UTERINE RECEPTOR FOR OXYTOCIN: EFFECTS OF ESTROGEN*

Melvyn S. Soloff

Department of Biochemistry
Medical College of Ohio
Toledo, Ohio 43614

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Summary: The effect of estrogen treatment on the affinity and concentration of uterine binding sites for oxytocin was studied. The oxytocin receptor was present in the uterus of the ovariectomized rat but the affinity for oxytocin increased more than four-fold 24 hr after a single injection of diethylstilbestrol. An increase in affinity was apparent 6 hr after estrogen. An increase in the number of oxytocin binding sites per uterus was apparent only 12 hr after estrogen, reaching twice the initial value by 24 hr. These results suggest that the enhanced sensitivity of the rat uterus to oxytocin following estrogen treatment is the result of an increase in the affinity and number of oxytocin receptors in the uterus. The affinity of uterine binding sites for (lysine)vasopressin was less than for oxytocin, whether or not the rats were treated with estrogen.

The uterus contracts in response to oxytocin when endogenous estrogen levels are high, as in proestrus (1,2) or in estrus (3-5). Oxytocin appears to be virtually inactive on uteri taken from rats during metestrus, when estrogen levels are relatively low (2). A number of workers (1,6-9) have shown that the sensitivity of the rat or guinea pig uterus to oxytocin increases with the administration of pharmacological doses of estrogen, particularly the synthetic estrogen diethylstilbestrol.[1]

Oxytocin-binding sites, presumably corresponding to oxytocin receptors, have been demonstrated in rat (10,11), sow (11) and human (12) uterus. The present studies examine the possibility that the basis of the increased uterine sensitivity to oxytocin by estrogen pretreatment results from an increased affinity and/or number of oxytocin receptors.

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[1]Diethylstilbestrol = DES

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EXPERIMENTAL PROCEDURES

Materials

Peptides. [Tyrosyl-^{3}H]oxytocin, 31 Ci per mmole (13) was synthesized by Schwarz-Mann. About 99% of the radioactivity was associated with oxytocin on high voltage electrophoresis. The [^{3}H]oxytocin had a potency of 452 International Units per mg in the isolated rat uterus assay (14). Synthetic oxytocin and (lysine)vasopressin were gifts from Dr. Maurice Manning of this department and Sandoz, Ltd., Basel, respectively.

Uteri. Rats (Sprague-Dawley, Spartan), 175-199 g, were ovariectomized twelve days prior to use. Groups of 8 animals were injected with 5 µg of diethylstilbestrol dipropionate in 0.2 ml of cotton-seed oil subcutaneously, and the uterine horns were removed 6, 12 and 24 hr later. Two control groups of 24 animals each were used. One group received 0.2 ml of vehicle alone 24 hr prior to hysterectomy. The other group was untreated. The results obtained with both control groups were identical.

Methods

Preparation of uterine particulate fraction. All procedures were performed at 4°. The uterine horns from each group were freed of fat, weighed and homogenized in 9 volumes of Tyrode's solution with an all-glass conical homogenizer. The homogenate was then blended further with a Polytron PT 10 ST (Brinkmann Instruments) for 15 sec at the maximum setting. Each homogenate was centrifuged at 500 x g for 10 min and the resulting supernatant was centrifuged at 165,000 x g for 30 min. The pellet was resuspended in Tyrode's solution and stored at -70° overnight.

Binding assay. The frozen samples were thawed rapidly, washed twice with 0.05 M Tris-maleate buffer, pH 7.6, containing 5 mM $MnCl_2$ and 0.1% gelatin (hereafter referred to as Tris buffer). The pellet was then homogenized in Tris buffer to give 4 to 7.8 mg of protein per ml. Protein concentrations were determined by the method of Lowry et al. (15), with bovine serum albumin as the standard (11).

Each assay tube contained 0.8 to 1.56 mg of particulate protein, about 9,000 dpm of [^{3}H]oxytocin (ca. 150 pg) and the appropriate amount of non-radioactive oxytocin or (lysine)vasopressin in a total volume of 250 µl of Tris buffer. Incubation was carried out for 1 hr at 20°, and was terminated by centrifugation of the tubes at 20,000 x g for 30 min. Determination of the bound and unbound radioactivity was carried out as described previously (11). The data were analyzed as described by Scatchard (16). Linear regressions were determined by the method of least squares.

RESULTS

The uterine binding of oxytocin by the control groups and at 6, 12 and 24 hr after estrogen treatment of ovariectomized rats was expressed as a Scatchard plot (Fig. 1). The linear regressions obtained indicate that there was a single class of binding sites in each preparation within the range of oxytocin concentrations examined, 0.53 to 3.2 nM. The apparent association constant, K_a, was estimated from the negative slope of each line.

