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OXYTOCIN RECEPTORS COUPLED TO UTERINE CONTRACTION IN ESTROGEN-DOMINATED RABBITS

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Summary

The present study investigated whether specific [^{3}H]oxytocin binding sites previously demonstrated in estrogen-dominated rabbit uterus have properties expected of physiologic receptors coupled to uterine contraction. Microsomal membranes from estrogen-dominated rabbit uterus were found to contain high-affinity specific oxytocin binding sites with K_d = 2—3 nM. These sites were predominantly myometrial in locus. Specific oxytocin binding exhibited a pH optimum between 7.5 and 8.0. Mg^{2+} or Mn^{2+} was necessary for maximal specific [^{3}H]oxytocin binding; in contrast, Ca^{2+} at submillimolar concentrations inhibited specific binding. Oxytocin binding sites were not detectable in microsomal membranes isolated from progesterone-dominated rabbit uterus.

Relative binding and uterotonic activities of 10 synthetic neurohypophyseal hormone analogues were determined in estrogen-dominated rabbit uterus. A qualitative correlation was observed between binding and uterotonic responses. Angiotensin II and insulin did not compete with [^{3}H]oxytocin for uterine binding sites. It is concluded that the specific high affinity [^{3}H]oxytocin binding sites demonstrated in estrogen-dominated rabbit uterus have the selectivity for neurohypophyseal hormone analogues expected for physiologic receptors coupled to uterine contraction.

Introduction

The action of oxytocin to promote uterine contraction in mammals involves a primary interaction of oxytocin with receptors at the surface of smooth-

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muscle cells; formation of the hormone-receptor complex is believed to lead to membrane depolarization, calcium influx, and increased cytoplasmic levels of Ca^{2+} which serves to activate the contractile system [1]. Although little is known about the precise process by which occupied receptors are coupled to activation of the contractile system, attempts have been made to study the uterine oxytocin receptor with labeled hormone ligands. Binding sites for [^{3}H]-oxytocin have been found in the uterus of the rat [2—4], sow [4], ewe [5] and human [6].

In our previous studies [7], we reported that estrogen treatment of immature rabbits induced the formation of high-affinity specific binding sites for radiochemically homogeneous [^{3}H]oxytocin, and that these sites virtually disappeared after progesterone treatment for 4 days; the number and affinity of these sites were unchanged if the oil vehicle was administered instead of progesterone. These effects of steroid treatment upon uterine oxytocin-binding sites paralleled the effects of estrogen and progesterone treatment upon the sensitivity of the rabbit uterus to oxytocin, providing support for the view that the specific binding sites detected with [^{3}H]oxytocin represent physiologically important uterine receptors for oxytocin. In the present report, further results are presented demonstrating that the specific [^{3}H]oxytocin binding sites in the estrogen-dominated rabbit uterus have properties expected for oxytocin receptors in uterine smooth-muscle cells.

Materials and Methods

Young (10—12 weeks; 2.5—3.0 kg) virgin female New Zealand white rabbits were obtained from Scientific Small Animal Farms (Arlington Heights, IL, U.S.A.). Progesterone and 17β-estradiol were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Bovine serum albumin was obtained from Armour Co. (Chicago, IL, U.S.A.) and was purified by charcoal adsorption [8]. Oxytocin was synthesized and tritiated (specific activity 31 Ci/mmol) as previously described [9,10]. [^{3}H]Oxytocin was purified by gel chromatography followed by affinity chromatography on neurophysin-sepharose columns [10]. The labeled hormone was radiochemically homogeneous in several thin-layer chromatography systems, and had activity identical with that of a synthetic standard of unlabeled oxytocin (500 U/mg) in the rat uterotonic assay of Holton [11]. Lysine vasopressin and arginine vasopressin were synthesized by the solid-phase method and were found to be fully active in the bovine renal medullary adenylate cyclase system [12] when compared to synthetic standards supplied by Dr. R. Walter. [Deamino^1]-oxytocinoic acid, [alanine^2]-oxytocin, [valine^4]-oxytocin, [valine^5]-oxytocin, [glycine^7]-oxytocin, [arginine^8]-vasopressinoic acid were generous gifts of Dr. R. Walter.

