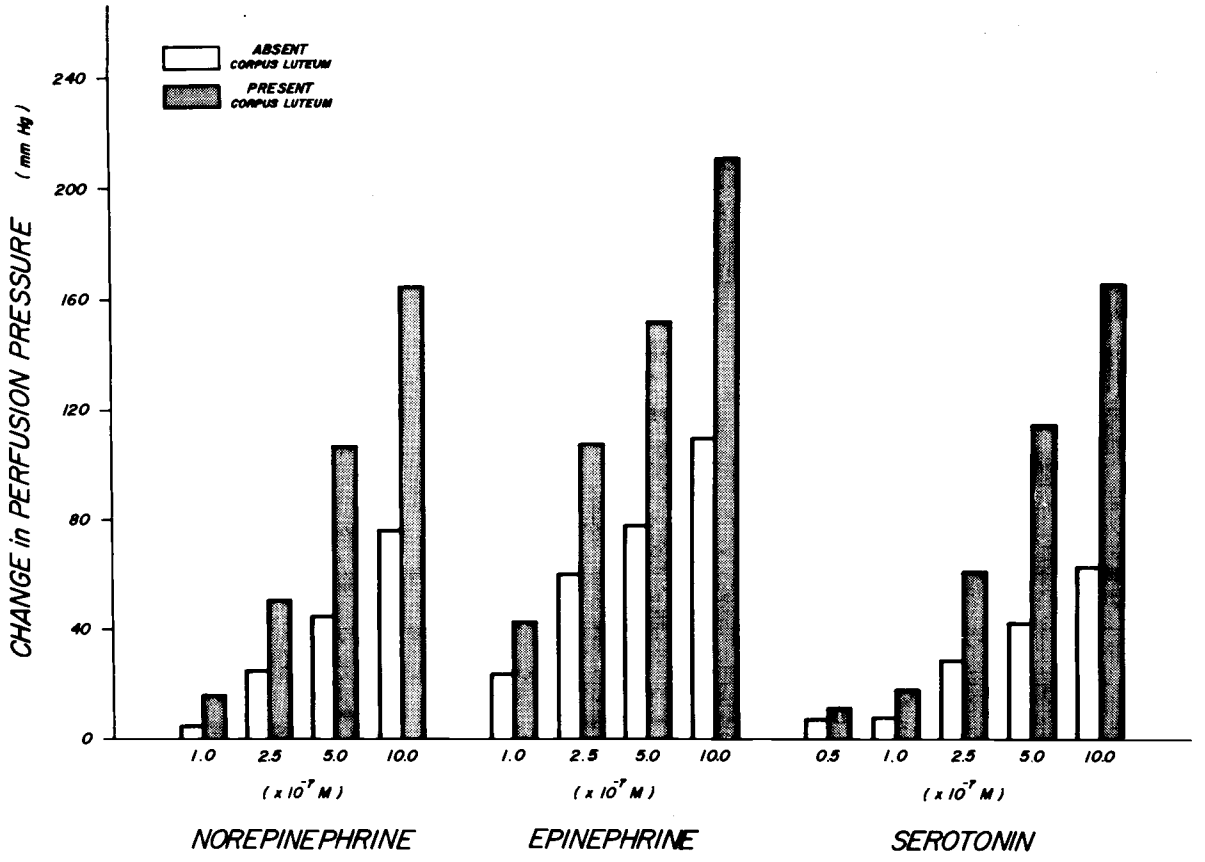

Figure 4. Vasoconstrictor responses of utero-ovarian arteries from the side adjacent to the ovary without a corpus luteum (open bars) and from the side with the ovary bearing a corpus luteum (shaded bars) to various doses of norepinephrine, epinephrine, and serotonin. Each bar represents the mean change in perfusion pressure (mm Hg) for six arteries. Differences in responsiveness between sides are significant ($p < 0.05$).

