AN ABSTRACT OF THE THESIS OF

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A local effect of endogenous ovarian hormones on the in vitro responses of the bovine infundibulum to the neurotransmitters norepinephrine (NE) and acetylcholine (Ach) was investigated. Prostaglandin $F_2\alpha$-tham salt was utilized to induce luteal regression in six heifers which were necropsied during proestrus. Six heifers were also necropsied during the mid-luteal phase (day 12) of the estrous cycle. A strip of each infundibulum from each heifer was placed in a tissue bath and exposed to NE (0.4, 0.8, 1.6 µg/ml) followed by Ach (0.1, 0.56, 1.0 µg/ml). Changes in frequency and amplitude of contractions and tissue tone were recorded.

Infundibula ipsilateral to the ovary bearing the largest follicle in proestrous heifers responded to all concentrations of NE with greater frequency of contractions than contralateral infundibula or both ipsilateral and contralateral infundibula of diestrous animals (stage

of cycle x tissue location interaction, $P < .01$). Ipsilateral infundibula of proestrous heifers appeared to respond to increasing concentrations of NE with a linear increase in frequency of contractions but the dose-response relationship was statistically nonsignificant. Amplitude of contractions induced by all concentrations of NE studied did not differ between stages of the cycle or between ipsilateral or contralateral infundibula. Mean tone of ipsilateral and contralateral infundibula of proestrous and diestrous animals increased linearly in response to all concentrations of NE ($P < .05$). Norepinephrine-induced increases in the mean tone of infundibula from diestrous animals were greater than for infundibula of proestrous heifers ($P \simeq .06$). Frequency of contractions in response to Ach was dependent upon the *in vitro* neurotransmitter concentration and tissue location within animal (Ach x tissue location interaction, $P < .01$). Acetylcholine at 0.1 µg/ml inhibited frequency of contractions regardless of stage or location of the infundibulum. In contrast, 0.56 and 1.0 µg/ml Ach increased frequency of contractions of ipsilateral but was without affect or further depressed this response of all contralateral infundibula. Amplitude of contractions in response to Ach was unaffected by cycle stage or infundibula location ($P > .05$). Ipsilateral and contralateral infundibula at both stages of the cycle exhibited a

reduction of mean tone in response to 0.1 µg/ml Ach. In contrast, 0.56 and 1.0 µg/ml Ach caused an increase in the mean tone.

These data demonstrate that the bovine infundibulum is responsive to NE and Ach and that these responses are governed by the concentrations of estrogen and progesterone to which the infundibulum is exposed.

THE RESPONSE OF THE BOVINE INFUNDIBULUM TO NOREPINEPHRINE AND ACETYLCHOLINE DURING ESTROGEN AND PROGESTERONE DOMINANCE

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REVIEW OF LITERATURE

Introduction

The ovarian end of the oviduct is the funnel-shaped infundibulum. Its function is to catch the ova extruded from the ovary and to transport them to the ampulla of the oviduct. On the free end of the infundibulum are a number of irregular processes called fimbriae. In the horse a few fimbriae are permanently attached to the end of the ovary and are called fimbria ovaricae (Nickel et al., 1973) whereas in sheep and other species the infundibulum partially envelops the ovary and the fimbriae are not attached to the ovary. In the rabbit and presumably other species the fimbriae appear to make sweeping motions over the surface of the ovary during estrus and especially during ovulation in order to secure the extruded ova.

The extent to which fimbriae envelop the ovary is dependent on the stage of the cycle and the species under consideration. In the guinea pig and rabbit the fimbriae and infundibulum form a bursa during ovulation but at other times of cycle they only partially cover

the ovaries (Blandau, 1969). It has been reported that during estrus the infundibulum of the ewe completely envelops the ovaries with the mucosa of the infundibulum forming ridges which protrude into the tubal lumen (Hadek, 1955). The fimbriae and infundibulum of the sow have also been reported to form a bursa during ovulation (Nalbandov, 1969). In the canid family a bursa is present throughout the cycle (Christensen, 1979). In some species the mesotubarium superius, a thin sheet of smooth muscle between the double serosal layer of the mesosalpinx is attached to the medial border of the fimbriae and during ovulation aids in the movement of fimbriae and infundibulum (Blandau, 1969; Halbert and Conrad, 1975). In the human and other species the mesotubarium ovarica (tubo-ovarian ligament) connects the fimbriae with the ovarian surface and during ovulation contracts and relaxes moving the fimbriae closer to the ovary (Sterin-Speziale et al., 1978). In species which have an ovarian bursa an opening connects the bursa with the peritoneal cavity while in all species the distal end of the infundibulum is connected to the ampulla via the abdominal ostium of the oviduct (Warwick and Williams, 1973; Nickel et al., 1973; Getty, 1975).

Anatomy of the Infundibulum

Smooth muscle and connective tissue layers of the infundibulum

The wall of the infundibulum, the thinnest of the oviduct, is composed of three layers: the outer connective tissue coat or tunica serosa, a middle muscular coat or tunica muscularis, and the inner mucosal coat or tunica mucosa which consists of the outer lamina propria and the inner lamina epithelialis (Nilsson and Reinius, 1969.)

The tunica serosa of most mammals consists of a single layer of squamous epithelium (mesothelium) covering a thin layer of loose connective tissue. This mesothelium is continuous with the general peritoneal lining. The connective tissue coat is highly vascularized and has several fairly large nonmyelinated nerve bundles of the autonomic nervous system (Beck and Boots, 1974). In addition, small smooth muscle fibers are found in the connective tissue of the serosa; these fibers are distributed in a highly irregular pattern (Beck and Boots, 1974). Also found within the connective tissue of the serosa are relatively large numbers of mast cells (Nilsson and Reinius, 1969). The major fibrous elements in the serosa are collagen, predomi-

nantly as a connective tissue fiber, and elastic fibers dispersed in a collagenous matrix. Reticular fibers are found associated with smooth muscle fibers and vascular elements.

Parallel to the infundibulum within the serosa of the mesosalpinx are several arteries, veins and lymphatic vessels that frequently branch off to penetrate the muscularis and mucosal layers (Beck and Boots, 1974). Between the serosa and muscularis are many arterioles and venules (Nickel et al., 1973).

The tunica muscularis lies under the serosa and is composed of smooth muscle fibers arranged in a variety of patterns. Depending upon the species the muscularis of the infundibulum has been found to consist of three patterns: 1) a central thick circular layer surrounded on both sides by a thin longitudinal layer, 2) a thick inner circular layer surrounded by a thin outer longitudinal layer, or 3) a thick outer circular layer surrounding a thin inner longitudinal layer. These patterns remain constant within any given species (Beck and Boots, 1974). In humans the outer longitudinal and inner circular layers are intertwined (Warwick and Williams, 1973) and, in contrast to the patterns mentioned above, the human infundibulum muscularis does not have a congruous arrangement (Paton et al., 1977).

The tunica mucosa lies under the muscularis and its anatomy is relatively constant in all mammals. The mucosa is thin and greatly folded to increase its surface area. The outer layer of the mucosa is the lamina propria; it is a thin circular layer of loose connective tissue which forms the framework for the mucosal folds. The propria is highly vascularized; it contains many arteries, veins, lymphatic vessels and nerve fibers. Although the propria is thin its actual depth depends on the stage of the cycle and on the degree of vascularity.

The matrix of the propria is primarily collagen; no elastic fibers have been demonstrated. In the region where the muscularis meets the propria of the mucosa a few reticular fibers may be found. None of these reticular fibers or any other types of fibers can be found in the folds of the mucosa.

The lamina propria of the infundibular wall is gland-free (except in certain marsupials) consisting primarily of fibroblasts, macrophages, formed elements of blood and "indifferent cells." Smooth muscle fibers not in association with the muscularis cannot be found (Beck and Boots, 1974). However, at least one researcher believes there are mucosal muscle fibers present, the demonstration of which requires better electron microscopy techniques (Nilsson and Reinius, 1969).

The inner layer of the tunica mucosa is the lamina epithelialis. The epithelial cells of the infundibulum fimbriae are simple columnar or pseudostratified columnar. There are basically two types of cells: ciliated and nonciliated and of the nonciliated there are secretory, peg (rod or intercalary) cells and basal (or indifferent) cells. The ciliated cells are the predominant cells in the infundibulum and fimbriae of most species. It has been reported that about 65% of the cells in the normal rabbit fimbriae are ciliated (Odor and Blandau, 1973). Nilsson and Reinius (1969) have reported that the majority of cells in the infundibulum and fimbria of the mouse, rat, guinea pig, cow and human are ciliated while those of the ewe are nonciliated. In contrast, other researchers disagree and report greater numbers of ciliated cells in the infundibulum and fimbriae of the ewe (Stalheim et al., 1975; A. Wu and F. Stormshak, unpublished). In the Rhesus monkey the extent of ciliation is dependent on the stage of the cycle suggesting that the oviduct may be influenced by changes in blood hormone levels (Brenner, 1969). Although the oviductal cilia of the cow, ewe, gilt and rabbit do not undergo cyclical changes, it has been observed that estrogen has a stimulatory effect on ciliogenesis (Brenner, 1969; Nayak et al., 1976a, b; Nayak and Ellington, 1977).