Steroid hormones dissolved in ethanol were added to peanut oil (final concentration, 1% ethanol) and administered by subcutaneous injection. Uteri in the 'estrogen-dominated' state were obtained by injecting rabbits daily with 17β-estradiol (200 μg/kg) for 4 days. To obtain progesterone-dominated uteri, rabbits were treated with estradiol (200 μg/kg) daily for 4 days and then with

progesterone (5.0 mg/kg) daily for 4 days. One group of estradiol-treated rabbits received the oil vehicle, instead of progesterone, for 4 days.

The uterotonic activity of oxytocin and other neurohypophyseal hormone analogues in rabbit uterus was assayed in Munsick's (plus Mg^{2+}) solution [13], as previously described [7]. Under these conditions (0.5 mM Ca^{2+}, 0.5 mM Mg^{2+}, 30°C) spontaneous, non-stimulated uterine contractions are eliminated. The isometric tension developed in response to addition of oxytocin or other peptides was monitored on a dynograph recorder. Uterine strips were 'washed out' at least four times with 10 ml of oxygenated Munsick's solution over a 10-min period berween addition of different peptides and between different doses of the same peptide, so that resting tension (0.5 g) was restored prior to the next addition. In neurohypophyseal hormone analogue studies, the concentrations of peptides producing half-maximal contractile responses (K_a values) were determined by graphical analysis of multiple dose-response curves.

Homogenate fractions were derived from the pooled uterine horns of at least three estrogen- or progesterone-dominated rabbits. Uterine horns were chopped at 2°C with a McIlwain chopper; uterine fragments were then extensively washed with ice-cold 0.9% NaCl/1.0 mM EDTA and then homogenized (20 strokes) in 4 vols. of ice-cold 0.25 M sucrose/1.0 mM EDTA using a 'loose', motor-driven Teflon pestle-glass homogenizer. Following filtration through gauze, the suspension was rehomogenized, first with 20 strokes in a 'loose' glass Dounce homogenizer and then with 20 strokes in a 'tight' glass Dounce homogenizer. The final homogenate was differentially centrifuged successively at $2000 \times g$ for 10 min (nuclear fraction), $10\,000 \times g$ for 20 min (mitochondrial fraction), and $95\,000 \times g$ for 60 min (microsomal fraction).

Specific [^{3}H]oxytocin binding was measured as previously described [7]. Unless otherwise noted, homogenate fractions (60 μg protein) were incubated with various concentrations of [^{3}H]oxytocin in a final volume of 0.1 ml containing 25 mM Tris-HCl (pH 7.6), 2.0 mM $MgCl_2$, and 0.1% bovine serum albumin. Incubations were carried out for 15 min at 30°C. Under these conditions, more than 95% of the ^{3}H radioactivity in the medium after incubation was found to be unmetabolized [^{3}H]oxytocin, as determined by thin-layer chromatography in several systems. Binding was terminated by addition of ice-cold Tris-HCl/$MgCl_2$-bovine serum albumin washing buffer, followed by rapid filtration (under reduced pressure) of the chilled suspension through 0.2 μm EDWP cellulose acetate Millipore filters. The filters then were washed twice in a cold room (2°C) by the addition and rapid filtration of 10 ml of ice-cold washing buffer. Washed filters were air-dried and then placed in 10 ml of scintillation fluid for tritium counting. Binding values were corrected for the efficiency of tritium counting, which averaged 40%. All binding values were corrected for nonspecific [^{3}H]oxytocin binding (obtained in the presence of 10 μM unlabeled oxytocin). At [^{3}H]oxytocin concentrations ranging from 0.1 to 10 nM, 75—80% of the total [^{3}H]oxytocin binding was specific binding. Blank [^{3}H]oxytocin binding to the filters was minimal and was not reduced in the presence of 10 μM unlabeled oxytocin. In neurohypophyseal hormone analogue studies, the concentrations of peptides producing 50% inhibition of specific [^{3}H]oxytocin binding (K_b values) were determined from multiple competitive-displacement curves.

