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A COMPARISON OF PROGESTERONE AND EPINEPHRINE INHIBITION ON THE MYOMETRIUM OF THE RAT

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Abstract—This study was executed to gain insight into the possibility of a relationship between the smooth muscle depressing effects of epinephrine and of progesterone.

The first part of the investigation considered the possibility that an adrenergic blocking agent, dichloroisoproterenol, may affect the ability of progesterone to exert its inhibitory effects on smooth muscle as the blocking agent does with epinephrine. The second was a comparative study on the oxygen-stimulating effects of epinephrine and progesterone. The rationale behind this last experiment was derived from observations of Bueding and Bulbring* which have led them to the conclusion that the epinephrine-induced relaxation of smooth muscle is mediated through an increase in oxidative metabolism.

Epinephrine (1·04 mg/ml) caused inhibition of the spontaneous contractions of rat uterine strips when bathed in Krebs original Ringer phosphate buffered solution. Progesterone, at a concentration of 20 μg/ml, also caused inhibition of spontaneous activity of the rat uterus under the same conditions. Dichloroisoproterenol, at 1 μg/ml, inhibited the depressing effect of epinephrine at the previously mentioned dose, but had no effect on the depressing effect of progesterone. Epinephrine caused an increase in the rate of oxygen consumption of the rat uterus at 5, 15, and sometimes 30 min, after which the rate diminished as the effect wore off. Progesterone caused a significant depression of oxygen consumption of the uterus over the entire hour measured, and especially during the last 15-min period.

The conclusion was drawn from these results that epinephrine and progesterone depress smooth muscle activity via independent mechanisms.

INTRODUCTION

EVIDENCE has accumulated suggesting that some similarities exist between the action of progesterone and epinephrine on spontaneously contracting smooth muscle preparations. Progesterone administration results in altered ionic movement across the muscle membrane, hyperpolarization, blocked impulse transmission, and inhibited spontaneous contraction in uterine[1, 2] as well as in extrauterine smooth muscle.[3] These same effects are reflected in smoooth muscle preparations treated with epinephrine.[4]

The objective of this investigation was to test for other similarities between these two hormones. This involved two phases of study. Initially, the influence of an adrenergic blocking agent, dichloroisoproterenol, on the ability of epinephrine or pro-

* E. Bueding and E. Bulbring, in *Pharmacology of Smooth Muscle*, pp. 37–54, Macmillan, New York (1964).

gesterone to exert its effects on smooth muscle was determined. This study was prompted by the results of Miller and Murry[5] and of Rudzik and Miller,[6, 7] who found that the effects of relaxin were mediated through release of epinephrine stores and could be prevented by adrenergic blocking agents.

Second, a comparative study was made of the effect of progesterone and epinephrine on the oxygen uptake of uterine strips. This approach was instigated to investigate the possibility that progesterone might bring about the same effect as that of epinephrine in causing an "active relaxation", which involves an increase in oxidative metabolism of the tissue.[4]

METHODS

Animals. Female white rats of the Sprague–Dawley strain were used. These animals were from 4–6 months old and weighed 200–300 g. They were given food and water *ad libitum* and exposed to a 12-hr on–12-hr off light cycle. To reduce variations in uterine responsiveness due to ovarian cycles, all animals were ovariectomized and treated daily with 5 μg of 17β-estradiol for 5 days prior to use.

Solutions. Krebs original Ringer phosphate solution (KRP) was used as the bathing medium for all experiments; it consisted of 100 parts 0·9% NaCl, 4 parts 1·15% KCl, 3 parts 1·122% $CaCl_2$, 1 part 3·8% $MgSO_4.7\ H_2O$, and 21 parts 0·1 m phosphate buffer, pH 7·4.

The following solutions were compounded in KRP in such a way that when diluted in the tissue bath the following concentrations resulted: dichloroisoproterenol (DCI, courtesy of Lilly Laboratories, Indianapolis), 1 μg/ml; epinephrine (K & K Special Chemicals), 1·04 mg/ml; progesterone (K & K Special Chemicals), 20 μg/ml in 3% ethanol solution; and also 20 μg/ml in a 0·06% albumin solution.

Tissue preparation. The rats were decapitated and entire uteri were removed as rapidly as possible and placed in a solution of chilled physiological saline. Fat and connective tissue were trimmed away, and the uterine horns were separated from the cervix. Segments of approximately 2 cm in length were cut from corresponding positions of each horn. One segment served as the control and the other as the experimental tissue.