Innervation of the Infundibulum and Oviduct

The innervation of the infundibulum is derived from both the sympathetic and parasympathetic divisions of the autonomic nervous system. All of the nerves in the sympathetic division communicate with the oviduct and infundibulum via the hypogastric nerve bundles in humans, cats and dogs. After the hypogastric nerve bundle, the sympathetic preganglionic fibers synapse in the ovarian plexus and postganglionic fibers innervate the fimbriae and infundibulum (Beck and Boots, 1974). Brundin and Wirsen (1964a, b) reported that the rabbit infundibulum and ampulla were lightly innervated by adrenergic nerve endings while the isthmus was richly supplied by these nerves. Similar observations were reported for the human oviduct (Owman and Sjöberg, 1966). In their review, Beck and Boots (1974) reported that in the rabbit, cat, rat and human the scarce adrenergic innervation of the infundibulum was confined to the walls of blood vessels. They also stated that the infundibular smooth muscle was probably under adrenergic control but as of yet this has not been confirmed. However, it was amply demonstrated using adrenergic agonists and antagonists that the human ampulla, isthmus (Seitchick et al., 1968) and utero-ovarian ligament (Sterin-Speziale et al., 1978) and the rabbit isthmus (Hodson and Pauerstein, 1974; Brundin,

1976) contain α-stimulatory and β-inhibitory adrenergic receptors.

The parasympathetic innervation of the infundibulum and oviduct is vaguely defined. It is believed that the proximal segment of the oviduct and hence the infundibulum is innervated by the vagus nerve via the ovarian plexus. In several species the only true cholinergic fibers are in the lamina propria and are thought to innervate the mucosal cells (Beck and Boots, 1974). It has been demonstrated, however, that acetylcholine stimulates muscular contractions in the human (Sandberg et al., 1960) and rabbit oviduct (Brundin, 1964). In the human oviduct longitudinal and circular muscles have similar responses to parasympathomimetic and sympathomimetic drugs (Sandberg et al., 1960; Hawkins, 1964); both classes of drugs stimulated the circular and longitudinal muscles. Acetylcholine also has been found to stimulate contraction of a circular muscle layer in both the isthmus and ampulla of the rabbit oviduct.

Vasculature of the Infundibulum

The blood supply to the infundibulum and fimbriae of cows and ewes is via several branches of the tubal branch of the ovarian artery. The caudal portion of the oviduct receives its blood via several branches of the

uterine branch of the ovarian artery which for the most part pass beneath the ovary in the broad ligament (Ginther, 1976). The cyclic hyperemic changes that take place within the infundibulum and fimbriae, therefore, are caused by the ovarian artery which anastomoses within the mesosalpinx and forms a strong vascular arch. The blood vessels are especially numerous within the fimbriae and infundibulum and are intertwined with muscle fibers (Stange, 1952), forming a tissue similar to that of erectile tissue. During ovulation the blood supply increases and causes the fimbriae to swell and increase their surface contact with the ovary (Hafez, 1961).

Lymph drainage from the infundibulum and oviduct is complex. Three separate lymphatic networks drain the mucosa, muscularis and serosa, respectively. The lymph drainage from the oviduct enters the mesosalpinx and eventually drains into the lumbar lymph nodes (Hafez, 1961) and the para-aortic nodes (Beck and Boots, 1974). Dubrueil (1944-45) postulated that intense lymph drainage into the infundibulum and oviduct established a current within the lumen which aided in ovum reception. It was later shown that if such a current existed it was not essential for normal ovum reception (Clewe and Mastroianni, 1958).

Hormonal Regulation of Oviductal Physiology

Ciliation

The extent of ciliation of the infundibulum, fimbriae and the entire oviduct is critically important for ova reception and transport. Odor and Blandau (1973) reported a direct correlation between the number of ciliated cells on the fimbriae of the rabbit and the transport of cumulus masses over the surface of the rabbit fimbriae; there was no transport of cumulus masses over the surface of the rabbit fimbriae with less than 44% ciliated cells. Increased ciliation resulted in increased efficiency and speed of cumulus transport. The importance of cilia in ovum reception and transport has been convincingly demonstrated. When muscular contractions of the rabbit oviduct were inhibited, the time required for cumulus masses to reach the ampulla-isthmic junction was unchanged when compared to control animals (Halbert et al., 1976). In a more recent series of experiments Eddy et al. (1978) reversed 1 cm segments of the rabbit isthmus or ampulla and found that in the ampullary reversed segments transport was completely stopped while in the isthmic reversed segments no change in transport was observed. This demonstrates that ciliary activity is not essential in all parts of the oviduct and exemplifies some of the complexities of the oviduct.

The effect of endogenous hormones on ciliated cells in the infundibulum and fimbriae is species specific. In the Rhesus monkey there is extensive deciliation during the progesterone dominated luteal phase and renewal of cilia during the early follicular phase when estrogens are the predominant hormone present (Brenner, 1969). In the cow and ewe changes of the ciliated cells corresponding to the changes in ovarian hormones during the estrous cycle do not occur (Nayak and Ellington, 1977). The epithelium of the infundibulum in the gilt and rabbit is similar to the ewe and cow with respect to cyclic changes and ciliation (Odor and Blandau, 1973; Nayak et al., 1976a; Nayak and Ellington, 1977).

The effect of exogenous ovarian hormones (estrogen and progesterone) on ciliation of the infundibulum has been studied utilizing ovariectomized animals. Rabbits ovariectomized 15-23 weeks showed a decrease but not a complete loss of ciliation. Estradiol benzoate for 2-5 days caused a variable deciliation followed by reciliation (Odor and Blandau, 1973). In another study (Rumery and Eddy, 1974), rabbits ovariectomized for 15-18 months showed extensive cilia loss and estradiol treatment for 10 days caused a marked increase in the number of cilia without the preliminary decrease mentioned by Odor and Blandau (1973).

Studies using ovariectomized Rhesus monkeys, gilts and ewes all showed similar results with estradiol treatment. In the Rhesus monkey ovariectomy resulted in a total loss of cilia on the fimbrial surface and estradiol treatment returned the cilia to a completely normal state (Brenner, 1967). In the 4 month-ovariectomized gilt the fimbrial epithelium was completely denuded of cilia. Treatment with estradiol for 1 or 2 days produced no change. Estradiol treatment for 3 days caused slight reciliation. After 4, 5, 6, and 7 days of estradiol treatment there was a marked increase in the number of cilia on the fimbrial surface (Nayak et al., 1976b). Following ovariectomy of the ewe only a slight deciliation of the fimbrial epithelium was observed. Estradiol treatment caused a slight reciliation. Ovariectomized ewes treated with progesterone showed no change in the number of ciliated epithelial cells of the fimbriae. Treatment of ovariectomized ewes with estradiol and progesterone simultaneously did not seem to affect cilia development (A. Wu and F. Stormshak, unpublished).

The results from intact and ovariectomized animals demonstrate that estradiol has a growth promoting effect on ciliation in the infundibulum and fimbriae while progesterone seems to have no effect on the ciliated cells of these tissues.

Secretory Activity

There have been very few studies characterizing the nature of the secretions from the secretory cells of the fimbriae and infundibulum in any species. Greenwald (1969) reported that the cells of the fimbriae in the rat contained lipid droplets. He also reported that in the rat fimbrial epithelium alkaline phosphatase activity was intense during estrus and metestrus with minimum activity observed during diestrus. It was concluded that alkaline phosphomonoesterase activity is greatest in the oviduct during proestrus and estrus. Several studies showed that estradiol generally caused hypertrophy and progesterone atrophy of the infundibular and fimbrial secretory cells (Nayak et al., 1976a, b; Nayak and Ellington, 1977).

In the 6-month-ovariectomized rabbit the processes on the secretory cells of the fimbriae had hypertrophied to such an extent that the secretory cells and not the ciliated cells were the predominant surface structure. The microvilli on the surface of the secretory cells varied in length and were generally reduced in numbers. In the 18-month-ovariectomized rabbit the normal bulbous shape of the fimbrial secretory cell was replaced by a flattened hexagonally shaped cell covered with short microvilli and a single prominent cilium in the center

of the cell. When the 15-18 month ovariectomized rabbit was treated with estradiol for 10 days the secretory cells resumed their bulbous shape, lost the central cilium but still lacked their full complement of microvilli (Rumery and Eddy, 1974).

In the ovariectomized gilt, administration of estradiol resulted in hypertrophy of the fimbrial secretory cells. Maximum differentiation of the secretory cells was seen after 3 days of estradiol treatment. The secretory granules within the cells increased dramatically between day 3 and days 5 and 7 of treatment. Administration of estradiol caused the rough endoplasmic reticulum (RER) to be highly developed and the cisternae dilated. The cisternae contained moderately dense fibrillar material similar to what was seen during the follicular phase, that is, during estradiol dominance. These data indicate that the secretory cells of the porcine fimbriae were probably increasing their protein synthesis in response to estradiol administration (Nayak et al., 1976b).

It was reported that in the intact cycling cow secretory cells of the fimbriae were under control of high plasma levels of estradiol (Nayak and Ellington, 1977). In the same study it was reported that the secretory granules were PAS-positive and diastase-resistant. Dur-

ing days 9 and 10 (luteal phase) of the estrous cycle when the fimbriae were under progesterone dominance, nucleated cytoplasmic projections were seen protruding into the tubal lumen. In addition, extruded nuclei and cytoplasmic organelles were observed floating free in the lumen (Nayak et al., 1976b).