Results

In initial studies we measured specific oxytocin binding to intact myometrial segments from estrogen- and progesterone-dominated rabbits. Specific oxytocin binding was assessed by two procedures: (a) incubation of tissue segments with a fixed concentration (3.0 nM) of [^{3}H]oxytocin plus varying concentrations of unlabeled oxytocin, and (b) incubation of segments with varying concentrations of [^{3}H]oxytocin in the absence and presence of excess unlabeled oxytocin (1.0 μM) for assessment of non-specific [^{3}H]oxytocin binding. The results of a representative experiment using procedure (a) are shown in Table I. In the presence of excess unlabeled oxytocin (1 μM), $HClO_4$-extractable [^{3}H]-oxytocin binding was significantly reduced (by 35%) in estrogen-dominated, but unchanged in progesterone-dominated myometrial pieces. Thus, significant specific [^{3}H]oxytocin binding was detected in estrogen-dominated but not progesterone-dominated myometrium. Identical results were obtained using procedure (b) to assess specific [^{3}H]oxytocin binding (data not shown). In both cases, high levels of nonspecific [^{3}H]oxytocin binding were noted. Because of this, intact myometrium was not suitable for the precise characterization of the kinetic parameters of the specific oxytocin-binding sites. We therefore studied [^{3}H]oxytocin binding to subcellular fractions of rabbit uterus.

TABLE I

[^{3}H]OXYTOCIN BINDING TO MYOMETRIAL STRIPS FROM ESTROGEN- AND PROGESTERONE-DOMINATED RABBITS

Myometrial pieces (2.5—3.5 mg total protein) were incubated with 3 nM [^{3}H]oxytocin plus varying concentrations of unlabeled oxytocin in 2.0 ml of Krebs-Ringer bicarbonate buffer (pH 7.4) containing 11.1 mM glucose and 1.0% bovine serum albumin. After an incubation period of 60 min at 30°C, the pieces were blotted on filter paper and added to tubes containing 1.0 ml of 1.0 N perchloric acid for extraction of bound [^{3}H]oxytocin. Tubes were centrifuged at 10 000 × g (5 min) and the acid extracts (supernatants) were directly assessed for ^{3}H-radioactivity. The residual pellets were solubilized by heating (80°C, 45 min) in 1.0 ml of 1.0 N NaOH. Aliquots of each NaOH-solubilized pellet were assayed for protein [14] and for ^{3}H-radioactivity. Values are the mean ± S.E. of binding to four myometrial pieces.

State of uterus	Unlabeled oxytocin (nM)	$HClO_4$-extractable [^{3}H]oxytocin binding (dpm/mg protein)		Residual [^{3}H]oxytocin binding (dpm/mg protein)
		Total	Δ due to unlabeled oxytocin	
Estrogen-dominated	none	2945 ± 267	0	1129 ± 361
	5	2606 ± 119	339	1110 ± 237
	50	1995 ± 110 [a]	950	646 ± 47
	1000	1916 ± 25 [a]	1029	794 ± 38
Progesterone-dominated	none	1738 ± 86	0	715 ± 68
	5	2010 ± 122	— [b]	1016 ± 282
	50	1081 ± 132	— [b]	788 ± 101
	1000	1834 ± 193	— [b]	902 ± 162

[a] Significantly less than the corresponding value obtained in the absence of unlabeled oxytocin.

[b] No detectable decrease in [^{3}H]oxytocin binding compared to the corresponding value obtained in the absence of unlabeled oxytocin.

