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Effects of Oxytocin on in vitro Ovarian Contractility During the Estrous Cycle of the Rat

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ABSTRACT

The effect of oxytocin on *in vitro* ovarian contractility was studied throughout the estrous cycle of the rat.

Oxytocin stimulated ovarian contractility in 29 of 44 animals. Doses of 0.5, 5 and 25 mU/ml were used in all experiments. Statistical analysis showed no significant differences in the sensitivity of the ovaries to oxytocin in the different phases of the estrous cycle. These results suggest that the action of the peptide is not influenced by the hormonal status of the animal.

INTRODUCTION

Many studies have been performed to investigate the role of the ovarian smooth muscle in reproduction. The participation of ovarian contractility in the process of ovulation and particularly in follicular rupture has been suggested (Virutamasen and Fuchs, 1974; Wallach et al., 1975). Oxytocin has a stimulating effect on ovarian smooth muscle (Diaz-Infante et al., 1974; Gimeno et al., 1973). The action of oxytocin has been studied in the periovulatory stages such as proestrus or estrus (Gimeno et al., 1973; Gimeno et al., 1974). The effect of oxytocin on the uterus and oviduct depends on the hormonal status of the animal (Chan et al., 1963; Coutinho and Maia, 1970). However, the hormonal influence on the action of oxytocin has not been thoroughly approached regarding the ovary. In this paper the effect of the peptide on rat ovarian contractility *in vitro* at all stages of the estrous cycle is described.

MATERIALS AND METHODS

Forty-four mature Wistar female rats weighing about 200 g were used. The sexual cycle was followed by vaginal smears; only animals with at least three consecutive normal cycles were used. The rats were kept in controlled lighting conditions (14 h light-10 h dark). Experiments concerning Diestrus I, Diestrus II, Proestrus and Estrus were started at 1400 h, those of late proestrus started at 2100 h.

The animals were killed by decapitation; both ovaries were removed and a cortical strip of each ovary was dissected. The strip was immediately placed in a 50 ml organ-bath containing Tyrode solution which had the following composition in mM: NaCl 140; KCl 2.7; $MgCl_2$ $6H_2O$ 0.5; $CaCl_2$ anhyd. 1.5; Na_2HPO_4 12 H_2O 0.2; $NaHCO_3$ 11.9; $C_6H_{12}O_6$ 5.6; pH was adjusted to 7.4 with 0.2 M HCl. One pole of the strip was attached by a hook to the bottom of the organ-bath and the other pole was tied by a cotton thread to a Statham strain gauge 0.15 oz. An eight-channel Sanborn Poly Viso was used as the recording system. The organ-bath was maintained at 37°C. Solutions were changed through a polyethylene catheter attached to the inner wall of the organ-bath. A tension of 400 mg was applied to the ovarian strip. The apparatus was calibrated to give a positive deflection of 4.5 cm on the recording paper for every 100 mg of tension applied.

After one hour of recording spontaneous contractions, oxytocin (Syntocinon, Sandoz) was added in doses adequate to obtain final concentrations in the organ-bath of 0.5, 5 and 25 mU/ml. Each dose was left in the organ-bath for 20 min, then the preparation was washed and after 30 min another dose was given. In

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some experiments (n = 8), Indomethacin (Merck, Sharp & Dohme) was added to the Tyrode solution (20 to 40 μg/ml) to avoid follicular biosynthesis of prostaglandins.

RESULTS

The contractile activity varied from one experiment to the other (Fig. 1). As a rule it was very difficult to make a quantitative analysis of spontaneous activity due to the irregularity of the contractions. Spontaneous activity was observed in 100 percent of the ovaries, i.e., it was present at all stages of the estrous cycle. In the experiments in which Indomethacin was added, no modifications of spontaneous activity were observed.

Oxytocin stimulated ovarian contractility, increasing the amplitude or frequency of contractions; in some experiments a rise in resting tension was also observed (Fig. 2). The latency varied from 30 sec to 4 min. One or both ovaries from 29 of the 44 rats responded to oxytocin. Of the 88 ovaries studied, 41 responded. The threshold dose was 0.5 mU/ml in 4.9 percent of the ovaries, and 5 mU/ml in 73.2 percent; the remaining 22.0 percent of the ovaries only responded to 25 mU/ml.

The quantification of the effect of oxytocin was carried out by measuring the areas under the response curves. Statistical analysis was performed for responses elicited by 25 mU/ml of oxytocin since this dose was the one which stimulated all 41 ovaries. The mean areas for the different stages are shown in Table 1. Analysis of variance for single variable of classification showed no significant differences in the value of the responses among the cycle stages ($F = 1.59$ while $F_{0.95,4,37} = 2.61$). The dose-response relationship was very similar for the various cycle stages. The mean for all the responding ovaries was 6.54 cm^2 for 25 mU/ml and 3.05 cm^2 for 5 mU/ml. The dose of 0.5 mU/ml of oxytocin was discarded because of the low number of responses elicited at this concentration. Regarding the number of positive responses at the different stages of the cycle (Table 1) the results were also analyzed carrying the proofs of independence by Chi square, introducing the Yates correction when necessary (α = 5 percent). No significant differences were observed in the amount of responses either among groups of ovaries ($X^2 = 0.78$) or among groups of rats ($X^2 = 1.96$) for the different cycle stages.