In the intact ewe, large glycogen deposits were seen in the cytoplasm of fimbrial secretory cells during the follicular phase while none were observed during the luteal phase (Nayak et al., 1976a). Maximum secretory cell differentiation was reported during the follicular phase; the cells were observed to have well developed RER, many ribosomes and secretory granules. During the luteal phase, cytoplasmic projections with nuclei protruded into the lumen and some had detached and were floating free in the lumen. In contrast to the many secretory granules observed during the follicular phase, during progesterone dominance few or no secretory granules were observed in the apical cytoplasm of the secretory cells. The observation of many secretory granules and a well developed RER during estrus indicate that secretion in the ewe fimbria is probably stimulated by estradiol.

Different results were observed in the ovariectomized ewe; simultaneous treatment with estradiol and progesterone resulted in increased secretory protrusions on the

secretory cells and seemed to enhance the general secretory activity of the fimbrial epithelium. This was contrasted to the lack of such an effect by progesterone or estradiol alone (A. Wu and F. Stormshak, unpublished). These results are contradictory to the observations regarding the fimbriae of the intact ewe and other animals and observations of the secretory activity in the ampullae of the ovariectomized ewe. Willemse (1975) reported that estradiol stimulated the secretory activity of the cells in the ampulla of the ewe while simultaneous administration of progesterone interfered with the action of estradiol.

Adrenergic Responses

Mature rabbit oviductal isthmic segments were shown to be more responsive to phenylephrine (an α-agonist) under estradiol treatment than after ovulation (Hodson and Pauerstein, 1974). The preceding study also demonstrated that the maximum contractions of circular versus longitudinal preparations were not significantly different in human chorionic gonadoptropin-treated (to induce ovulation) in comparison to estradiol-treated rabbits. However, longitudinal preparations of the oviduct were significantly more responsive to phenylephrine than were circular preparations in both hormonal regimens.

In addition, oviducts of rabbits treated with estradiol were much less responsive to isoproterenol (a β-agonist) than were those removed after ovulation; a maximum of 50% inhibition was achieved in the estradiol-treated group while in the post-ovulatory group complete inhibition was possible. These results indicate that the decreased sensitivity for α-agonists after ovulation probably reflected an increased β-adrenergic receptor activity rather than a change in α-adrenergic receptor activity. It was concluded that ovulation and the ensuing increase in progesterone probably caused formation of or increased sensitivity of β-adrenergic receptors and that under an estradiol regimen α-adrenergic receptors are dominant. These results are not in agreement with those of Brundin (1976) and Spilman (1974) who concluded that estradiol increased α-adrenergic receptor activity via enhanced sensitivity while progesterone enhanced β-or decreased α-adrenergic receptor sensitivity. Another interesting experiment showed that heifers treated with progesterone had increased rates of tubal transport when compared to estradiol-treated animals (Crisman et al., 1980). These results agree with those of Hodson and Pauerstein (1974) in that estradiol-treated animals probably had greater contraction of the oviduct, especially of the isthmus, resulting in the "tubal block" while the ovi-

ducts in progesterone-treated animals were probably more relaxed and allowed the ovum to pass through more quickly. Paton et al. (1977) reported a dose dependent response of estradiol on oviductal contractions; small doses increased tubal transport while larger doses caused "tube locking".

Research on the effects of neurotransmitters and contractile activity within the infundibulum and fimbriae of any species is scarce. Sterin-Speziale et al. (1978) reported that the human fimbriae had frequent fluctuations in the rhythm and amplitude of the contractile cycles. However, the time course in terms of the average activity of isometric developed tension (IDT = $\bar{x}$ amplitude for 10 min) and frequency of contraction (FC = number of contractions per 10 min) was stable for periods exceeding 1 hour. Indomethacin (a prostaglandin synthetase inhibitor), significantly decreased spontaneous IDT and FC of the human fimbrial musculature during the follicular and luteal stage of the cycle, therefore, indicating that prostaglandins may influence these actions. The subject of prostaglandins will be discussed below. Bradykinin stimulated frequency and tension in fimbrial strips taken from women during both stages of the cycle. Acetylcholine increased IDT and had no effect on FC during any cyclic phase.

Fimbria taken from women during the follicular phase showed an increase in IDT and FC with exposure to norepinephrine (NE) while follicular fluid only increased IDT and had no effect on FC. During the luteal phase of the cycle NE slowly increased IDT and FC but not to as great an extent as during the follicular phase. Follicular fluid elicited the same responses during the luteal phase as it produced during the follicular phase; an increase in IDT with no change in FC.

The responses to NE suggest that this neurotransmitter causes contraction of the fimbrial musculature probably via α-adrenergic receptors. It was also suggested by Sterin-Speziale et al. (1978) that after ovulation the high progesterone levels may induce β-adrenergic receptors or may unmask β-inhibitory mediated reactions to such an extent as to diminish the contractile effect of NE exerted through α-adrenergic receptor sites. Black (1974) reported that in the rabbit oviduct the relative dominance of either α or β-adrenergic receptors was under hormonal control. Estradiol potentiated α-adrenergic receptors and progesterone increased β-adrenergic receptor sensitivity. It was also emphasized that for the previous reactions on the fimbriae, different or opposite reactions were taking place within the tubo-ovarian ligament - a structure critical for ovary-fimbriae association.

Often while the fimbriae were increasing in contraction the tubo-ovarian ligament was decreasing in contraction and (or) frequency.

Sterin-Speziale et al. (1978) concluded that NE, some prostaglandin and follicular fluid released at ovulation may cause the fimbriae to move towards the ovary by increasing contraction of the tubo-ovarian ligament. After ovulation high progesterone levels relaxed the tubo-ovarian ligament.

The general results of the previous work with human fimbriae and the tubo-ovarian ligament are not in agreement with other research on the oviductal isthmus in rabbits. Estradiol withdrawal caused increased spontaneous activity of the isthmus in response to epinephrine (an α-agonist) and isoprenaline (a β-agonist). Phenoxybenzamine (an α-blocker) inhibited movement of the ovum through the oviduct. Increased muscle tonus might explain why high doses of estradiol retard the rate of egg transport (Howe and Black, 1973). In another experiment using rabbits, the pattern and amplitude of contraction of the mesotubarium superius (an adjacent structure) were not altered with tetrodotoxin (a sodium uptake blocker) treatment, therefore, indicating that its contraction was probably myogenic in origin (Halbert and Conrad, 1975).

Ovum Reception

It has been reported that the hormonal requirement of ovum reception is different from that of ovum transport within the oviduct. In estradiol-treated rabbits, ova viabilities were higher when the ova were within the ovarian bursa than when they were inside the oviduct (Hafez, 1961). This may indicate that the ovum must be at specific stages of development when inside specific segments of the oviduct in order to fully appreciate developmental potential. In addition to hormonal influences on ova viabilities and the response to neurotransmitters the ovarian hormones play other roles in ovum reception and transport. It has been reported that the concentrations of NE in the rabbit isthmus increase with estradiol alone or with estradiol and progesterone together (Bodkhe and Harper, 1973). In another experiment Bodkhe and Harper (1972) showed that rabbits pretreated with estradiol subsequently had greater NE levels and had greater egg retention in the isthmus. An interesting facet of this experiment is that rabbits pretreated with reserpine (an NE depleter) exhibited similar ovum retention responses. If these results hold true for the infundibulum, the adrenergic mechanism of ovum reception cannot be viewed as simply a neurotransmitter-smooth muscle response, but as a complicated

interaction between neurotransmitter concentrations, receptor sensitivities, and smooth muscle responses, all under hormonal influence.

Other Factors of Possible Importance in Ovum Reception

Electrostatic Interactions

It has been recently suggested that the presence of sialic acid in association with proteinacious complexes on the ciliary membranes of the tips of cilia cling to cumulus masses via electrostatic charges.

Norwood et al. (1978) showed that upon the addition of any polycationic macromolecule onto the fimbriae of rabbits, the cumulus mass lost its ability to adhere to the fimbriae. It was proposed that these cationic macromolecules formed a bond with the anionic molecules on the tip of fimbrial cilia forming a neutral charge. Therefore, the fimbria ceased to bind to the cations of cumulus masses because the fimbriae lacked unbound anionic charges.

Estradiol Induced Edema

Overström et al. (1980) have shown that ovariectomized rabbits treated with estradiol had increased water retention in all segments of the oviduct. This estra-

diol-induced edema was similar to what was observed in the 4-hour postcoitum rabbit. In comparison, ovariectomized rabbits treated with estradiol and progesterone had a less pronounced edema while rabbits treated with progesterone alone exhibited no water retention. If estrogens have a similar effect on the infundibulum the increased edema of this structure could result in closer apposition of the fimbriae to the surface of the ovary and thereby enhance ovum reception and transport.