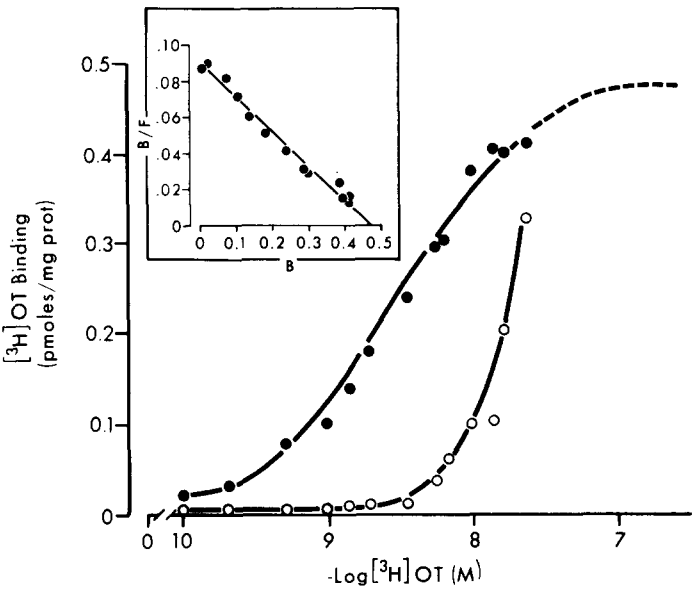

Fig. 1. Specific (•) and non-specific (○) [^{3}H]oxytocin binding to microsomal membranes from estrogen-dominated rabbit uterus. The dashed line represents an extrapolation of specific [^{3}H]oxytocin binding to the maximum value determined by Scatchard analysis. Inset: Scatchard plot of specific [^{3}H]oxytocin binding values. Specific binding (B) is expressed as picomoles bound per milligram of protein.

Specific [^{3}H]oxytocin binding sites were detected in the mitochondrial and microsomal fractions of estrogen-dominated rabbit uterine homogenates. Significant specific oxytocin binding was not detectable in the nuclear fraction of these homogenates. Although the K_d values for specific oxytocin binding to these fractions were similar (2.5 vs. 2.2 nM), the concentration of binding sites was considerably lower in the mitochondrial fraction than in the microsomal fraction (0.16 vs. 0.37 pmol/mg protein). The microsomal fraction contained more than 50% of the total number of specific oxytocin binding sites in the uterine fractions. Electron microscopic evaluation demonstrated a predominance of smooth- and rough-membrane vesicles in the microsomal fraction, with few contaminating mitochondria. The mitochondrial fraction, as expected, consisted of numerous mitochondria and some muscle fibers, as well as membrane vesicles. Accordingly, the microsomal fraction was used in subsequent binding experiments.

Significant specific [^{3}H]oxytocin binding could not be demonstrated in any of the homogenate fractions from progesterone-dominated rabbit uterus.

Specific [^{3}H]oxytocin binding to estrogen-dominated uterine microsomal membranes was dependent upon the medium concentration of [^{3}H]oxytocin between 0.1 and 20 nM (Fig. 1). Saturation of specific binding sites was approached but not achieved at the highest concentration of [^{3}H]oxytocin studied (20 nM). It was not possible to measure specific binding at [^{3}H]oxytocin concentrations in excess of 20 nM because of the sharp increase in non-specific binding which occurred at these high [^{3}H]oxytocin concentrations.

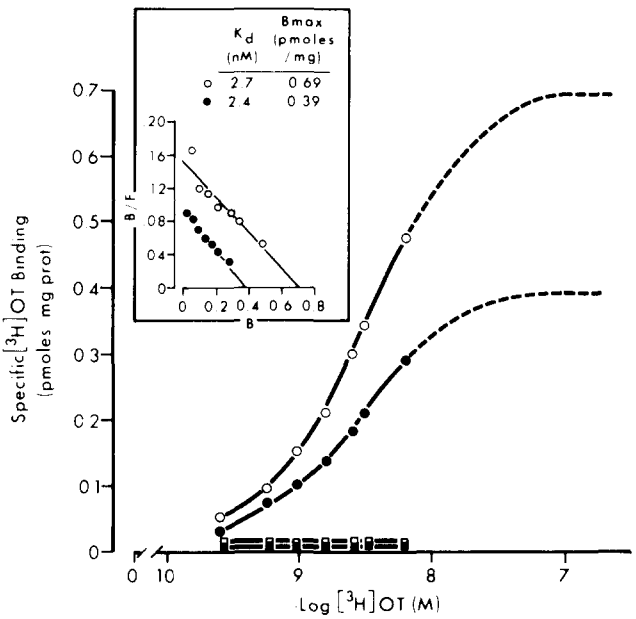

Fig. 2. [^{3}H]oxytocin binding to microsomal membranes isolated from estrogen-dominated whole uterus (●), estrogen-dominated myometrium (○), progesterone-dominated whole uterus (■), and progesterone-dominated myometrium (□). Dashed lines represent extrapolation to the maximum specific binding values as determined by Scatchard analysis. Inset: Scatchard plots of specific [^{3}H]oxytocin binding. Specific binding (B) is expressed as picomoles bound per milligram of protein. The equilibrium constants of dissociation (K_d values) were calculated from the negative reciprocal of the slopes, and the number of binding sites (B_{max} values) was estimated from the x-intercept of the Scatchard plots.