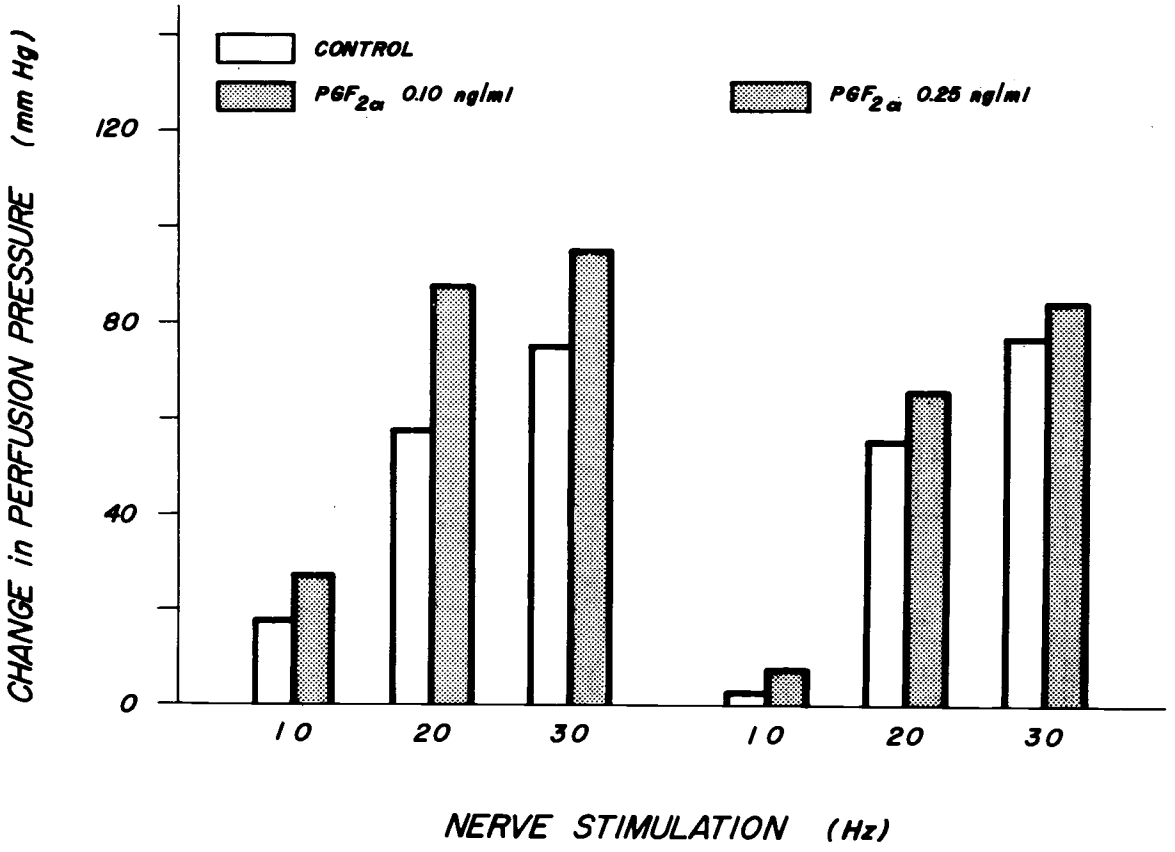

Figure 5. Vasoconstrictor responses of utero-ovarian arteries to various frequencies of periarterial sympathetic nerve stimulation alone (open bars) and during the continuous infusion of PGF_{2a} (shaded bars). Each bar represents the mean change in perfusion pressure (mm Hg) for six arteries. Differences in responsiveness between control and treatment were significant ($p < 0.05$).

elicited to the electrical pulses are enhanced in the presence of both of the concentrations of $PGF_{2\alpha}$. These differences were significant ($p < 0.05$) for the three different frequencies delivered during infusion of each of the doses of $PGF_{2\alpha}$.

Figure 6 illustrates the comparison of control values of changes in perfusion pressure to norepinephrine alone and of the values recorded when the same doses of the biogenic amine were administered during continuous infusion of one of the two concentrations of $PGF_{2\alpha}$. The experimental data indicates that for this tissue, vasoconstrictor responses to each of the doses of norepinephrine were facilitated when $PGF_{2\alpha}$ was present in the perfusate. These observed differences were significant ($p < 0.05$) for each of the doses of norepinephrine administered during infusion of either of the concentrations of $PGF_{2\alpha}$.

Discussion

The results of this investigation strongly imply that in cattle the corpus luteum exerts some influence on the vascular properties of the utero-ovarian artery adjacent to the ovary that bears it. This greater responsiveness was demonstrated not only for periarterial sympathetic nerve stimulation but also for administered biogenic amines, norepinephrine, epinephrine, and serotonin. Of interest, in addition, is the finding that the initial baseline tone of the arterial segments from the luteal side is also elevated over that of its