Differing Responses of the Various Muscle Layers and Segments of the Oviduct and Nonadrenergic Neuronal Mechanisms

In order to appreciate the complexity of ovum reception and transport the different and sometimes contrasting responses of the oviduct should be considered. Lindblom et al. (1979) separated the inner circular and external longitudinal muscle layers of the ampullary-isthmic junction of the human oviduct. These researchers found that the circular muscles had a greater frequency but shorter duration of spontaneous contractions than did the longitudinal muscles. The responses to NE and epinephrine of the muscle layer were also different; the longitudinal muscle strips were stimulated while the circular muscle layers were inhibited. In comparison, NE stimulated the circular muscle in the rabbit isthmus

but inhibited the circular muscle layer in the ampulla (Brundin, 1964). As previously mentioned, rabbit isthmus longitudinal preparations were more responsive to phenylephrine than were circular preparations under both estrogen and progesterone dominance (Hodson and Pauerstein, 1974). It was also demonstrated that transmural field stimulation (TFS) of the human ampullary-isthmic junction produced the same results as did NE, that is, stimulation of longitudinal and inhibition of the circular muscle layers (Lindblom et al., 1979). A very exciting aspect of this research was that nerve mediated inhibition (TFS-induced) of the circular muscle layer could not be blocked by propranolol (a β-receptor blocker), atropine (a muscarinic receptor blocker), or guanethidine (NE depleter), but was abolished by tetrodotoxin (a sodium uptake blocker). The authors contended that these data suggested the existence of an unknown nonadrenergic or noncholinergic neurotransmitter of an inhibitory nature.

Effects of Prostaglandins

The importance of prostaglandins in the neurological control of ovum transport and ovum reception was established when it was reported that prostaglandins were involved in NE-release mechanisms within mammalian adrenergic nerve terminals (Hedqvist and Brundin, 1969; Hedqvist,

1970; Swedin, 1971). Additional research showed that prostaglandins E were released from autonomic effector cells upon nervous stimulation (Swedin, 1971; Hedqvist and Von Euler, 1972).

As mentioned above, indomethacin decreased spontaneous IDT and FC of human fimbrial strips during both stages of the cycle indicating that prostaglandins may influence these phenomena (Sterin-Speziale et al., 1978). The fact that prostaglandins affect oviductal contractions is well documented. Prostaglandin $F_2\alpha$($PGF_2\alpha$) has been observed to stimulate contractions in the human (Brundin, 1976) and rabbit oviduct (Spilman and Harper, 1974; Brundin, 1976); unfortunately the stage of the reproductive cycle was not determined. More recently it was reported that $PGF_2\alpha$ greatly increased FC and IDT of the human fimbria with an equal magnitude during both follicular and luteal phases of the cycle (Sterin-Speziale et al., 1978).

Prostaglandin E_1(PGE_1) was observed to inhibit IDT and FC in the human fimbria with the inhibition observed during the luteal phase being of much greater magnitude than during the follicular phase (Sterin-Speziale et al., 1978). Brundin (1968, 1976) reported that PGE_1 inhibited human and rabbit oviduct contractions and decreased or abolished oviductal contractions elicited by hypogastric

nerve stimulation in the rabbit. Prostaglandin E_2 (PGE_2) was reported to slightly increase IDT and FC in the human fimbria with equal magnitude during both follicular and luteal phases (Sterin-Speziale et al., 1978).

In other research, PGE_2 was shown to inhibit contractile responses elicited by adrenergic nerve stimulation in the rabbit isthmus (Moawad et al., 1975; Paton and Johns, 1975). It was also reported that PGE_2 inhibited transmurally stimulated contractions to a much greater magnitude than NE-induced contractions (Paton and Johns, 1975). This, the authors suggested, was due to a possible presynaptic block of NE. Moawad et al. (1975) agreed with this by reporting that PGE_2 inhibited ^3H-NE release.

Clearly, prostaglandins play a critical role in the autonomic control of ovum reception and the mechanisms of their involvement deserves further investigation.

EXPERIMENT:
LOCAL EFFECT OF OVARIAN HORMONES ON RESPONSES OF THE BOVINE INFUNDIBULUM TO NEUROTRANSMITTERS

Introduction

Ovum reception by the infundibulum is a critical step in the sequence of events leading to the reproduction of viviparous mammals. Westman (1926), using rabbits prepared with an abdominal window, observed that the fimbriae of the infundibulum swept across the surface of the ovary at the time of ovulation. This close anatomical association was attributed to contractility of the mesotubarium superius causing adovarian movement of the oviduct and to contraction of the mesovarium which brought about changes in the position of the ovary relative to the fimbriae. It generally has been assumed that similar muscular contractions contribute to the ability of the infundibulum to capture the ovum in other species lacking an ovarian bursa. However, the possibility that rhythmic contractions of the fimbriae aid in ovum reception cannot be discounted (Blandau, 1969).

Oviducts of mammals, are innervated primarily by sympathetic nerves with a possible contribution by the parasympathetic system in some species (Brundin, 1965; Black, 1974; Paton et al., 1977). According to Paton

et al. (1977), the oviducts of all species studied possess α-and β-adrenergic receptors. Indeed, adrenergic neurotransmitters and agonists as well as stimulation of sympathetic nerves evoke a marked response by smooth muscle of the isthmus and ampulla (Black, 1974; Johns and Coons, 1981). Spontaneous activity and neurotransmitter-induced responses of isthmic and ampullary smooth muscle have also been shown to be affected by estrogen and progesterone (Howe and Black, 1973; Higgs and Moawad, 1974; Kennedy and Marshall, 1977; Lindblom et al., 1980).

Although the responses of the isthmus and ampulla to neurotransmitters and ovarian hormones have been extensively studied, comparatively little is known about the role of these agents in regulating the muscular activity of the infundibulum. Sterin-Speziale et al. (1978) reported that norepinephrine (NE) increased the frequency and strength of isometric contractions of fimbria removed from women during the follicular phase to a greater extent than those of fimbria during the luteal phase of the menstrual cycle. Acetylcholine (Ach) induced only a small increase in the isometric contractility of fimbria removed at both stages of the cycle. In a subsequent study from the same laboratory, Borda et al. (1980) found that fimbria of the sow immediately prior to and after ovulation also responded to NE with an increase in frequency

and strength of isometric contractions. These data suggest that the musculature of the infundibulum, like that of the isthmus and ampulla, is responsive to neurotransmitters and that these responses may be affected by ovarian hormones.

The present study was conducted to examine the in vitro response of the bovine infundibulum to NE and Ach during proestrus and diestrus of the estrous cycle. A local effect of endogenous ovarian hormones was also evaluated by comparing neurotransmitter-induced responses of the infundibulum ipsilateral to the ovary bearing the largest follicle (proestrus) or corpus luteum with those of the contralateral infundibulum.

Materials and Methods

Twelve 2-year-old nulliparous Hereford and Angus crossbred heifers exhibiting normal estrous cycles of 20.06 $\pm$ .42 days ($\bar{x} \pm$ SE) were utilized in this study. Estrus (day 0) was determined with twice daily checks with a vasectomized bull. As the heifers were detected in estrus, they were assigned alternately into two groups. Six heifers were injected intramuscularly with 25 mg prostaglandin $F_2\alpha$-tham salt ($PGF_2\alpha$; Upjohn Co.) on days 8, 9, or 10 and necropsied 3 to 4 days later on days 11, 12,or 13 of the cycle. This treatment regimen was

employed to cause luteal regression and thus ensure that infundibula of these animals be exposed to predominantly estrogen at the time of necropsy. The other six heifers were killed on day 12 of the cycle when progesterone is the predominant ovarian hormone secreted. On the morning of the day of necropsy, jugular blood samples were taken by venipuncture. Serum was separated and frozen at -20°C until radioimmunoassayed for progesterone (Koligian and Stormshak, 1976, 1977) and estradiol-17β(Zelinski et al., 1982).

Immediately following necropsy, infundibula were carefully removed noting which was ipsilateral and which was contralateral to the ovary bearing the corpus luteum or largest follicle. The tissue was transported to the laboratory immersed in Krebs-Ringer Bicarbonate (4°C) containing glucose (11mM) that had previously been gassed with 95% O_2 - 5% CO_2 (Umbriet et al., 1959). At the laboratory all the experimental procedures discussed below were performed first on the ipsilateral infundibulum. The contralateral infundibulum was kept in oxygenated KRB-glucose solution (4°C) until utilized.

The tissue was placed in a petri dish containing the same oxygenated KRB-glucose solution used in transport and a strip of infundibulum parallel to the fimbria measuring 6 to 8 x 38 to 42 mm was excised. Infundibular segments distal to the strips and portions of the fimbria

ovarica were placed in Bouins fluid, sectioned at 5 μm, stained with Mallory's phosphotungstic acid hematoxylin and examined for the presence of smooth muscle by light microscopy. The segment of infundibulum was placed into a glass tissue bath containing 12 ml of KRB-glucose solution which was continually gassed with 95% O_2 - 5% CO_2 maintained at 37°C. One end of the strip was attached with suture to a Pasteur pipet fashioned into a holder while the other end was fastened, also by means of a suture, to a micro-scale accessory (Gould, U15) coupled to a force transducer (Gould UC2 universal transducing cell) which was connected to a recorder (Beckman Dynograph RB3) via a strain gauge coupler (Beckman type 9853) and an amplifier (type 474A).

During a period of 15-min the tissue was adjusted to a basal resting tension of 250 mg using a micrometer mounted adjusting slide (Velmex unislide A1500). An additional equilibration period of 10-min was allowed before the treatments began.