The number of binding sites was therefore estimated by Scatchard analysis [15] of specific [^{3}H]oxytocin binding (Fig. 1, inset), which yielded a linear plot, suggesting the presence of a single non-cooperative set of binding sites over the concentration range of [^{3}H]oxytocin tested. The equilibrium constant of dissociation (K_d) for these binding sites, as determined from the negative reciprocal of the slope of the Scatchard plot, was 3.0 nM. The binding capacity of these membranes, as calculated from the extrapolated x-intercept of the Scatchard plot, was 0.47 pmol/mg protein.

[^{3}H]oxytocin binding was measured using a microsomal membrane fraction derived from myometrical strips and compared to binding obtained with a microsomal fraction derived from the contralateral whole-uterine horns of the same uterus (containing endometrium plus myometrium) (Fig. 2). For estrogen-dominated animals, consistently greater specific [^{3}H]oxytocin binding was obtained using the membrane fraction derived from myometrial strips as compared to the whole uterus. Scatchard analysis of these data (Fig. 2, inset) demonstrated that removal of the endometrium resulted in a nearly 2-fold increase in the concentration of specific [^{3}H]oxytocin binding sites (from 0.39 to 0.69 pmol/mg protein), with little or no change in binding-site affinity. Progesterone-dominated rabbit uterine membranes, whether derived from myometrium or whole uterus, contained no detectable specific [^{3}H]oxytocin binding.

Specific [^{3}H]oxytocin binding to microsomal membranes from estrogen-dominated rabbit uterus had a rapid onset at 30°C; half-maximal binding was obtained at 4 min, and steady-state binding was reached after 15 min of incubation. At this temperature, nonspecific [^{3}H]oxytocin binding reached a maximum at 3 min. At 4°C, specific as well as nonspecific [^{3}H]oxytocin binding was slower; specific binding did not attain the steady-state value observed at 30°C even after 60 min of incubation, while nonspecific binding reached a maximum after 5 min. Specific [^{3}H]oxytocin binding to uterine microsomal membranes from estrogen-dominated rabbits was undetectable at medium pH value of 5.0 to 6.0 and increased to maximum between pH 7.5 and 8.0. The magnitude of nonspecific [^{3}H]oxytocin binding was essentially independent of medium pH.