In the uterine twitch studies, one end of the uterine segment was firmly anchored in a warm chamber while the other end was attached to a myograph. Fifty ml KRP was contained in the bath, which was maintained at 37° $\pm$ 0·1° and continuously gassed with oxygen. Tension on the uterine strip was adjusted to 0·2 g in the uncontracted phase and recordings were registered on a Grass polygraph. An initial series of contractions was recorded for 15–20 min to allow for stabilization of contractile activity; then the various compounds were added to the bathing solution. When the blocking agent, dichloroisoproterenol, was added, 5 min was allowed to elapse before addition of the hormones. After each experiment the tissue was washed and activity was allowed to return to normal. For these studies 10 animals were used for each dose level, and usually two experiments were obtained from each set of uterine horns.

In the respiration studies, the uterine horns were prepared in a similar manner, but in addition were cut longitudinally to assure adequate access to the bathing medium. The tissues were then placed into Warburg flasks each of which contained 2·8 ml of chilled KRP. Methods of Umbreit *et al.*[8] for the determination of oxygen uptake were followed throughout the experiment. Manometer readings were taken 5, 15, 30, 45,

and 60 min after the contents of the side arm had been delivered to the medium. At the end of 60 min, the tissue was dried at 120° for 12 hr to obtain the dry weight. This was used to derive the Q_{O_2} of the tissue, which is defined as the number of cubic milliliters of oxygen consumed per milligram (dry wt.) of tissue per hour.[8]

Two types of statistics were used. For the initial studies, Student's *t*-test was employed, but later, as paired samples became available, paired sample analysis was used to compare the two tissues from the same animal.

RESULTS

Uterine twitch studies. Due to the insolubility of progesterone in water, an adequate solvent was needed which by itself would not affect spontaneous contractions of the uterus. Propylene glycol is one of the standard progesterone solvents in use, but in our hands it was found to be depressing to the uterine preparation (Fig. 1a). A solution of 3% ethanol in KRP was tried, and although there was usually a slight depression immediately upon addition, no alteration in frequency of contraction or tension was recorded (Fig. 1b). The original experiments were carried out with 3% ethanol as the solvent for progesterone. As can be seen in Fig. 1 (c, d, and e), an adequate dose response effect at 5, 10, and 20 μg/ml of progesterone was obtained. However, due to subsequent effects of the alcohol vehicle on tissue respiration, the above experiments were repeated with a 0·06% albumin carrier for the progesterone. Figure 1f shows that, at the 20 μg/ml dose, the progesterone was equally effective in the albumin solution.

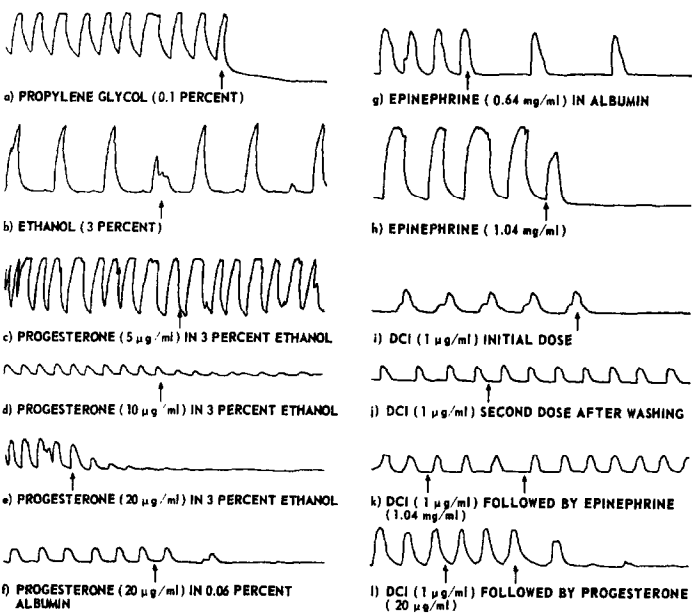

FIG. 1. The effects of solvents and drugs on the spontaneous contractile patterns of rat uterine strips.

Dose response studies were also determined with epinephrine. Since the effects were not apparently different with the alcohol and albumin solvents, only the results of the latter solvent are shown (Fig. 1g and h). The effective dose for complete inhibition

of the muscle was determined as 1·04 mg/ml, whereas 0·64 mg/ml resulted in partial inhibition.

Initial experiments with DCI showed some depression of spontaneous activity at a dose (1 μg/ml) which effectively blocked epinephrine (Fig. 1i). Subsequent experiments, however, demonstrated that washing of the tissue after the initial introduction of DCI prevented depression by the second addition of this drug at the same dose (Fig. 1j). This procedure did not seem to alter the effectiveness of DCI as a blocking agent in our experiments and so was the method used.