The neurotransmitters to which each infundibulum was exposed, the sequence of treatments and the final concentration of the neurotransmitter in the medium were as follows: norepinephrine (0.4, 0.8 and 1.6 μg/ml; Breon Co., Palo Alto, CA), and then acetylcholine chloride (0.1, 0.56 and 1.0 μg/ml; Sigma Chemical Co., St. Louis,

MO). After addition of the neurotransmitter to the medium the tissue was allowed to attain maximum response of contraction or relaxation as measured by changes in milligrams of tension. Data during a 2-min interval of maximum response was compared to that of a 2-min interval immediately preceding the addition of neurotransmitter. Response of the infundibulum to each concentration of neurotransmitter was followed by a 72 ml wash of the tissue with KRB-glucose solution for 2 to 4 min and subsequently a 2 to 4 min equilibration period before further treatment. After the infundibula had been subjected to all concentrations of each of the neurotransmitters they were individually weighed, oven dried for 48 h ($50^{o}C$) and then reweighed.

Tissue response to each concentration of neurotransmitter was evaluated by determining frequency and amplitude of contraction and mean tissue tone. Frequency of contraction was determined by obtaining the difference between the number of recorded contractions during the 2-min maximum response and those during the 2-min equilibration immediately prior to the particular treatment in question. Change in mean amplitude of contractions was determined by measuring the height of each recorded contractile response during the 2-min treatment period followed by subtraction of the mean amplitude of contraction

observed during the preceding 2-min equilibration. A formula similar to that used in calculating mean cardiovascular blood pressure as derived from phasic blood pressure recordings was utilized to determine the difference in mean tissue tone between the 2-min treatment and the 2-min equilibration. The formula utilized was $\bar{x}_T = \bar{x}_B + \bar{x}_A / 3$ with T = tissue tone, B = base of contraction and A = amplitude of contraction.

Data for each characteristic response to each neurotransmitter were analyzed as a factorial-split-plot by use of analysis of variance. The factors were stage (proestrus and diestrus), location of infundibulum (ipsilateral and contralateral) and dose (concentrations of the neurotransmitter). Linearity and slope of the dose-response curves for each characteristic were determined by orthogonal comparisons. Because portions of the data on responses of the infundibula to acetylcholine consisted of negative values the data were converted to positive integers prior to analysis of variance by coding but decoded for presentation herein. Data for wet and dry weights of infundibula were analyzed as an experiment of 2^2 factorial design by use of split-plot analysis of variance. Differences in ovarian characteristics and serum hormone concentrations were tested for significance by use of Students unpaired t test.

Results

The ovarian characteristics and serum concentrations of ovarian hormones of all experimental animals are presented in table 1. Although treatment with prostaglandin to induce luteal regression was effective, only two of six heifers exhibited behavioral estrus and none of the animals had ovulated prior to necropsy. The mean diameter of the largest follicle in proestrous heifers was greater than in diestrous animals but the difference was not significant statistically. In all but two animals, the follicle of largest diameter was present in the ovary with the regressing corpus luteum (proestrus) or functional corpus luteum (diestrus phase). As anticipated, systemic serum concentrations of estradiol-17β were greater ($p \simeq .06$) in heifers during proestrus than during diestrus while the opposite situation prevailed for serum concentrations of progesterone ($P < .01$).

Stage of the cycle, more specifically, the predominant ovarian hormone secreted during the stage, was without affect on the wet weight of the infundibulum. Mean wet weight of the samples of infundibula of proestrous heifers tended to weigh more than those of diestrous heifers (276.5 ± 17.5 vs 233.5 ± 15.5 mg, respectively) but the difference was not significant statistically. Similarly, on a dry weight basis there was no significant

difference between infundibula of proestrous and diestrous animals (33.3 ± 1.5 vs 27.4 ± 2.2 mg). On a within-animal basis, the ipsilateral infundibulum did not differ in either wet or dry weight from the contralateral infundibulum for either proestrous or diestrous animals. Because weights of infundibula did not differ significantly within or among animals no adjustment of the response data for this characteristic was considered to be necessary before statistical analysis.

Histological preparations of the infundibula of heifers were examined for the presence of smooth muscle but none was observed.

Responses of Infundibula to Norepinephrine. Stage of the cycle and location within the animal affected the frequency of contractions of infundibula provoked by *in vitro* exposure to norepinephrine (stage x location interaction, $P < .01$). This interaction was caused by the infundibulum ipsilateral to the ovary bearing the largest follicle in proestrous heifers responding to all concentrations of norepinephrine with greater frequency of contractions than the contralateral infundibulum or both ipsilateral and contralateral infundibula of distrous animals (figure 1). Although ipsilateral infundibula of proestrous heifers appeared to respond to increasing concentrations of norepinephrine with a linear increase in

frequency of contractions, the overall dose-response relationship for infundibula was nonsignificant statistically.

Amplitude of the contractions of infundibula induced by norepinephrine was similar for all concentrations of neurotransmitter studied and did not differ between stages of the cycle or between locations (ipsilateral vs contralateral) within animal (figure 2). In contrast, ipsilateral and contralateral infundibula of proestrous and diestrous animals responded to all concentrations of norepinephrine with corresponding linear increases in mean tone ($P < .05$; figure 3). However, mean tone of the ipsilateral and contralateral infundibula during proestrus was less than those of infundibula during diestrus ($P \simeq .06$).

Responses of Infundibula to Acetylcholine. Frequency of contractions of infundibula in response to acetylcholine was dependent upon the *in vitro* concentration of neurotransmitter and the location of the tissue within animal (Ach x location interaction, $P < .01$). Regardless of the stage of the cycle, 0.1 µg/ml of Ach inhibited the frequency of contractions of infundibula (figure 4). However, 0.56 and 1.0 µg/ml of Ach stimulated frequency of contractions of the ipsilateral but, in general, was without effect or further depressed

frequency of contractions of the contralateral infundibula of proestrous and diestrous heifers.

Concentration of acetylcholine had an effect on amplitude of the contractions of infundibula with the low concentration of neurotransmitter causing a greater reduction in amplitude than the intermediate or high concentration ($P < .01$; figure 5). Amplitude of contractions was not affected by stage of the cycle or location of the infundibulum within animal ($P > .05$). Mean tone of infundibula increased when exposed to increasing concentrations of acetylcholine ($P < .01$) but the magnitude of the responses differed depending upon the location of the infundibulum within the animal ($P < .05$). Ipsilateral and contralateral infundibula at both stages of the cycle responded to 0.1 µg/ml Ach with a reduction in mean tone (figure 6). However, in vitro exposure to 0.56 and 1.0 µg/ml Ach caused a greater increase in mean tone of ipsilateral compared with contralateral infundibula of proestrous and diestrous animals.

Discussion

Results of the present experiments demonstrate that the bovine infundibulum is responsive to norepinephrine and acetylcholine. However, the responses evoked by these neurotransmitters are governed by the ovarian

hormone to which the infundibulum is exposed. Hormones secreted by the bovine ovary can affect the function of distant target tissues via the systemic circulation and can also act locally on tissues of the reproductive tract. Ford et al. (1976) have shown that ovarian hormones in the cow act locally to alter the contractility of uterine arterial smooth muscle induced by nerve stimulation. Experimental results suggest that estrogen secreted by the ovary of the cow during proestrus may act locally to enhance the contractility of the ipsilateral infundibulum. Frequency of contractions of the infundibulum adjacent to the ovary bearing the largest follicle in proestrous heifers was increased by norepinephrine compared with that of the contralateral infundibulum or the infundibula of animals exposed predominantly to progesterone.

These data are in general accordance with the results of previous investigations demonstrating that frequency of contractions of oviductal smooth muscle is greater when under the influence of exogenous or endogenous estrogen than progesterone (Howe and Black, 1973; Spilman, 1974; Higgs and Moawad, 1974; Gimeno et al., 1976; Sterin-Speziale et al., 1978; Lindblom et al., 1980). Tonic release of norepinephrine from adrenergic nerve terminals in the bovine infundibulum exposed to increased local concentrations of estrogen may enhance the contractility of

this structure and thus promote its ability to capture the ovum. The local pathway by which ovarian estrogen is able to reach the infundibulum of the cow may be similar to that found to exist in the primate by Beachy et al. (1980). These researchers demonstrated that tissue attaching the ovary to the adjacent fimbriae contained venous anastomoses.

Ipsilateral and contralateral infundibula of heifers during diestrus responded to norepinephrine with an increase in tone compared with that of infundibula from heifers in proestrus. The increased systemic concentration of progesterone to which the contralateral infundibulum was exposed precluded detection of a local effect of the steroid, if any, on this infundibular response. The mechanism by which progesterone enhances the ability of norepinephrine to increase infundibular tone is not known. Norepinephrine-induced increase in infundibular tone during progesterone dominance was not due to an increase in amplitude of contractions because this infundibular response to the neurotransmitter did not differ between stages of the cycle studied.