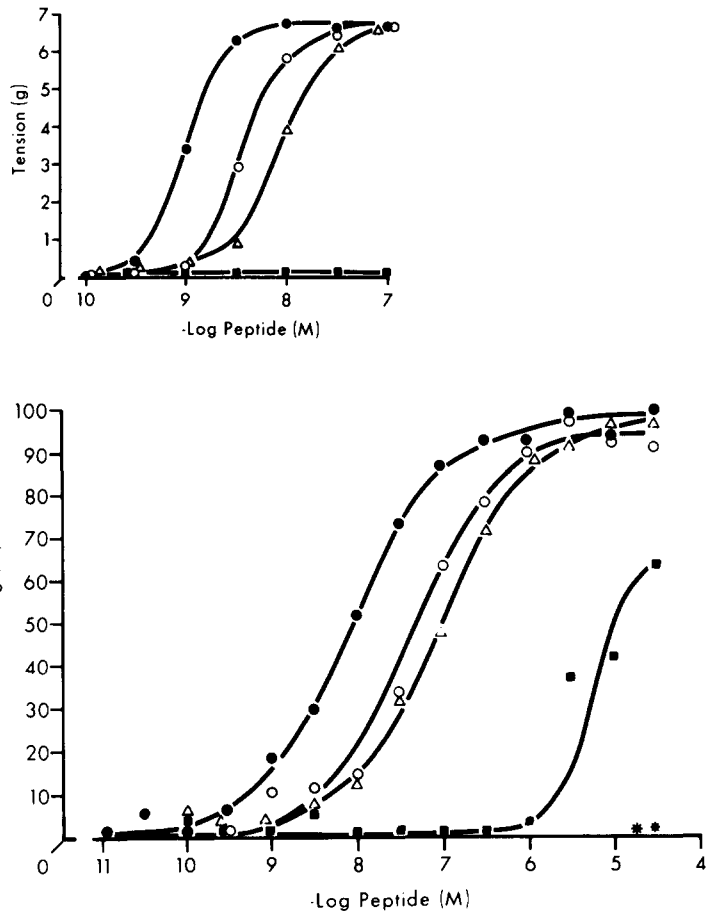

Fig. 3. (a) Isometric tension (grams) developed by estrogen-dominated rabbit uterine strips in response to various concentrations of oxytocin (●), [gly^7]-oxytocin (○), [val^4]-oxytocin (△), and [val^5]-oxytocin (■). Resting tension (0.5 g) was subtracted from each value. (b) Inhibition of specific [^{3}H]oxytocin binding to estrogen-dominated rabbit uterine microsomal membranes produced by various concentrations of unlabeled oxytocin (●), [gly^7]-oxytocin (○), [val^4]-oxytocin (△), [val^5]-oxytocin (■), insulin (*), and angiotensin II (*).

A small amount of specific [^{3}H]oxytocin binding was obtained in estrogen-dominated uterine microsomal membranes in the absence of divalent metals. Optimal concentrations of Mg^{2+} (2.0—10 mM) prompted a 2- to 3-fold increase in specific [^{3}H]oxytocin binding. Mn^{2+} was as effective as Mg^{2+}, the concentrations for half-maximal stimulation of specific binding being 0.5 and 0.7 mM, respectively. Ca^{2+}, in contrast to Mg^{2+} and Mn^{2+}, produced a concentration-dependent inhibition of specific [^{3}H]oxytocin binding. Half-maximal inhibition was obtained at a Ca^{2+} concentration of 0.2 mM. Ca^{2+} likewise inhibited specific [^{3}H]oxytocin binding in the presence of 0.4 mM $MgCl_2$; the Ca^{2+} concentration required for half-maximal inhibition of specific binding in the presence of 0.4 mM $MgCl_2$ was 0.5 mM.

The uterotonic activity of a set of synthetic neurohypophyseal hormone analogues was determined in estrogen-dominated rabbit uterine strips and compared to specific binding ativity as evaluated by competitive binding assays with [^{3}H]oxytocin in estrogen-dominated rabbit uterine microsomal membranes. Fig. 3a shows a representative uterotonic study comparing the contractile potency of oxytocin to three synthetic analogues, where the order of potency was found to be oxytocin > [gly^7]-oxytocin > [val^4]-oxytocin; [val^5]-oxytocin was inactive at concentrations up to 100 nM. The activity of these peptides in competing with [^{3}H]oxytocin for binding sites showed the same order of potency (Fig. 3b); [val^5]-oxytocin displayed slight binding activity, but only at concentrations greater than 1.0 μM. Fig. 3b also shows

TABLE II

CORRELATION BETWEEN THE BINDING AND UTEROTONIC ACTIVITIES OF SEVERAL NHH ANALOGUES IN ESTROGEN-DOMINATED RABBIT UTERUS

The concentrations of peptide eliciting a half-maximal uterotonic response (K_a values) were determined by graphical analysis of log dose-response data. The concentrations of peptide that reduced specific [^{3}H]-oxytocin binding by 50% (K_b values) were determined by competitive binding experiments. For each peptide, activities are expressed relative to the activity of oxytocin (OT), which is given an arbitrary value of 1. Also shown are the uterotonic and binding activities (as a percent of the maximal oxytocin-induced activities) elicited by maximal concentrations of each peptide.

Peptide	% Maximal contractile response	Relative [a] uterotonic activity (range)	Relative [b] binding activity (range)	% Maximal inhibition of specific [^{3}H]oxytocin binding
OT	100 (7) [c]	1	1	100
[deamino]OT	100 (3)	1 (0.5—1.6)	1.2 (1.0—1.4)	100
Arginine vasopressin	100 (5)	1 (0.7—1.7)	1.3 (1.1—1.5)	100
Lysine vasopressin	100 (5)	2 (1.3—2.5)	2.0 (1.6—2.4)	100
[gly^7]-7OT	100 (3)	4 (3.2—4.2)	6.0 (5.6—6.4)	100
[val^4]-4OT	100 (3)	9 (8.5—10)	7.7 (7.4—8.0)	100
[deamino]-OT acid	15 at 1 μM [d] (3)	>200	225 (200—250)	100
[val^5]-5OT	0 at 100 nM [d] (3)	—	900 (800—1000)	80 at 10 μM [d]
Arginine vasopressinoic acid	0 at 100 nM [d] (3)	—	1000 (800—1200)	75 at 30 μM [d]
[ala^2]-2OT	0 at 1 μM [d] (3)	—	8000 (7000—9000)	50 at 30 μM [d]