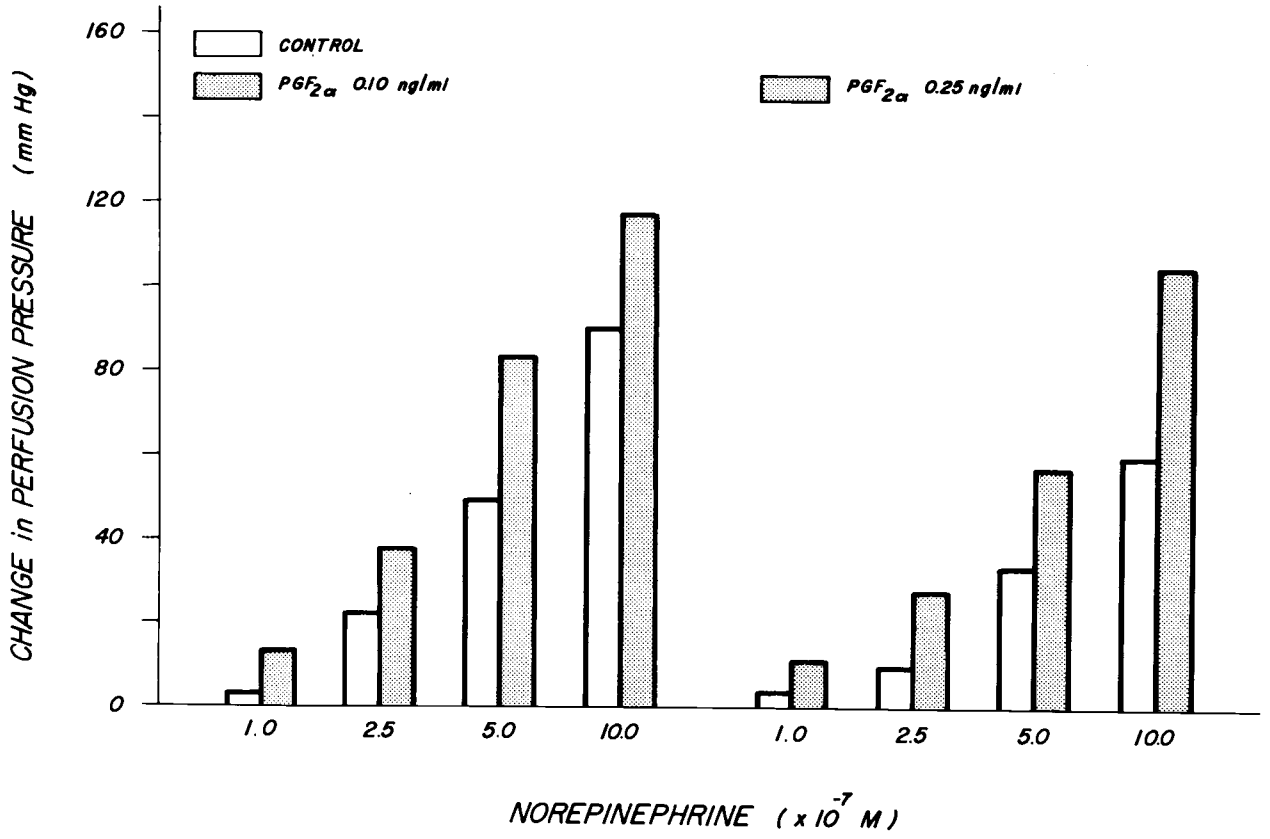

Figure 6. Vasoconstrictor responses of utero-ovarian arteries to various doses of norepinephrine alone (open bars) and during the continuous infusion of $PGF_{2\alpha}$ (shaded bars). Each bar represents the mean change in perfusion pressure (mm Hg) for six arteries. Differences in responsiveness between control and treatment were significant ($p < 0.05$).

counterpart. The above findings are in agreement with those reported for sheep utero-ovarian arteries in Section II. This suggests, another apparent similarity between the ovine and bovine reproductive systems.

The portion of this study devoted to examination of the effects of $PGF_{2\alpha}$ on bovine utero-ovarian arterial responsiveness was based on reports in the literature which regard this agent as a luteolytic substance in domestic animals (9-13) as well as a vascular smooth muscle modifier (16-21). Evidence has been presented that the administration of $PGF_{2\alpha}$ at doses lower than that which alter vascular tone itself will facilitate the vasoconstrictor response to sympathetic nerve stimulation (16-21). With regard to the effect of $PGF_{2\alpha}$ on response to exogenously administered norepinephrine, some workers have reported enhancement (16-17, 21), whereas others have found no effect (18-20). This study was done using two different doses of $PGF_{2\alpha}$, both of which were considered to be well within physiologically normal limits (24-27). It appears that for bovine utero-ovarian arteries, $PGF_{2\alpha}$ is exerting apparently similar influences on both sympathetic nerve stimulation (16-21) and norepinephrine (16-17, 21) as reported by other groups. The enhancement of vasoconstrictor responses to both nerve stimulation and to added norepinephrine would tend to suggest that $PGF_{2\alpha}$ is somehow modifying smooth muscle response at the post-neuronal level for this particular tissue.