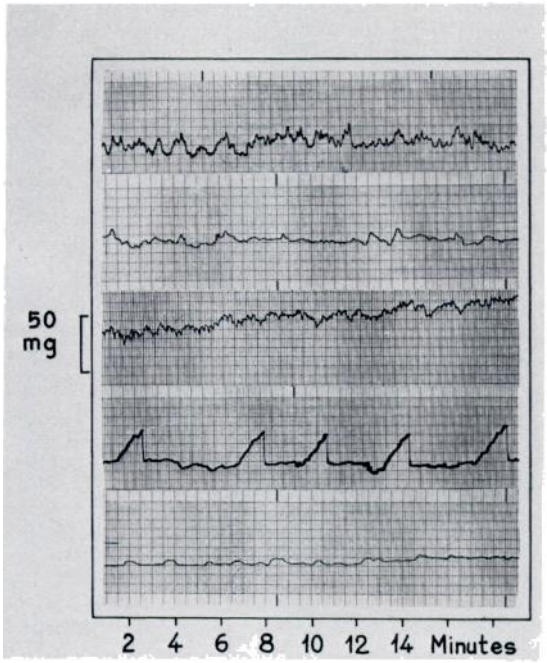

FIG. 1. Recordings of spontaneous contractility in five ovaries. Note the dissimilar contractile patterns.

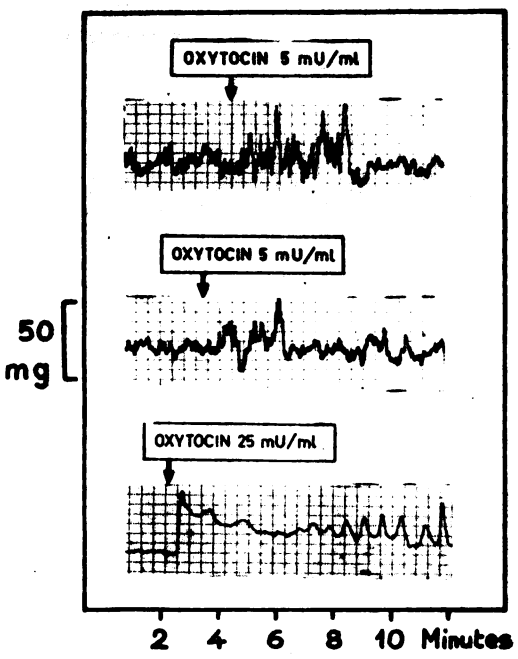

FIG. 2. Different types of ovarian responses elicited by oxytocin.

DISCUSSION

Spontaneous contractility in the monkey ovary is modified by the administration of high

TABLE 1. Ovarian contractile responses to oxytocin.

Cycle phase	No. of animals	No. of positive responses	No. of ovaries	No. of positive responses	Area of responses Mean ± SE (cm^2)*
Diestrus I	8	5	16	6	7.66 ± 4.07
Diestrus II	10	5	20	10	5.39 ± 1.46
Proestrus	11	8	22	10	6.23 ± 0.99
Late proestrus	6	4	12	6	7.09 ± 2.15
Estrus	9	7	18	9	5.63 ± 1.49
Total	44	29	88	41	

*The mean areas correspond to responses elicited by 25 mU/ml of oxytocin.

doses of ovarian steroids (Díaz-Infante et al., 1975). In women, increased ovarian activity following administration of human chorionic gonadotropin has also been described (Coutinho et al., 1974). In our experiments in the rat, spontaneous contractility was observed in all of the ovaries studied. This suggests, at least for the rat, that spontaneous activity is not greatly influenced by the hormonal status. Indomethacin was used to avoid any possible interactions of prostaglandins in the experiments. As the compound was used in only a few experiments no conclusions can be reached about its action on ovarian contractility.

This study confirms previous findings describing a stimulating effect of oxytocin on ovarian contractility in the rat (Gimeno et al., 1973). Gimeno et al. (1974) found that the rat ovary was relatively insensitive to oxytocin except in late proestrus. However, our studies showed that oxytocin stimulated ovarian contractility throughout the entire estrous cycle. Furthermore no single stage was more responsive than the others. Even taking into account the limitations of *in vitro* studies, our results suggest that the effect of the peptide on ovarian smooth muscle does not depend on the levels of sex steroids.

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