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Inhibition of Oxytocic Responses to Oxytocin in Pregnant Rats by [1-L-Penicillamine]oxytocin and [1-β-Mercapto-β,β-diethylpropionic acid]oxytocin [1] (38105)

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Some years ago, [1-L-penicillamine]oxytocin and [1-deaminopenicillamine]oxytocin were synthesized and found to be potent inhibitors of the oxytocic response to oxytocin of rat uterine horns *in vitro* (1). Subsequently they were shown to be specific competitive inhibitors of the oxytocic response to oxytocin in both *in vitro* and *in vivo* uterine preparations from virgin rats in natural estrus (2, 3). These two compounds differ from oxytocin (Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2)

1	2	3	4	5	6	7	8	9

and deaminooxytocin ([1-β-mercaptopropionic acid]oxytocin) only by the presence of two methyl groups replacing hydrogen on the β-carbon of the residue in position 1 of the parent molecules. It may be recalled that deaminooxytocin is a more potent oxytocic agent than is the parent hormone (4, 5).

A series of compounds closely related to [1-deaminopenicillamine]oxytocin (βMe_2-deaminooxytocin) has been prepared (6–8), of which [1-β-mercapto-β, β-diethylpropionic acid]oxytocin (βEt_2-deaminooxytocin) is the most potent antioxytocic agent. For the *in vitro* rat uterus it is approximately twice as potent an inhibitor as [1-deaminopenicillamine]oxytocin and more than twice as potent as [L-penicillamine]oxytocin (βMe_2-oxytocin) (8).

In this paper the work on penicillamine-oxytocin and βEt_2-deaminooxytocin has been extended to study the effect of these compounds on the uterine contractions of pregnant rats *in vivo*.

Materials and Methods. Sherman albino rats were used. Dates of conception were determined, taking as the first day of pregnancy the first day of appearance of sperm in the vaginal smear that is followed by the absence of the next expected estrus. Rats of 12- to 16-day gestation were used. On the day of the experiment, the rat was anesthetized with urethane given in two doses, 100 mg/100 g ip followed by another 50 mg/100 g sc. Atropine, 0.1 mg/100 g sc, was also administered. Tracheotomy was performed to insure a patent airway. Both jugular veins were cannulated for intravenous infusions. The pregnant uterus was exposed through a midabdominal incision. A segment of the uterus (approximately 50 mm) was isolated, arranged medially along the abdominal incision, and ligated with a suture at both ends. The proximal suture was tied horizontally to a fixed post and the distal suture attached horizontally to a force-displacement transducer with a basal tension of 1.0 g. The incision was then covered by a piece of gauze sponge kept moist with isotonic saline. Isometric contractions of the uterus were induced by oxytocin infusion and recorded on a polygraph. Only uteri exhibiting no spontaneous contractions were used. When rhythmic contractions had been induced by a continuous infusion of oxytocin at a suitable rate, one of the inhibitors was infused simultaneously from the other jugular vein to sup-

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press the oxytocin-induced contractions. The rate and duration of infusion varied from experiment to experiment depending on the sensitivity and the response of the animal.

The oxytocin used was Pitocin (Parke-Davis). Penicillamine-oxytocin and βEt_2-deaminooxytocin were synthesized and purified in our laboratories.

Results and Discussion. The sensitivity of the pregnant rat uteri to oxytocin and to oxytocin inhibitors varied widely from animal to animal and thus precluded accurate quantitative measurements. Although 10 pregnant rats were used, only six gave preparations in which there were no spontaneous uterine contractions. Of these six rats, one was in the 12th day of gestation and the remaining five were in the 16th day of gestation. Rhthmic contractions were induced in these animals by a continuous infusion of oxytocin throughout the entire experiment at a rate varying from 10 to 30 mU/min depending on the sensitivity of each animal preparation. In all of these six preparations, a simultaneous infusion of βEt_2-deaminooxytocin at a rate varying from 0.005 to 0.010 mg/min for 5–20 min suppressed completely or nearly completely the oxytocin-induced contractions. The inhibitory effect was never immediate. The inhibition might require 5–10 min of βEt_2-deaminooxytocin infusion to develop. Once the inhibition was established, the effect lasted for 30–60 min depending on the rate and the duration of the infusion of the inhibitor (Fig. 1).