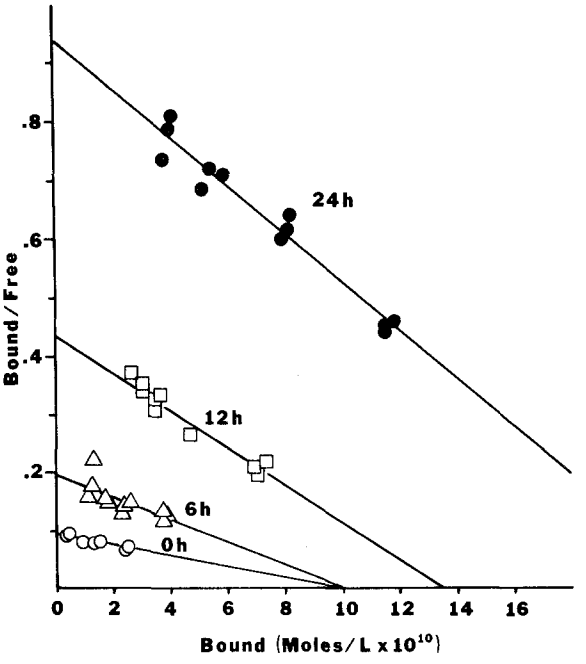

Figure 1: Scatchard plot of [^{3}H]oxytocin binding to the uterine particulate fraction from ovariectomized rats untreated (o—o), or treated with DES 6 h (Δ—Δ), 12 h (□—□), or 24 h (●—●) earlier. The assay tubes for each group contained 800, 1060, 960 and 1560 μg or particulate protein, respectively. The apparent K_a was estimated from the negative slope of each line. The concentration of binding sites was estimated from the x-intercept.

The concentration of binding sites was estimated from the x-intercept.

The affinity of uterine binding sites for oxytocin increased progressively at 6, 12 and 24 hr after a single injection of 5 μg of DES (Fig. 1 and 2, Table I). By 24 hr the apparent K_a for oxytocin binding was 4.2 times greater than the control. There was no change in the number of oxytocin binding sites per uterus at 6 hr after DES (Fig. 2, Table I). However, the number of sites was 2.1 times greater than the control by 24 hr (Table I).

The concentration of binding sites is expressed per mg of particulate protein and per uterus in Table I. The increase in receptor sites, however, is only apparent if the concentration of sites is expressed per uterus

TABLE I. The effect of a single injection of DES on the apparent K_a and number of binding sites for oxytocin in rat uterus

Time after DES (hr)	K_a ($M^{-1}x10^{-8}$)	Increase in affinity	No. binding sites per mg protein (moles x 10^{13})	Increase in no. of binding sites per mg	No. binding sites per uterus (moles x 10^{13})	Increase in no. of binding sites per uterus	Increase in oxytocin bound per uterus (column B x column F)[1]
	(A)	(B)	(C)	(D)	(E)	(F)	(G)
0	1	1	3.3	1	4.9	1	1
6	1.9	1.9	2.5	0.76	5.0	1	1.9
12	3.1	3.1	3.6	1.1	6.8	1.4	4.3
24	4.2	4.2	3.9	1.2	10.5	2.1	8.8

[1] at oxytocin concentrations below saturation of binding sites

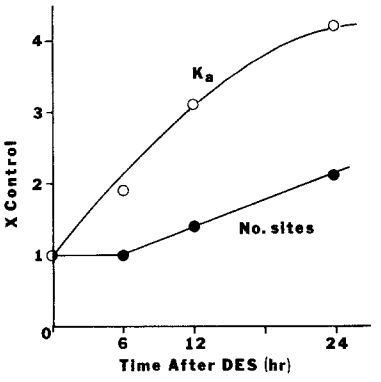

Figure 2: Rate of increase in the apparent K_a (o——o) and number of oxytocin binding sites per uterus (●——●) after treatment of ovariectomized rats with DES. The control group received no DES.

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rather than per mg of particulate protein. This can be explained by the estrogen-stimulated synthesis (17-22) of non-receptor proteins which appear to precede the increase in oxytocin receptors.

The product of the increase in affinity and the increase in the number of sites reflects the increased amount of oxytocin than can be bound to receptor when the hormone is present in subsaturating (i.e. physiological) concentrations. As shown in Table I, the concentration of oxytocin bound per uterus increased to 190, 430 and 880% at 6, 12 and 24 hr, respectively, after DES administration. The binding of [^{3}H]oxytocin by uterine particles from ovariectomized rats was not affected by the direct addition of either 10^{-6}M or 10^{-8}M DES to the incubation mixture (data not shown).