Whether measuring frequency or amplitude of contractions or mean tone, the bovine infundibulum responded

to increasing concentrations of acetylcholine with a stereotypical pattern. The low concentration of acetylcholine always caused inhibition while responses to the intermediate and high concentrations of acetylcholine were generally stimulatory. In general, exposure of the infundibulum to estrogen or progesterone did not appear to affect these characteristic responses to acetylcholine, but a local effect of the steroid may be important in modulating the action of acetylcholine. Ipsilateral infundibula at both stages of the cycle studied were more responsive to acetylcholine than contralateral infundibula, at least in terms of changes in mean tone, and although not significant statistically, in frequency of contractions. The paradoxical effects of acetylcholine are difficult to explain. It is not known why low concentrations were inhibitory whereas higher concentrations were less inhibitory or stimulatory. However, the pattern of contractility of arterial smooth muscle evoked by increasing concentrations of acetylcholine (Vanhoutte, 1974) is remarkably similar to that observed for the infundibulum. Vanhoutte (1977) proposed that acetylcholine has a dual effect on vascular smooth muscle with low concentrations causing relaxation and high concentrations inducing contraction. Both the inhibitory and excitatory component are inhibited by atropine suggesting the presence of two

muscarinic receptors in vascular smooth muscle. Perhaps the smooth muscle in the infundibulum of the cow is also endowed with similar muscarinic receptors. On the other hand, a report by Merrigan and Lais (1981) indicates that high doses of acetylcholine cause contraction in rat vasculature by an action on the smooth muscle. In contrast, lower doses of acetylcholine, which did not produce a direct smooth muscle effect, are capable of inhibiting completely the release of norepinephrine as a result of field stimulation of the sympathetic nerve plexus indicating a prejunctional site of action for this neurotransmitter. If there is tonic release of norepinephrine in the bovine infundibulum, the general depression of contraction amplitude and frequency and also of mean tone at 0.1 µg/ml acetylcholine vs an enhancement of these characteristics at higher doses may be an expression of the presence of sensitivity differences in pre- and postjunctional acetylcholine receptors in this tissue. These data would then be similar to those described for other tissues with sympathetic innervation (Vanhoutte, 1977; Merrigan and Lais, 1981). The failure of this relationship in the ipsilateral infundibulum from cows in proestrus may indicate a specific action of estrogen on the prejunctional acetylcholine receptor. Such a specific alteration in the prejunctional acetylcholine receptor

following environmental stress has been previously reported (Merrigan and Lais, 1981).

It is generally acknowledged that the smooth muscle present in the infundibulum of all mammals is sparse compared with the more proximal portions of the oviduct. Reports on the presence of circular and(or) longitudinal smooth muscle in the bovine infundibulum are equivocal. Lombard et al. (1950) reported finding no smooth muscle in the infundibulum of the cow. However, according to Beck and Boots (1974) the bovine infundibulum is characterized by the presence of an inner longitudinal and an outer circular layer of smooth muscle. Examination of histological preparations of the infundibula of heifers in the present study failed to reveal the presence of smooth muscle which was not associated with arteriole walls. However, if smooth muscle in the bovine infundibulum exists in spiral bundles such as found in the ampulla (Schilling, 1962) it could have been missed because serial sections were not examined. Thus, the nature of the smooth muscle in the bovine infundibulum that responded to norepinephrine and acetylcholine is not known. Alternatives to typical circular or longitudinal smooth muscle may be the smooth muscle of arterioles or the mesotubarium superius, portions of which may have been included in the samples removed for study. The

latter possibility seems remote because care was taken to remove adhering ligaments from the samples utilized for *in vitro* experiments.

Based upon the results of this study it is proposed that the bovine infundibulum is not merely a passive structure but rather one that undergoes rhythmic contractions, particularly when under the influence of estrogen, and thus plays an active role in ovum reception.

TABLE 1. OVARIAN CHARACTERISTICS AND SERUM HORMONE CONCENTRATIONS (MEAN ± SE)[a]

Item	Stage of the cycle	
	Proestrus	Diestrus
Corpus luteum wt., (g)	.852 ± .074	3.223 ± .267**
Largest follicle, dia.(mm)[b]	17.9 ± 1.0	13.7 ±1.2
Serum progesterone (ng/ml)	.20 ± .02	2.51 ± .15**
Serum estradiol-17β(pg/ml)	7.88 ± 1.66	4.95 ± .31*

[a]Means are based upon samples from six heifers at each stage of the cycle.

[b]Only the diameter of the largest visible follicle on both ovaries was measured.

*P = .06

**P< .01

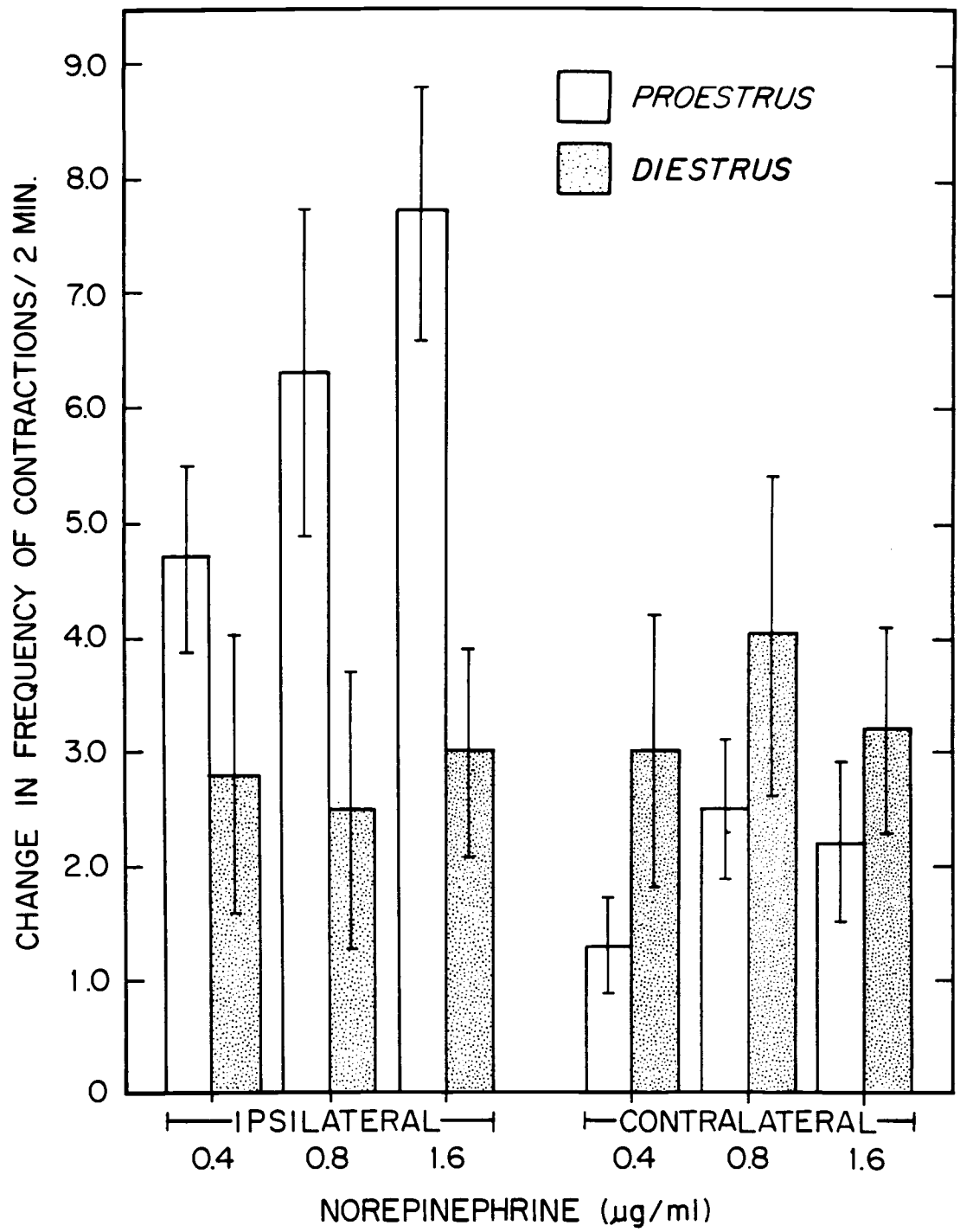

Figure 1. Changes in the frequency of contractions of bovine infundibula induced by norepinephrine in vitro. Ipsilateral and contralateral infundibula were from six heifers each in proestrus or diestrus of the estrous cycle. Each bar represents the mean ± SE.