Fig. 1k illustrates the effectiveness of 1 μg/ml DCI in blocking the depression of uterine activity by epinephrine. As can be seen, instead of depressing the uterine activity, epinephrine apparently slightly stimulated the frequency of contraction in the presence of DCI. Progesterone, however, in the same procedure was still able to completely depress uterine activity (Fig. 1l), with the same gradual pattern of inhibition seen in the absence of DCI.

Respiration studies. The second approach to the overall problem was to investigate the relative effects of epinephrine and progesterone on the oxidative metabolism of the uterus. In order to discover if the change in oxygen consumption was related to inhibition of spontaneous activity, a low dose that had been ineffective in depressing physical activity was compared to the dose that caused complete inhibition of activity. Figs. 2 and 3 present the results of this study and do indeed exhibit a definite

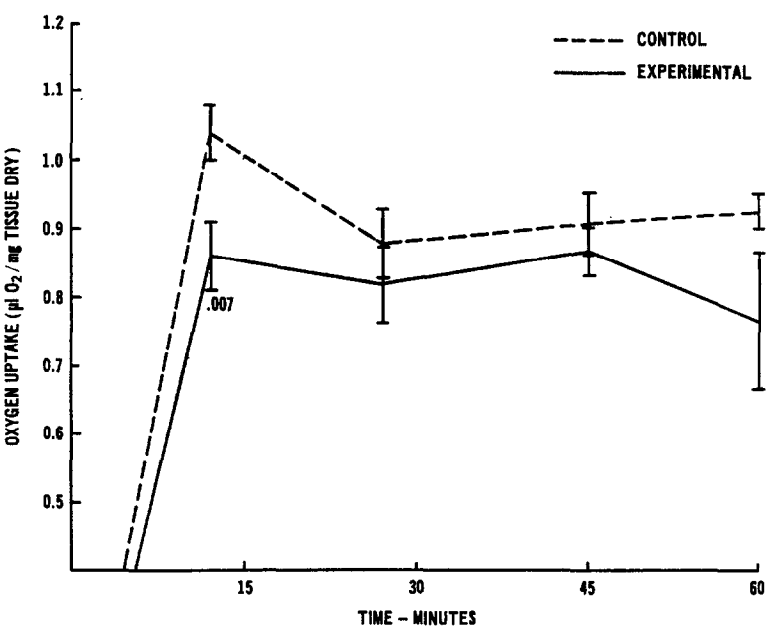

FIG. 2. Mean effects of epinephrine (0·18 mg/ml) on Q_{O_2} of rat uterine strips. Epinephrine added at 0 time. I indicates S.E; figures indicate P values.

relationship between increased oxygen utilization and activity depression. At the lower dose (0·18 mg/ml) there was no stimulation of oxygen uptake but a definite depression at the 15-min period. At the higher dose (1·04 mg/ml) there was a regular

significant increase in oxygen consumption by the treated tissue at the 15-, 45- and 60-min periods.

Since the original progesterone and epinephrine work was done in weak alcoholic solutions, the effect of epinephrine in ethanol on uterine respiration was measured. After 30 min in the presence of ethanol, the control tissue was found to respond with

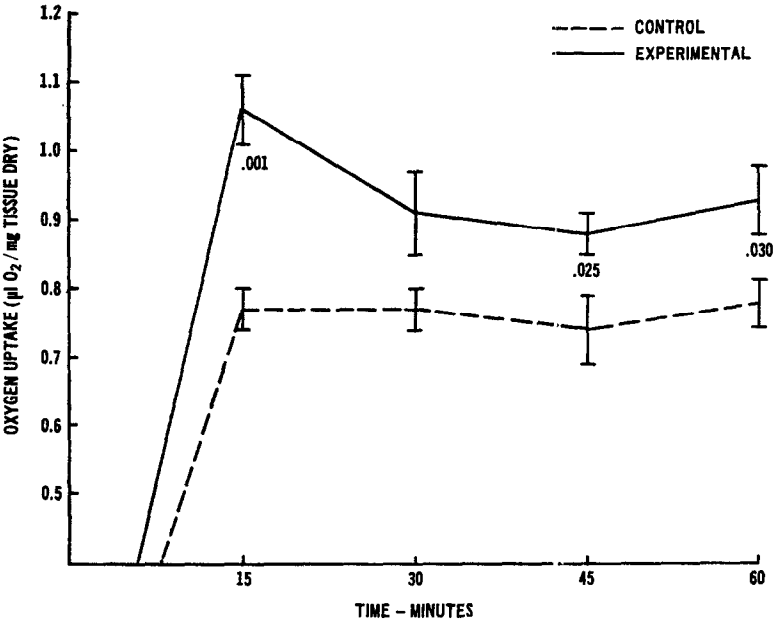

FIG. 3. Mean effects of epinephrine (1·04 mg/ml) on Q_{O_2} of rat uterine strips. Epinephrine added at 0 time; I indicates S.E; figures represent P values.