Oxytocin and (lysine)vasopressin reduced the binding of [^{3}H]oxytocin to uterine particles from ovariectomized rats treated with DES 24 hr before (Fig. 3). The decreased binding was proportional to the log of the concentrations of oxytocin and (lysine)vasopressin, and the regressions were parallel, indicating a common set of binding sites for oxytocin and (lysine) vasopressin. The potency of vasopressin was about 20% that of oxytocin, whether the uteri were taken from ovariectomized rats or from ovariectomized rats treated with DES.

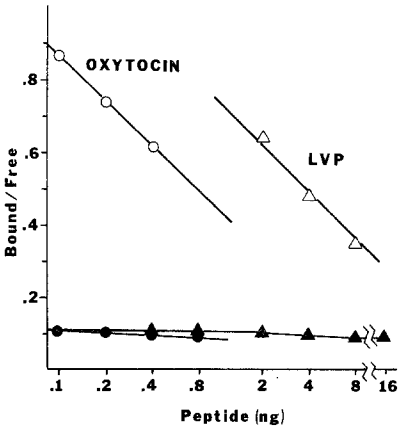

Figure 3: Competition for [^{3}H]oxytocin binding sites by (lysine)vasopressin (LVP, triangles) and oxytocin (circles) in uterine particles from ovariectomized rats, untreated (closed symbols) or treated with DES 24 hr prior (open symbols).

DISCUSSION

Estrogen treatment results in an increase in affinity and in the number of binding sites for oxytocin by rat uterus. The data are more meaningful if expressed as sites per uterus rather than per mg of protein, because DES treatment stimulates uterine protein synthesis. Provided that oxytocin is present in nonsaturating concentrations, the amount of hormone that could be bound per uterus was increased almost 9-fold by 24 hr after DES treatment. This increase is close to the 10-fold increase in uterine sensitivity to oxytocin resulting from treatment of rats with pharmacological doses of DES for 3 days (8). Therefore, DES appears to affect the response of the uterus to oxytocin by increasing the potential for binding oxytocin.

Estrogens also have been shown to raise the resting membrane potential of uterine smooth muscle close to threshold levels so that the muscle is more readily stimulated, either spontaneously or by depolarizing substances such as oxytocin (23). Oxytocin itself can either depolarize or repolarize the membrane to bring the potential into the range most suited for activity (23). Estrogens, therefore, not only sensitize the myometrium to oxytocin

by bringing about an increase in the number of oxytocin-receptor interactions, but also affect the electrochemical processes which appear to be coupled to the occupancy of receptor sites by hormones.

Munsick and Jeronimus (24) have shown that DES can significantly change the uterotonic potencies of several oxytocin analogues with respect to oxytocin. Whereas vasopressin was not examined by these workers, several laboratories have reported that the myometrium from nonpregnant women was more sensitive to vasopressin than to oxytocin (cf. 25). However, there are different opinions on the relative effects of oxytocin and vasopressin on the uterus during the phases of the reproductive cycle in which estrogens or progestins predominate (cf. 25). In the present study (lysine)vasopressin was about 20% as potent as oxytocin in competing with [^{3}H]oxytocin for binding to uterine particles from DES-treated rats. This potency was not appreciably different in the untreated ovariectomized rat. Therefore, the specificity of uterine binding sites for oxytocin apparently does not change with DES administration, at least with respect to (lysine)vasopressin and oxytocin.

The present experiments do not tell us whether the DES-induced increase in oxytocin receptors is the result of the de novo synthesis of sites or the unmasking of existing sites. The increase in sites was not apparent until later than 6 hr after DES. On the other hand, the increase in apparent K_a was noticeable by 6 hr. The rates of increase in sites and K_a also were distinct from each other. The increase in sites was linear at 6, 12 and 24 hr after DES, whereas the increase in K_a with DES treatment was leveling off by 24 hr. The increase in K_a, therefore, does not appear to be associated solely with the presence of new building sites. Rather, DES appears to bring about a modification of existing oxytocin receptors, at least in part. The inability to demonstrate an effect of DES in vitro suggests that its effect may be more complex than one of direct interaction with the oxytocin receptor site.

Because the increased sensitivity of the uterus to oxytocin due to DES is paralleled by an increase in oxytocin binding, these experiments further support the conclusion (11) that the oxytocin binding sites are part of the receptor complex.

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