[a] Calculated as K_a (x)/K_a (OT).
[b] Calculated as K_b (x)/K_b (OT).
[c] Numbers in parentheses are the number of uterine strips tested.
[d] Highest peptide concentration tested.

that angiotensin II and insulin, at concentrations in excess of 10 μM, did not compete with [^{3}H]oxytocin for binding sites.

Examination of a variety of neurohypophyseal hormone analogues revealed a close correlation between uterotonic activity and binding potency (Table II), providing strong evidence that the observed binding sites represent oxytocin receptors coupled to uterine contraction.

Discussion

We have previously reported that treatment of young rabbits with estradiol increases the number of high-affinity specific uterine [^{3}H]oxytocin binding sites, and that progesterone treatment of estradiol-pretreated rabbits reduces the number of binding sites to undetectable levels [7]. Administration of these steroids produced parallel effects upon oxytocin-induced contraction of rabbit uterus, suggesting that the [^{3}H]oxytocin binding sites in rabbit uterus may represent physiologic receptors. The present results provide additional evidence for this conclusion.

The specific [^{3}H]oxytocin binding sites in estrogen-dominated rabbit uterus appear to be localized primarily in the myometrium, as expected of receptors coupled to the contractile system in smooth-muscle cells. Thus, the number of specific [^{3}H]oxytocin binding sites per milligram of protein in microsomal membranes isolated from myometrial strips (after removal of endometrium) was increased nearly 2-fold as compared to membranes isolated from the contralateral whole uterine horns. When progesterone was administered to estradiol-pretreated rabbits, specific [^{3}H]oxytocin binding to myometrial microsomal membranes was virtually abolished. These findings establish that if specific endometrial binding sites for [^{3}H]oxytocin exist, they are present in much lower concentration (or have much lower affinity) than the sites in myometrium. These results in rabbit uterus are not in agreement with the report that the concentration of [^{3}H]oxytocin binding sites is higher in endometrium than in myometrium [5] in the estrogen-dominated ewe.

Specific binding sites in estrogen-dominated rabbit uterus exhibited the high affinity for oxytocin expected of physiologic receptors. Thus, the K_d values obtained by Scatchard analysis of specific [^{3}H]oxytocin binding (2—4 nM) were similar to the concentration of oxytocin (K_a) which produced half-maximal contraction of the estrogen-dominated rabbit uterus in vitro (3 nM). Thus, oxytocin binding to specific uterine sites occurred over the same concentration range that elicited contraction of the estrogen-dominated rabbit uterus.

The specific binding sites detected with [^{3}H]oxytocin in estrogen-dominated rabbit uterus had a pH optimum between 7.5 and 8.0; specific binding was near maximal at pH 7.4. These results are similar to those reported with sow myometrium where optimal [^{3}H]oxytocin binding was obtained at pH 7.4—7.8 [4]. As reported for sow myometrium [4], divalent cations were required for maximal specific [^{3}H]oxytocin binding to rabbit uterine microsomal membranes. Mn^{2+} and Mg^{2+} both potentiated specific [^{3}H]oxytocin binding; the concentrations of Mg^{2+} and Mn^{2+} that gave half-maximal stimulation of specific binding were 0.7 and 0.5 mM, respectively. Ca^{2+}, when present at 0.2—10 mM, markedly inhibited specific oxytocin binding both in the presence and absence

of Mg^{2+} in the external medium. These findings are of interest since it is well known that both Mg^{2+} and Mn^{2+} potentiate the rat uterotonic activity of neurohypophyseal hormone analogues [16]; the effect of these metals has been ascribed to increased affinity of uterine receptors for these peptides [16]. The present data demonstrate that Mg^{2+} and Mn^{2+} indeed promote specific [^{3}H]-oxytocin binding, although the mode of this action, as well as the inhibitory action of Ca^{2+} remains obscure *. It should be mentioned that the present findings with Ca^{2+} in rabbit uterine membranes are in contrast to results reported for sow myometrium where Ca^{2+} (in concentrations as high as 5 mM) did not influence [^{3}H]oxytocin binding [4].

