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HORMONAL ALTERATION OF THE RESPONSE OF THE RAT UTERUS TO CATECHOLAMINES

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Ahlquist[1] has postulated that all uteri possess both β-inhibitory and α-stimulatory adrenergic receptors. Evidence supporting this hypothesis has been obtained in several species.[2-7] In addition, changes in the hormonal state of the uterus have been shown to alter the response of the uterus to catecholamines in the cat[2] and guinea pig [3, 8] and to alter the uterine response to nerve stimulation in the rabbit.[5] However, no reports have yet appeared concerning the influence of ovarian hormones on the uterine response to catecholamines in the rat. Therefore, the present study was undertaken in order to investigate the effects of estradiol and progesterone on the response of the rat uterus to norepinephrine and epinephrine.

METHODS

Female Holtzman rats weighing from 180 to 220 grams were used throughout this investigation. All animals were ovariectomized and allowed to recover for 7-8 days before hormone injections were begun. The animals were divided into 4 groups which were treated as follows: Group A was ovariectomized, but did not receive any hormone injections. Group B animals

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were ovariectomized and injected subcutaneously with 50 μg of estradiol benzoate in peanut oil 48 hours before sacrifice. Animals in Group C were ovariectomized and received a priming dose of 50 μg of estradiol benzoate followed 48 hours later by a combination dose of 25 μg of estradiol benzoate and 3 mg of progesterone daily for 3 days. The animals were sacrificed 24 hours after the last injection of estradiol and progesterone. Group D animals were ovariectomized and received a priming dose of 50 μg of estradiol benzoate followed 48 hours later by 3 mg of progesterone daily for 3 days. They were sacrificed 24 hours after the last progesterone injection.

All animals were anesthetized with diethyl ether and the uteri were removed and suspended in muscle baths for recording of isometric tension as previously described.[7]

The following drugs were used: l-norepinephrine (Levophed, Winthrop Laboratories); l-epinephrine (Adrenalin, Parke, Davis and Co.); propranolol HCL (Alderlin, Imperial Chemical Industries, Ltd.); phentolamine HCL (Regitine, Ciba, Inc.); estradiol benzoate and progesterone (Nutritional Biochemicals Corp.).

RESULTS

Figure 1 illustrates representative tracings for the 4 groups of animals tested.

The spontaneous contractions in the untreated ovariectomized group (tracing A) were rapid, irregular and of low amplitude. In every case (10 experiments) these uteri were relaxed upon addition of norepinephrine or epinephrine to the bath. This relaxing effect was β-adrenergic in character and was completely blocked by pretreating the muscles with the β-adrenergic blocking agent, propranolol. It was difficult to demonstrate any α-stimulatory

effect in these uteri as shown by the lack of effect seen after norepinphrine in the presence of propranolol. Four times the usual concentration of norepinephrine still did not consistently stimulate the uteri in the presence of propranolol, although weak stimulation was seen in a few experiments. The fact that these muscles were capable of being stimulated was demonstrated by their response to oxytocin, but the amplitude of contraction was never very large.

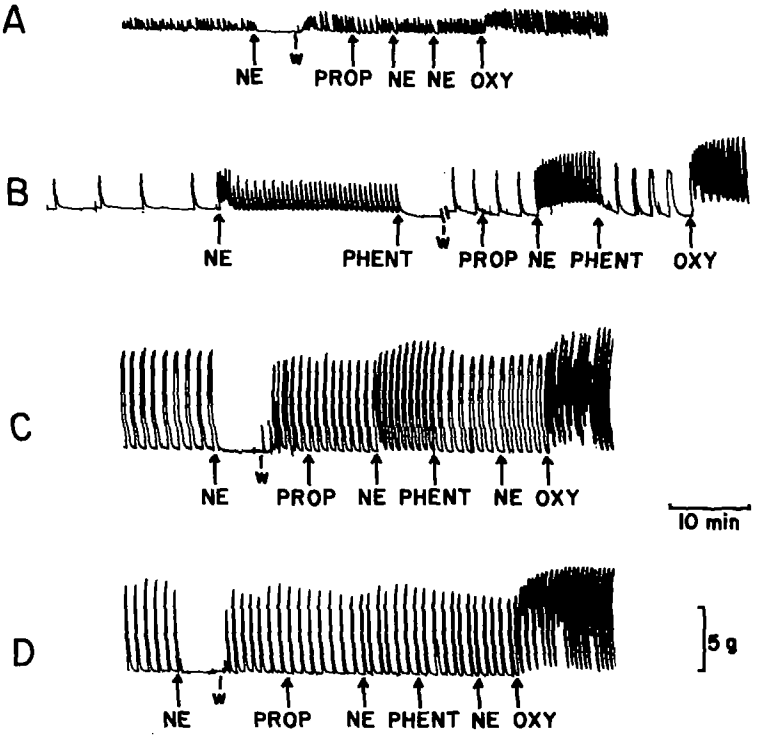

Fig. 1

Influence of estrogen and progesterone on the response of the rat uterus to norepinephrine.

Concentrations of the drugs used were as follows: norepinephrine (NE), $3x10^{-6}$M; propranolol HCL (PROP), 1 μg/ml; phentolamine HCL (PHENT), 20 μg/ml; oxytocin (OXY), 2 mU/ml. W's indicate points at which drugs were washed from the preparation. Tracings A, B, C and D represent results from 4 different groups of animals. See methods section for details of the treatment.