The mechanism by which the corpus luteum apparently exerts a local effect on the artery serving it is as yet undefined. It is interesting to note that $PGF_{2\alpha}$ seems to be a natural substance circulating in the utero-ovarian vascular system of domestic animals and that, at least in cattle, physiological concentrations of this agent can facilitate arterial responses to sympathetic nerve stimulation and norepinephrine administration. Whether there is any interrelationship between the experimental findings of this study is as yet speculative. Certainly a possible explanation for the unilaterally greater responsiveness of utero-ovarian arteries in beef and sheep is that the corpus luteum, during the luteal phase of the cycle, is itself producing $PGF_{2\alpha}$ and releasing it into the adjacent ovarian vein where it is somehow able to alter the physiological properties of the closely situated artery.

Summary

Some physiological properties of bovine utero-ovarian arteries were examined in vitro with perfusion chamber preparations which allow measurement of changes in vascular tone in response to periarterial sympathetic nerve stimulation and to exogenously administered pharmacological agents. Vascular response characteristics to sympathetic nerve stimulation delivered at different frequencies and to various doses of norepinephrine, epinephrine, and serotonin were

analyzed with reference to arterial association to ovaries with or without a luteal body. As was demonstrated in sheep (Section II), experimental evidence demonstrates that arteries serving the ovaries bearing functional corpora lutea respond with significantly greater increases in perfusion pressure to sympathetic nerve stimulation and to each of the vasoactive biogenic amines than do arteries adjacent to ovaries not bearing corpora lutea. Initial baseline perfusion pressures were significantly higher for arterial segments from the corpus luteum side of the uterus as well. Vascular responsiveness of these arteries to sympathetic nerve stimulation and to norepinephrine during a continuous infusion of $PGF_{2\alpha}$ was investigated. $PGF_{2\alpha}$ itself did not alter perfusion pressures, but responses to both periarterial sympathetic nerve stimulation and norepinephrine were significantly increased.

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IV. A PRELIMINARY FLUORESCENCE HISTOCHEMISTRY STUDY OF BOVINE UTERO-OVARIAN ARTERIES

Introduction

A correlation between the physiological characteristics of ovine and bovine utero-ovarian arteries and the presence of the corpus luteum in the adjacent ovary has been demonstrated (Sections II and III). For both beef and sheep, the utero-ovarian artery which delivers blood to the corpus luteum-containing ovary is more responsive to periarterial sympathetic nerve stimulation and to administered vasoactive biogenic amines than is its counterpart. Some investigators have reported that transmitter content of adrenergic nerves may be altered by chronic treatment with sex steroids (1). Because the corpus luteum is the primary progesterone producing body during the estrous cycle (2), and because of the ipsilateral influence noted on utero-ovarian arteries, a preliminary investigation was undertaken to determine if there is a demonstrable difference in transmitter content between arteries removed from each side of the uterus.

Materials and Methods

Pairs of utero-ovarian arteries and their adjacent ovaries were obtained at a slaughterhouse from heifers of mixed breeds ranging in age from one to three years. Based on weight and appearance of the

corpus luteum (3-4), two pairs of arteries were selected from animals sacrificed in the luteal phase of the estrous cycle. Note was made of which ovary contained the corpus luteum.

Shortly after removal of the vasculature from each animal, each utero-ovarian artery was quickly and carefully dissected from its associated utero-ovarian vein and the surrounding connective tissue and then rapidly frozen in liquid Freon. The arteries were then transferred to liquid nitrogen for storage. Fluorescent micrographs were prepared according to the method described by Falck and Owman (5).

Results

Using the technique described by Falck and Owman (5), biogenic amine-containing nerve fibers were not visualized in either the vascular wall or its surrounding connective tissues. Bundles of non-fluorescent myelinated nerves were occasionally seen at the periphery of the vascular adventitia. The adventitia was also seen to be heavily populated with highly fluorescent mast cells, as pictured in Illustration 1. These appeared to completely ring the vessel in all sections examined. Illustration 2 pictures especially well the cytoplasmic granulation characteristic of mast cells. There was no observable difference in the appearance of the two arteries removed from each side of the uterus for either animal.

Illustration 1. Fluorescence micrograph of bovine utero-ovarian arterial segment showing heavy population of highly fluorescent mast cells (20x).

Illustration 2. Fluorescence micrograph of bovine utero-ovarian arterial segment showing the cytoplasmic granulation of mast cells (200x).

Discussion

Failure to visualize biogenic amine-containing nerve fibers cannot be interpreted as unequivocal evidence that adrenergic innervation to these blood vessels is absent. Certainly, before any definitive statement can be made in this regard, other analytic methods should be tried. Also, pretreatment of the animal with a monoamine oxidase inhibitor prior to sacrifice should be considered.

Since the adrenergic nerve fibers were not visualized for either utero-ovarian artery, no statement can be made as to whether the corpus luteum may be exerting a local effect on transmitter concentrations. The histamine content of the mast cells appeared to be the same for arterial segments from each side of the uterus.

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