Penicillamine-oxytocin was also effective in inhibiting oxytocin-induced uterine contractions in pregnant rats. Its potency was less than that of βEt_2-deaminooxytocin. In two experiments the two compounds were compared in the same animal (16-day-pregnant rats). In one rat, the inhibitory activity of penicillamine-oxytocin was tested first. After the oxytocin-induced contractions recovered a steady rhythmic pattern, the inhibitory activity of βEt_2-deaminooxytocin was then tested. In the other rat the order of testing of the inhibitors was reversed. In these two experiments, oxytocin was infused throughout the experiment at 20 mU/min. Nearly complete suppression of the oxytocin-induced contractions was achieved with 0.005 mg/min of βEt_2-deaminooxytocin or 0.01 mg/min of penicillamine-oxytocin infused for 5 min. Although in both cases full recovery to oxytocin-induced contractions was observed between 25 and 30 min, low-amplitude and low-frequency contractions were observed within 2 to 3 min after the cessation of penicillamine-oxytocin infusion whereas no uterine contractions were observed during the 5 min immediately after the cessation of administration of βEt_2-deaminooxytocin. It will be recalled that penicillamine-oxytocin was also found to be a less potent inhibitor than βEt_2-deaminooxytocin in *in vitro* studies on the nonpregnant uterus (8).

It is clear from our preliminary experiments reported here that penicillamine-oxytocin and βEt_2-deaminooxytocin are effective in suppressing oxytocin-induced uterine contractions

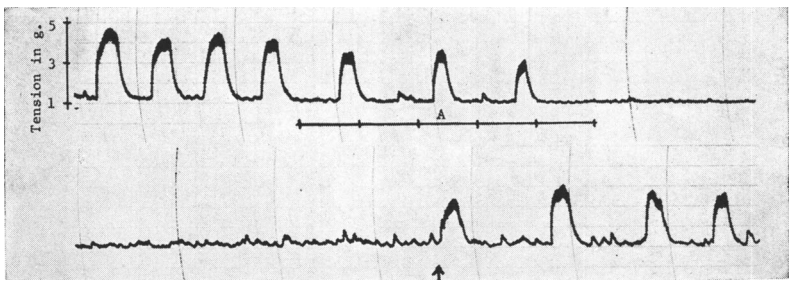

FIG. 1. *In vivo* inhibition of uterine contractions by [1-β-mercapto-β,β-diethylpropionic acid] oxytocin (βEt_2-deaminooxytocin) in a pregnant rat. Rhythmic contractions of the uterus were induced by a continuous infusion of oxytocin at 10mU/min throughout the experimental period. During the 5-min period marked by A, the inhibitor was infused simultaneously with oxytocin at a rate of 0.010 mg/min. Note the complete inhibition of uterine contractions and the long duration of action of the inhibitor. Recovery began at 40 min (marked by arrow) after the cessation of infusion of inhibitor. Rat weighed 280 g, 16 days pregnant.

in pregnant rats. However, because of the small number of experiments and the variability in sensitivity of these *in vivo* preparations both to oxytocin and the inhibitor, the data obtained do not permit a precise quantitative comparison between the potencies or the duration of action of these inhibitors.

The experimental technique used in previous *in vivo* studies involving the measurement of changes in intrauterine fluid pressure is satisfactory for the study of antioxytocic agents in the nonpregnant uterus. However, it proved to be unsatisfactory for studies on the pregnant uterus because much of the intraluminal space is occupied by the fetuses. By the use of the new technique described above, which involves no intrusion into the uterus, it was possible to record oxytocin-induced contractions in the pregnant uterus and to demonstrate their inhibition by antioxytocic agents. So far as we know, the present work is the first time that an antioxytocic compound has been tested on the pregnant uterus *in vivo*. It remains to be seen, of course, whether penicillamine-oxytocin and βEt_2-deaminooxytocin have an inhibitory effect on contractions of the pregnant human uterus, but the potential clinical applications in the treatment of threatened abortion or premature labor are obvious.

Summary. The ability of [1-L-penicillamine]oxytocin and [1-β-mercapto-β,β-diethylpropionic acid]oxytocin to suppress the contractions induced by oxytocin in the uteri of 12- to 16-day-pregnant rats has been demonstrated, using a new technique for recording uterine activity *in vivo*.

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