Tracing B illustrates the effect of norepinephrine on the motility of uteri from ovariectomized rats injected with estradiol benzoate. Spontaneous contractions, in these samples were infrequent, and in some cases the muscles were completely quiescent. However, spontaneous contractions, when they did occur, were of greater amplitude than those in Group A and the muscles were capable of strong contractions when stimulated. In most cases the response to norepinephrine was reversed in these animals. Instead of the relaxation seen in the untreated ovariectomized group, a stimulation of uterine motility was seen in the uteri from the estrogen-treated rats. This stimulation was abolished by addition of the α-adrenergic blocking agent, phentolamine, and the β-inhibitory effect of norepinephrine was observed. In only 3 of the 21 samples tested in this group did norepinephrine produce relaxation as its major effect. It is possible that if larger doses of estrogen had been used, these samples would also have been stimulated. Epinephrine, in contrast to norepinephrine, caused a relaxation of uteri from estradiol injected animals. However, when the tissues were pretreated with propranolol, epinephrine, as well as norepinephrine, produced a marked stimulation of uterine motility. This stimulation was again abolished by addition of phentolamine, and further addition of norepinephrine was without effect. However, a marked stimulation of the muscle could still be obtained by adding oxytocin to the bath at this point.

Tracings C and D illustrate the effects of norepinephrine and adrenergic blocking drugs on the motility of uteri taken from rats pretreated with estrogen plus progesterone (group C) and with progesterone alone (group D) for 3 days prior to sacrifice. All of these uteri exhibited a higher degree of spontaneous motility than did those from rats which received estrogen only.

Contractions were stronger, more regular and more frequent in the progesterone treated group. The response to norepinephrine was also different from that seen in the estrogen treated group. In all of the uteri from progesterone treated animals, norepinephrine produced an inhibition of spontaneous motility, even in those animals which had received both estradiol and progesterone for the last 3 days. Some stimulation could be seen in these uteri when they were treated with norepinephrine in the presence of propranolol, but this stimulation was much less marked than that seen in uteri from estrogen treated rats (group B). This stimulation was again abolished by phentolamine. Oxytocin, in the concentration used, was still capable of producing a marked stimulation of uteri from progesterone injected animals.

Although complete dose response curves were not done in this study, experiments with blocking agents and low doses of norepinephrine indicate that the estrogen dominated uteri are more sensitive to both the stimulatory and the relaxant effects of catecholamines than are the progesterone dominated or the untreated ovariectomized uteri.

DISCUSSION

Since uteri of several species have been shown to possess both β-inhibitory and α-excitatory adrenergic receptors,[2-7] the response of the uterus to norepinephrine and epinephrine (which are capable of stimulating both types of receptors) may depend upon which receptor predominates in the muscle at a given time. Tsai and Fleming[2] have reported that in the virgin cat the uterus is relaxed by epinephrine (a β-adrenergic effect) whereas it is stimulated by epinephrine in cats under the influence of progesterone (an α-adrenergic effect). Reports in other species, however, differ from these observations in the cat. For example, in the rabbit[5] it has been shown that

the response to nerve stimulation in the estrogen treated myometrium is predominately an excitatory one (and is blocked by α-blockers) while the response in the progesterone treated muscle is inhibitory (and is blocked by β-blockers). Similarly, in the guinea pig after premedication with estrogens, epinephrine causes a contraction of uterine muscle, but after treatment with progesterone, epinephrine relaxes the uterus in that species.[8] On the basis of these latter results, it was assumed hypothetically that progesterone possesses the ability to sensitize the β-adrenergic receptors in the guinea pig uterus.[8]

Our results in the rat are compatible with the above reports for rabbit and guinea pig uterus. In our experiments, uteri from untreated ovariectomized rats, or from ovariectomized rats treated with progesterone or progesterone plus estrogen, were all relaxed by norepinephrine (a β-adrenergic effect). However, in ovariectomized rats treated with estrogen alone, the uterine smooth muscle was stimulated by norepinephrine (an α-adrenergic effect). Thus, in this species, the levels of circulating ovarian hormones can influence the response of the myometrium to norepinephrine. Estradiol benzoate appears to influence the α-adrenergic receptors of the rat myometrium in some way, so that the response to norepinephrine is reversed, from one of relaxation in ovariectomized animals to one of contraction in ovariectomized estrogen treated animals. Progesterone, on the other hand, appears to antagonize this action of estrogen, so that in rats which have received both estrogen and progesterone, the uterus is relaxed by norepinephrine (just as it is in the ovariectomized controls). Progesterone does not appear to act by sensitizing the β-adrenergic receptors of the rat uterus as suggested by Cieciorowska for the guinea pig,[8] since the progesterone dominated rat

uterus is less sensitive to the β-adrenergic or relaxing effects of norepinephrine. Miller and Marshall have shown that the levels of circulating ovarian hormones can alter the uterine response to nerve stimulation in the rabbit.[5] It would be interesting to see whether similar effects could be demonstrated in other species. Such an interaction may play a role in the initiation of labor in pregnant animals at term.

SUMMARY

The influence of circulating ovarian hormones on the uterine response to catecholamines in the rat has been studied. Uterine smooth muscles from untreated ovariectomized rats, or from ovariectomized rats under the influence of progesterone, are relaxed by norepinephrine. This response is blocked by β-adrenergic blocking drugs. Samples taken from estrogen-dominated rat uteri, on the other hand, are usually stimulated by norepinephrine, a response which can be prevented by α-adrenergic blocking agents.

Thus, the response of the rat uterus to norepinephrine appears to depend on a balance between α and β adrenergic receptors in the myometrium, and this balance is in turn regulated by the ovarian hormones.

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