a rapid uptake of oxygen (Fig. 4); this finding compared to the response of experimental tissue covered up any increase in oxygen consumption that might have occurred in it at that time. Due to the large variations the experiment was altered and the albumin carrier was selected. Epinephrine (1·04 mg/ml) in the presence of albumin, however, stimulated an increase in oxygen consumption (Fig. 5) which lasted 30 min.

In the first experiments with progesterone in ethanol, no significant changes occurred, although the experimental values were consistently below those of the control tissues for the first 45 min. No effect was elicited when the experiment was repeated with progesterone carried on albumin until the 60-min reading, where a slight but definite depression in oxygen consumption resulted (Fig. 6).

DISCUSSION

This evidence seems to exclude two possible relationships between the actions of epinephrine and progesterone. One of these possibilities is that progesterone might act in a way similar to that of relaxin. In a series of experiments, Miller and Murry[5] and Rudzik and Miller[6, 7] found a definite relationship between uterine epinephrine stores and reactivity of the uterus to relaxin-containing ovarian extracts. Evidence gained by these investigators led them to advance the theory that relaxin causes relaxation of the uterus by releasing stored epinephrine so that the hormone in this

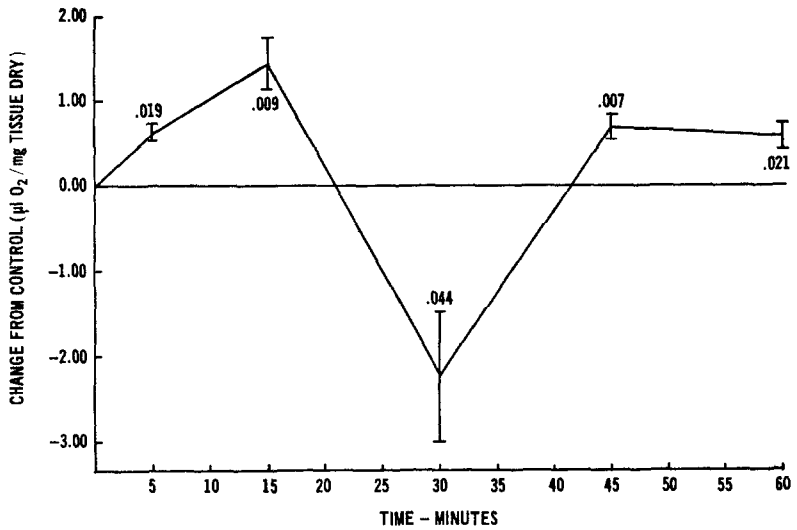

FIG. 4. Changes from control Q_{O_2} values induced by epinephrine (1·04 mg/ml in 0·3% ethanol). I indicates S.E; figures represent maximum P values.

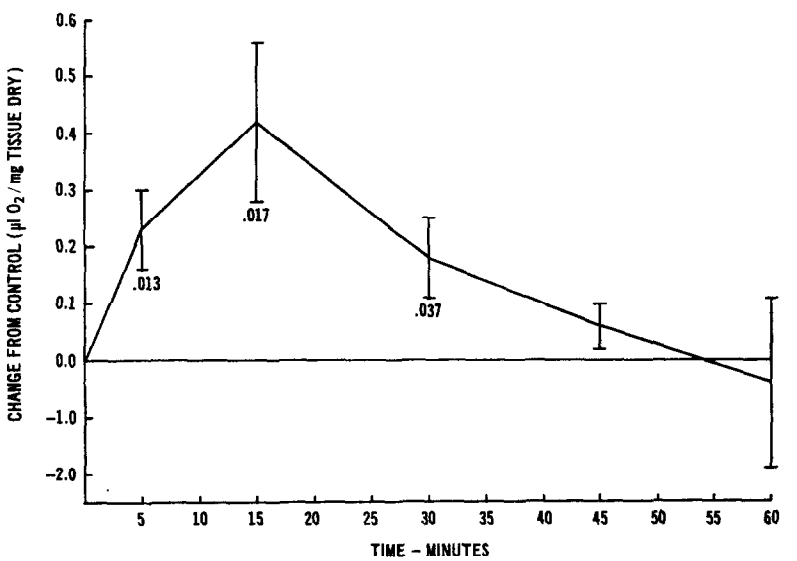

FIG. 5. Changes from control Q_{O_2} values induced by epinephrine (1·04 mg/ml in 0·06% albumin). I indicates S.E; figures are maximum P values.

way effectively inhibits activity of the uterus. Failure of DCI to prevent the effect of progesterone would lead one to believe that the mechanism underlying progesterone inhibition is not mediated through the epinephrine stores. Also, repeated treatment with estrogens depletes uterine epinephrine stores,[7] preventing the effect of relaxin. Since this procedure did not affect progesterone inhibition in our animals, additional evidence is provided against an action here identical to that of relaxin.