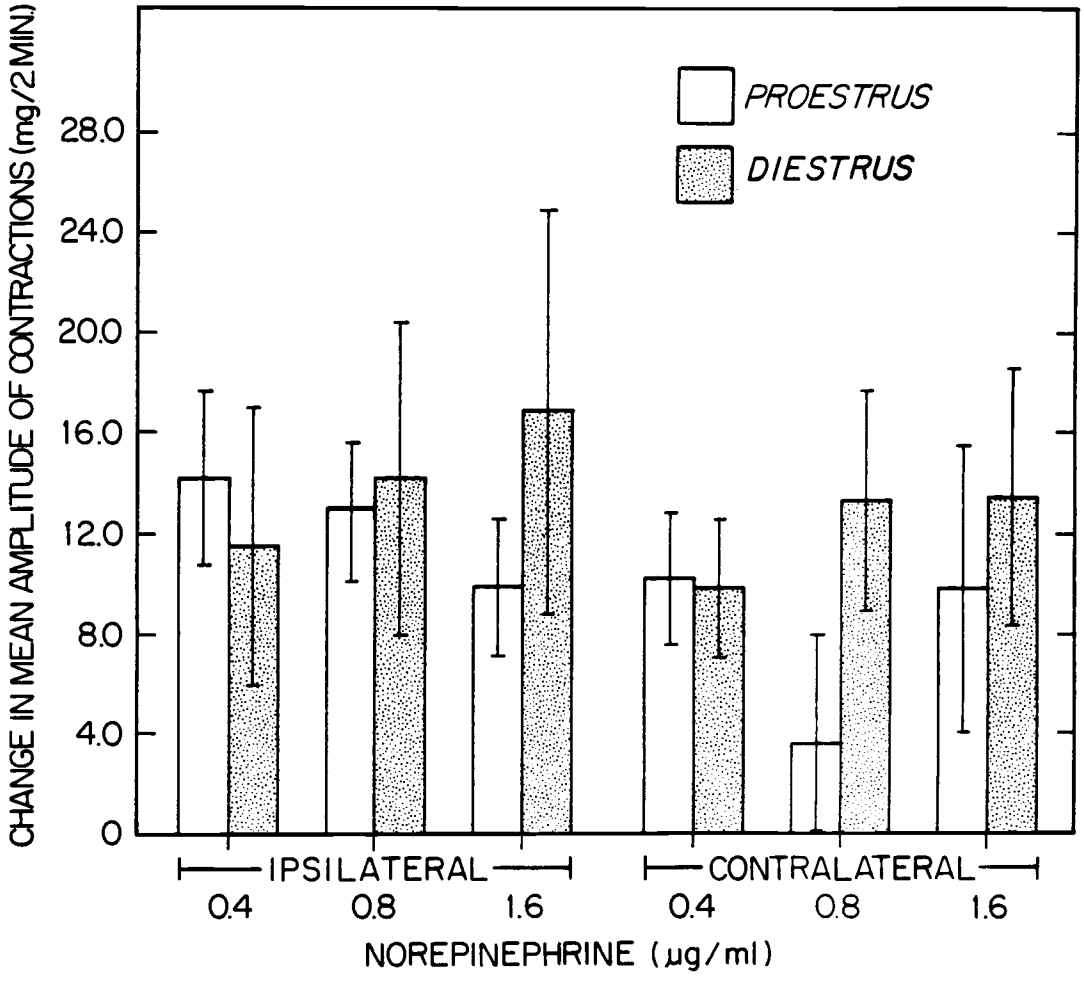

Figure 2. Amplitude of contractions of bovine infundibula after exposure to norepinephrine in vitro. Ipsilateral and contralateral infundibula were from six heifers each in proestrus or diestrus of the estrous cycle. Each bar represents the mean ± SE.

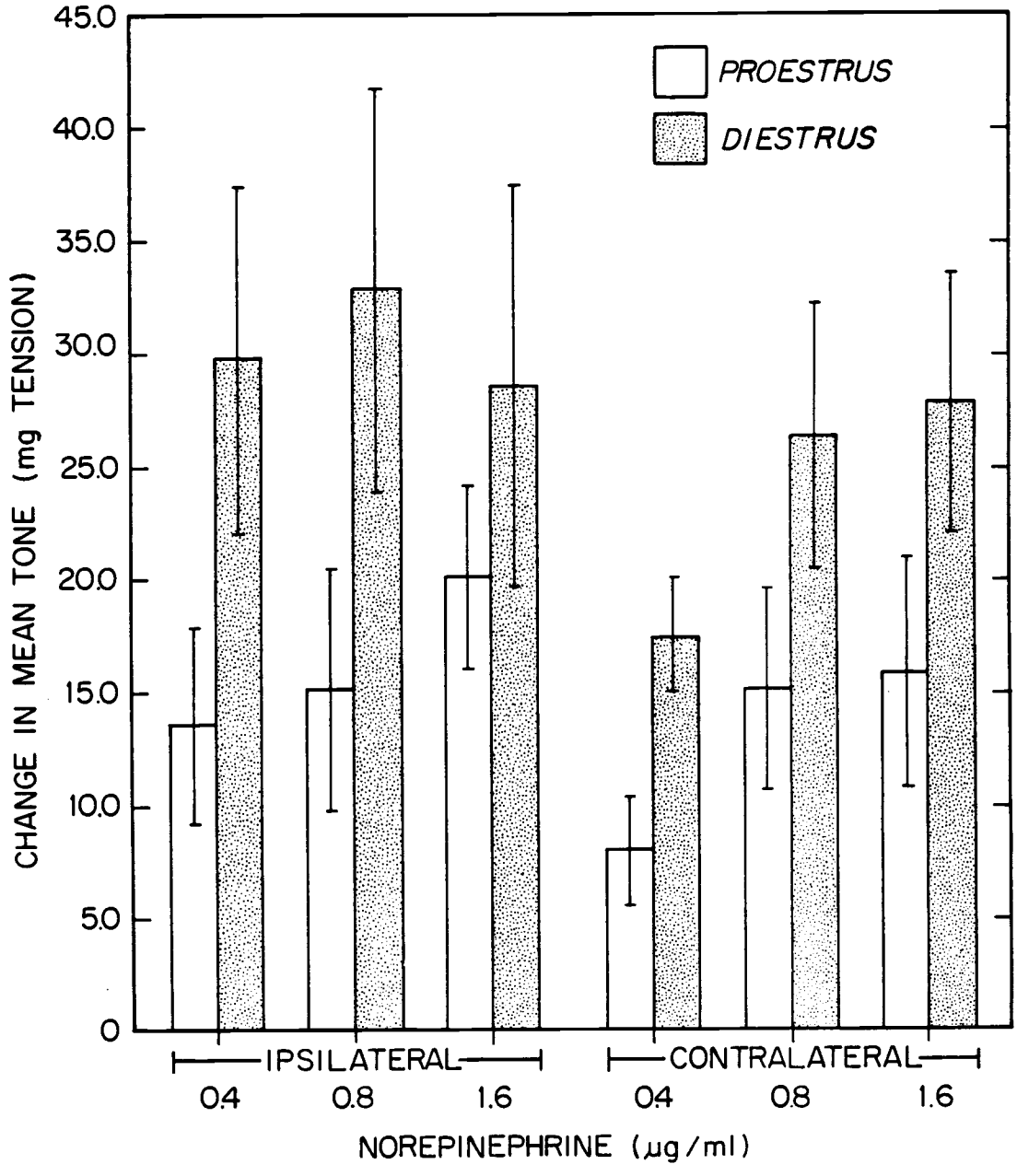

Figure 3. Norepinephrine-induced changes in infundibular tone as determined in vitro. Ipsilateral and contralateral infundibula were from six heifers each in proestrus or diestrus of the estrous cycle. Each bar represents the mean ± SE.

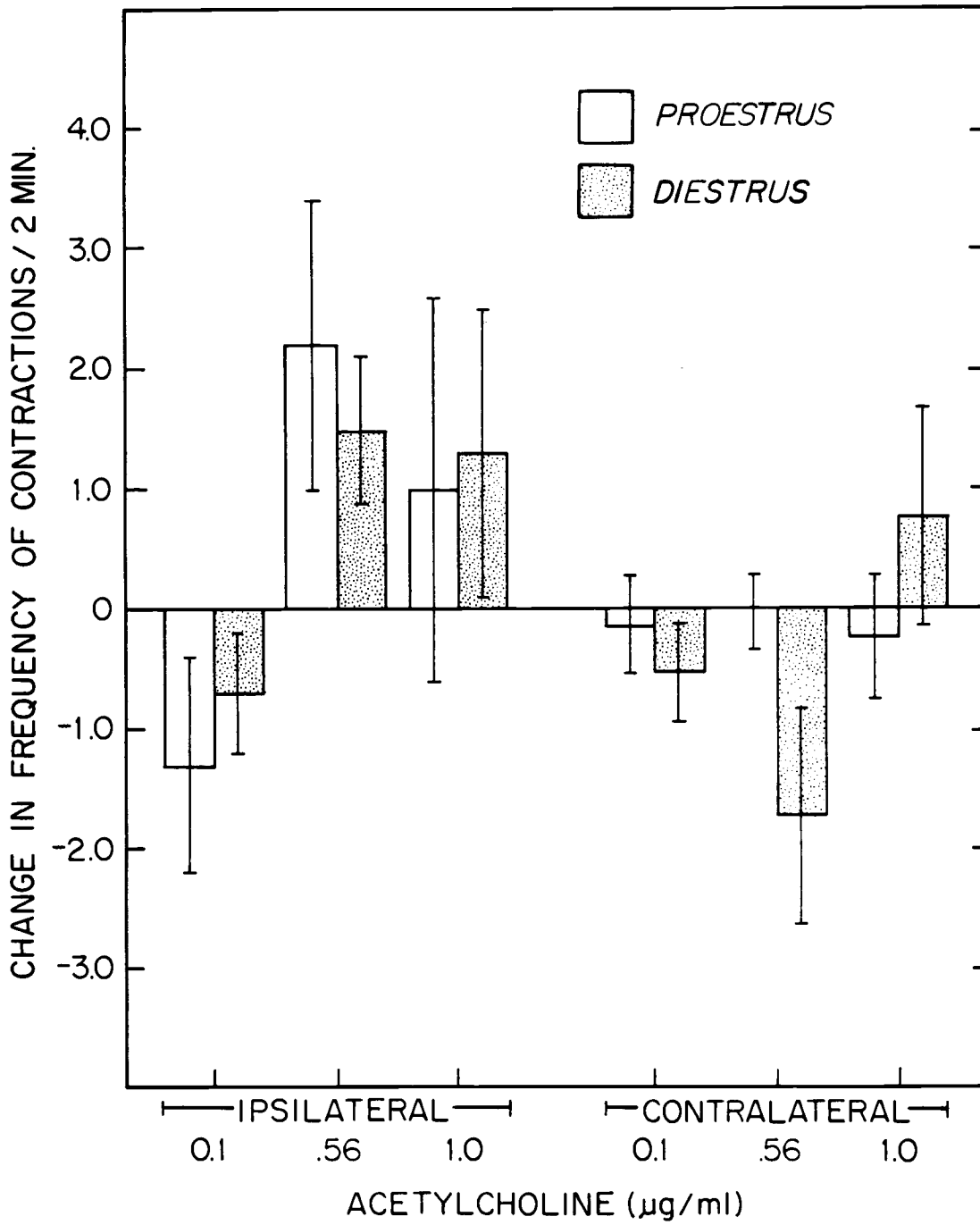

Figure 4. Effect of acetylcholine on in vitro frequency of contractions of ipsilateral and contralateral infundibula of six heifers each during proestrus and diestrus of the estrous cycle. Each bar represents the mean ± SE.