Study of structure-activity relationships using a set of neurohypophyseal hormone analogues demonstrated that binding activity paralleled uterotonic potency (Table II), indicating that the [^{3}H]oxytocin binding sites have specificity characteristics expected of physiologic receptors in estrogen-dominated rabbit uterus. The correlation between binding and uterotonic activities was observed with neurohypophyseal analogues that differ in affinity but are agonists in the uterotonic assay (oxytocin, arginine vasopressin, lysine vasopressin, [gly^7]-oxytocin, [val^4]-oxytocin, [deamino^1]-oxytocin, and [deamino^1]-oxytocinoic acid), as well as with analogues with little uterotonic potency ([val^5]-oxytocin, [ala^2]oxytocin, and [arg^8]vasopressinoic acid). The agonists [gly^7]-oxytocin, [val^4]-oxytocin, and [deamino^1]-oxytocinoic acid exhibited a progressive decrease in both binding and uterotonic potency; their K_b values were respectively increased 6-, 8- and 225-fold relative to oxytocin, whereas the K_a values were increased 4-, 9- and 200-fold, respectively. Structural modifications of the oxytocin molecule that markedly reduced uterotonic activity likewise produced parallel effects upon binding activity in estrogen-dominated rabbit uterus. Thus, [ala^2]-oxytocin did not induce contraction of the estrogen-dominated rabbit uterus at the highest concentration tested (1.0 μM); similarly, this analogue was about 8000 times less active than oxytocin in competing for specific [^{3}H]oxytocin binding sites. Analogous results were obtained with [val^5]-oxytocin, which showed a paralellel loss of uterotonic and binding activity. [Arg^8]-vasopressinoic acid, which had no contractile activity at concentrations up to 1.0 μM, likewise had binding activity about 1000 times lower than oxytocin. Two peptides structurally unrelated to neurohypophyseal hormones, angiotensin II and insulin, were ineffective in competing for [^{3}H]oxytocin binding sites.

Previous studies on the isolated rat uterus have demonstrated that [gly^7]-oxytocin and [val^4]-oxytocin are respectively 2—7 times and 4 times less active than oxytocin in eliciting contractile responses [18,20], while [deamino^1]-oxytocin has been found to be slightly more potent than oxytocin [21]. Similar relative activities were obtained with these analogues in the rabbit uterotonic assay in the present studies. In contrast, a marked discrepancy was found between the contractile potencies of arginine vasopressin and lysine vasopressin

* It has been suggested that the formation of an active peptide hormone-receptor complex may require a ternary intermediate involving divalent metals [17]. In this view, Ca^{2+} might compete with Mg^{2+} (or Mn^{2+}) for this site and thereby inhibit the formation of the hypothetical metal-hormone-receptor complex. It is also possible that Mg^{2+} (and Mn^{2+}) influence [^{3}H]oxytocin binding to uterine membranes, at least in part, by replacing Ca^{2+} present in the membranes.

in the rabbit uterus, as compared to results reported for rat uterus. Thus, Munsick [13] reported that arginine vasopressin and lysine vasopressin were respectively 20 and 30 times less active than oxytocin in contracting the isolated rat uterus. In the present study, lysinevasopressin and arginine vasopressin were respectively 50 and 100% as active as oxytocin in contracting the estrogen-dominated rabbit uterus, and were respectively 50 and 80% as active as oxytocin in binding to rabbit uterine neurohypophyseal hormone receptors. The non-gravid human uterus appears to resemble the rabbit, rather than the rat, in that oxytocin and vasopressin appear to be equipotent as contractile agents in human uterine segments [22]. Indeed, some evidence suggests that the non-gravid human uterus may be more responsive to vasopressin than to oxytocin [23,24].

In summary, specific [^{3}H]oxytocin binding sites in estrogen-dominated rabbit uterus have characteristics including high affinity and neurohypophyseal analogue specificity that are expected of physiologic oxytocin receptors coupled to uterine contraction. As such, this system should be useful in the further study of the factors that regulate uterine oxytocin sensitivity during pregnancy and parturition.

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