The possibility that progesterone and epinephrine have the same active site is also discouraged by this evidence, which is similar to the conclusions of Hava and Helfert.[9]

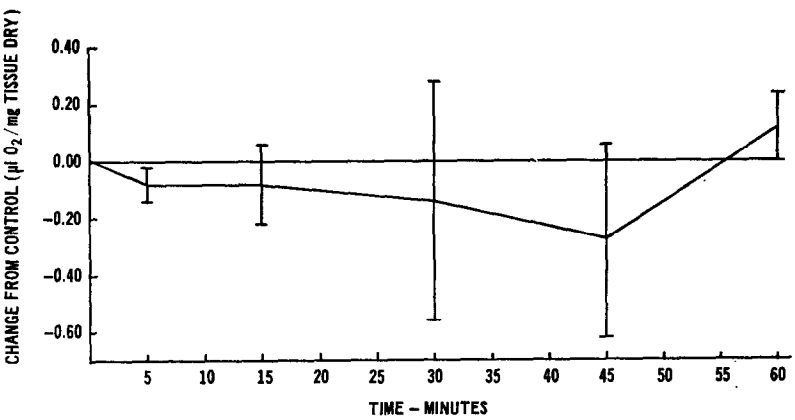

FIG. 6. Changes from control Q_{O_2} values induced by progesterone (20 μg/ml in 0·06% albumin). I indicates S.E; figure represents maximum P value.

Since DCI is thought to tie up the site of action of epinephrine irreversibly, then this agent should also prevent the effect of progesterone. This does not happen, so one can assume that epinephrine and progesterone do not mediate their effects by acting on the same receptor site.

The second approach to the overall problem was investigation of the relative effects of epinephrine and progesterone on the oxidative metabolism of the uterus. One of the proposals used as a basis for this work is that the inhibition of smooth muscle by epinephrine is a function of increased metabolism. The various experiments of Bueding and Bulbring[4] lead one to believe that there is a definite relationship between oxidative metabolism and inhibition of smooth muscle. Although on the surface this appears to be an enigma, the eivdence that ATP is needed for both contraction and inhibition of contraction of smooth muscle has been amply proven.[4, 10–12] The hyperpolarizing effect of epinephrine on smooth muscle is documented, and it is assumed that this effect alone is enough to inhibit spontaneous contractions. It would seem then, since hyperpolarization is an active process calling for more ions to be moved against their concentration or electrical gradient, that this would require more ATP and therefore more oxidative phosphorylation.

Therefore the depression of smooth muscle activity by epinephrine is probably an active process, requiring an increase in the oxygen consumption of the muscle. If this is the case, then epinephrine, in the concentration that caused uterine depression in our media, should also cause an increase in the oxygen uptake in the time period corresponding to the depression of contractile activity. The first investigation was designed to determine if this would happen in our uterine preparation and if so, to compare this effect with the results obtained from repeating the experiment with progesterone. Also, since the dosage found necessary for uterine inhibition in the original experiments differed from that used by other workers in this field, both the concentration of epinephrine needed to inhibit uterine activity in our preparation and that used by Bulbring[13] (2×10^{-2} μg/ml) were compared.

The fact that there is a correlation with epinephrine between increased oxygen consumption and inhibitory effects at the high dose and absence of either of these effects at the low dose is presented as evidence that there is correlation between these

two phenomena in our preparation. Progesterone failed to mirror epinephrine as far as an increase in oxygen consumption is concerned. Progesterone not only tended to decrease oxygen consumption, but also actually antagonized the stimulation usually brought about by ethanol when it was used as a solvent. The observation that progesterone relaxes and presumably hyperpolarizes smooth muscle[1, 2] without an increase in oxygen consumption appears to be inconsistant with relaxation being an energy dependent process. It is concluded from this study that, although progesterone and epinephrine exhibit some similarities in inhibiting spontaneous uterine activity, they cause this inhibition via different mechanisms.

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