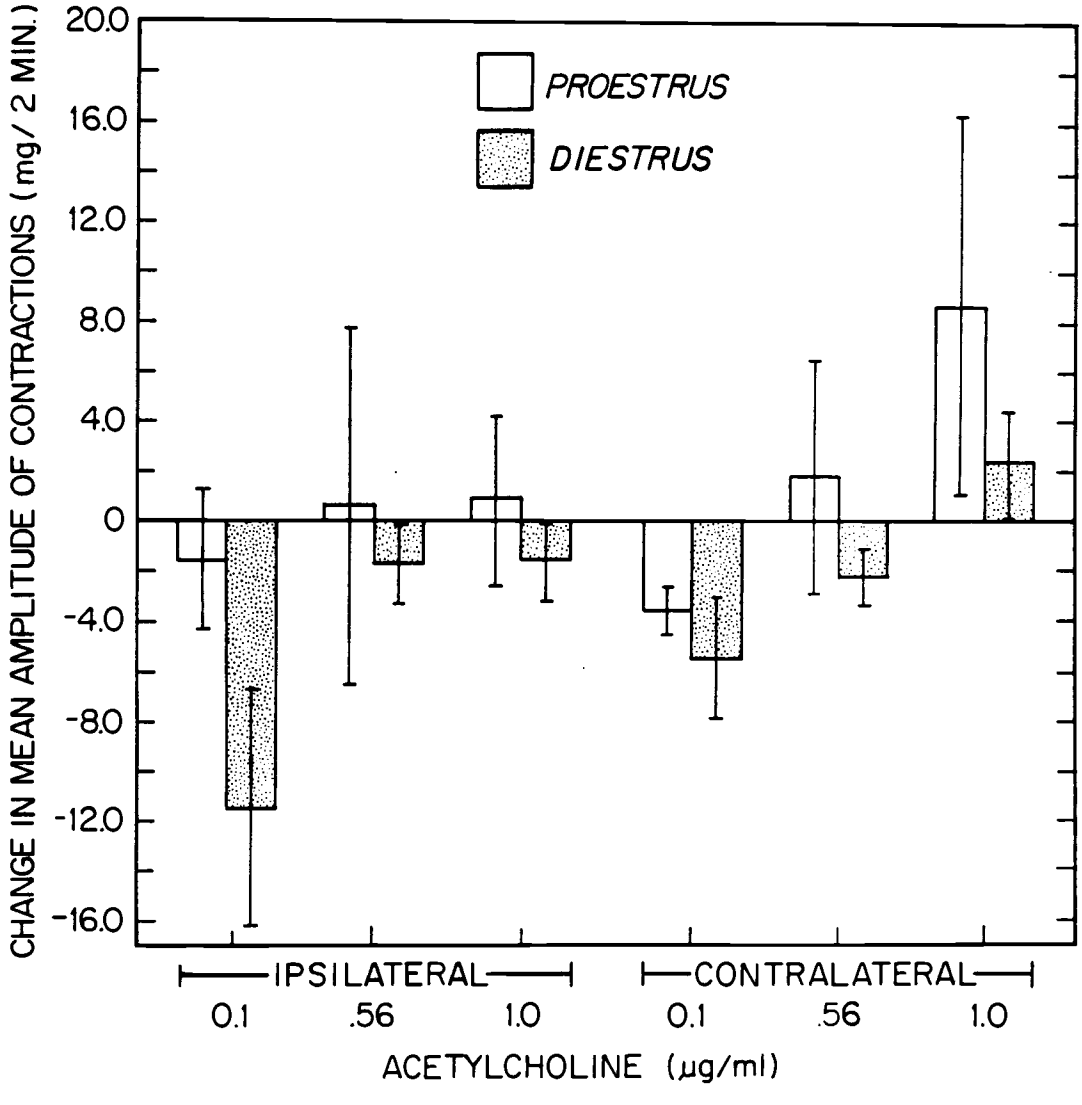

Figure 5. Changes in amplitude of contractions of bovine infundibula induced by acetylcholine in vitro. Ipsilateral and contralateral infundibula were from six heifers each in proestrus or diestrus of the estrous cycle. Each bar represents the mean ± SE.

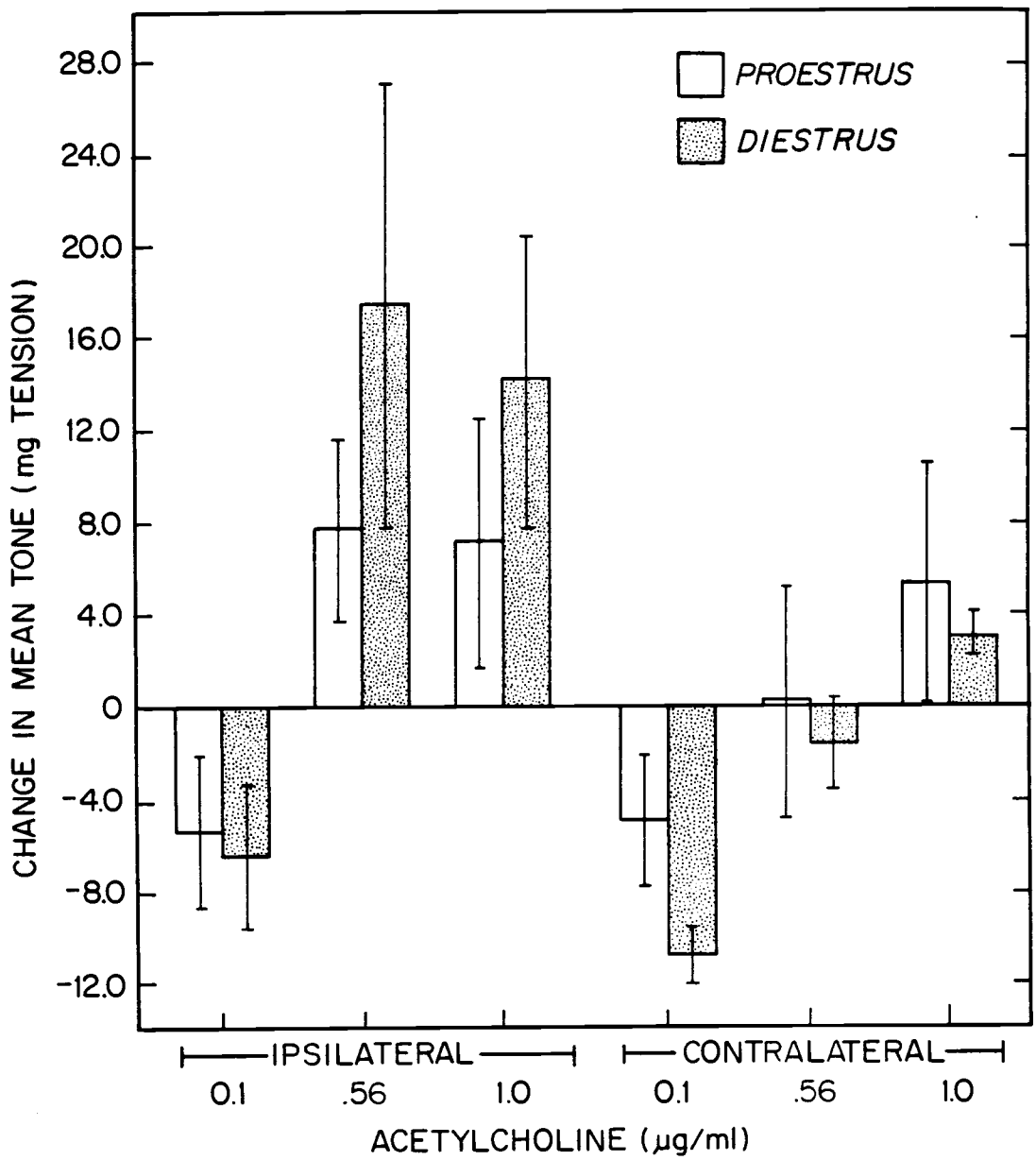

Figure 6. Infundibular tone as affected by acetylcholine in vitro. Ipsilateral and contralateral infundibula were from six heifers each in proestrus or diestrus of the estrous cycle. Each bar represents the mean ± SE.

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