Characterization of a Proposed Oxytocin Receptor in the Uterus of the Rat and Sow*

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SUMMARY

[3H]Oxytocin, incubated with rat uterine segments, was bound specifically, and about equally, to the three particulate fractions obtained by homogenization and centrifugation at 1,000, 20,000, and 105,000 × g. There apparently was no binding in the cytosol fraction.

[3H]Oxytocin, added directly to rat uterine particles sedimenting between 1,000 and 20,000 × g, was bound with an apparent Kd of 1.8 × 10⁻⁸ m; 0.18 pmole of oxytocin was bound per mg of protein. For the 20,000 × g particulate fraction of the uterus from a pregnant sow, the apparent Kd for oxytocin binding was 1.5 × 10⁻⁹ m; 0.15 pmole of oxytocin was bound per mg of protein. The affinity of oxytocin for the 105,000 × g particles from sow uterus was about 3 times greater than for the 20,000 × g particles. The binding constants are of the same order as the concentration of oxytocin which elicits a half-maximal uterine response.

The binding of [3H]oxytocin by sow uterine 20,000 × g particles was maximal by 40 min at 20°C. Less binding occurred at 37 and 0°C than at 20°C.


Binding was optimal in the range of pH 7.4 to 7.8, was enhanced by Mn²⁺ and Co²⁺ > Mg²⁺ > Zn²⁺, and was absent with 1 mM ethylenediaminetetraacetate. The concentrations of Mn²⁺ giving maximal and half-maximal augmentation of binding were 5 and 0.4 mM, respectively. Ca²⁺, 5 mM, did not affect binding.

A portion of the [3H]oxytocin appeared to be metabolized during the incubation with sow uterine particles. The uterine oxytocin receptor site is protein, at least in part, because binding was reduced by trypsin, —SH reagents such as N-ethylmaleimide, p-chloromercuribenzoate, and by thioglycolate. Phospholipases A and C inhibited binding; however, their inhibitory activities appeared to be separate from their catalytic activities. Neuraminidase, phospholipase D, butanedione, and sodium fluoride did not affect [3H]oxytocin binding. Binding was depressed by guanosine triphosphate, cytosine triphosphate, and uridine triphosphate.

However, 1 mM ATP inhibited binding by more than twice as much as the other nucleoside triphosphates.

The high affinity and ligand specificity of the uterine binding sites for oxytocin indicate that the binding protein is part of the oxytocin receptor system.

Oxytocin is an octapeptide hormone which is synthesized by the paraventricular neurons of the hypothalamus (see Ref. 1 for a review). The hormone, in the form of neurosecretory vesicles, passes along the nerve axons to the neural lobe of the pituitary, where it is stored. Upon the appropriate stimulus, oxytocin is released from the posterior pituitary and enters into the circulation. The hormone exerts at least two major physiological actions involving contractile elements in its target tissues in mammals. It causes milk ejection from the mammary gland and contraction of the uterus.

Oxytocin was the first biologically active peptide hormone to be synthesized (2). In the 20 years following the elucidation of its structure, almost 200 related peptides have been synthesized (3). These analogs have been used in attempts to define the chemical requirements for oxytocic activity and to elucidate the hormone receptor site (4). Whereas the relative importance of functional groups on the oxytocic peptides has been elucidated (3, 5), the general nature of the receptor site is unknown. However, now that [3H]oxytocin of high specific activity has been synthesized (6), it has been shown to be bound by particulate fractions from lactating rat mammary gland (7) and to epithelium from frog skin (8, 9) and bladder (10).

We have observed that radioactivity from [3H]oxytocin is accumulated by segments of rat uterus in vitro (11). Several synthetic analogs of oxytocin depressed the accumulation of radioactivity in direct proportion to their uterotonic potencies (11). This specific uptake of oxytocin indicated that oxytocin-binding substances would be present in the rat uterus. The present studies show that [3H]oxytocin is bound specifically and with high affinity to particulate fractions from rat and sow uterus.

EXPERIMENTAL PROCEDURES

Materials

Peptides—[tyrosyl-3H]Oxytocin (28 Ci per mmole) (6) was purported by the manufacturer, Schwarz-Mann, to be fully active
in the rat uterus bioassay. More than 90% of the radioactivity migrated with authentic oxytocin upon thin layer chromatography (7). Aliquots of [H]oxytocin were put in plastic tubes and stored at -70°. No change in the purity of the hormone was apparent during 1 year.

Oxytocin, [4-proline]oxytocin, [B-valine]oxytocin, and [4-threonine]oxytocin were provided by Dr. Maurice Manning of this department. Oxytocin (Systocin) and [B-lysine]oxytocin were gifts from Sandoz, Ltd.

Enzymes and Chemicals—Neuraminidase from Clostridium perfringens, 1.1 units per mg; trypsin from bovine pancreas, 10,000 units per mg; phospholipase A from Vipera russelli, 7.7 units per mg; phospholipase C from Clostridium welchii, 2.5 units per mg; phospholipase D from cabbage, 31 units per mg; and protease from Streptomyces griseus, 3.5 units per mg, were purchased from Sigma. L-α-Leucine (5-dimethylallyl L-α-leucine, type I, synthetic, 96% pure), iodoacetamide, N-ethylmaleimide, p-hydroxymercuribenzoate, 2,3-butanedione, thioglycollic acid, ATP, GTP, CTP, and IFP were purchased from Sigma.

Methods

Subcellular Distribution of Radioactivity from [3H]Oxytocin

Uterine horns were taken from rats (CFE, Carworth Farms, Inc.) 200 to 220 g, as described previously (11). The horns were cut into segments, about 20 mg each, and each segment was incubated separately for 1 hour at 20° with shaking in a capped plastic tube containing 2.3 × 10^{-3} Ci of [3H]oxytocin, and either 0 or 5 µg of oxytocin or 5 µg of [4-proline]oxytocin in 1 ml of Tyrode's solution, pH 7.6. Two similarly incubated pieces were blotted lightly with filter paper, combined, and homogenized in 1 ml of Tyrode's solution in a conical glass homogenizer at 4°. All subsequent operations were performed at 4°. The homogenate was fractionated by differential centrifugation into particles sedimenting at 1,000, 20,000, and 105,000 g, as described previously (7). The radioactivity from [3H]oxytocin in each fraction and in the 105,000 g supernatant was determined (7).

Preparation of 20,000 × g Particles—Rat uterine horns were homogenized in 9 volumes of Tyrode's solution in an all glass conical homogenizer at 4°. All subsequent operations were at 4°. The homogenate was centrifuged at 1,000 × g for 10 min and the resulting supernatant was centrifuged at 20,000 × g for 10 min. The 20,000 × g pellet was washed in the original volume of Tyrode's solution and then resuspended in 1/3 volume. The suspension was stored at -70° for several weeks without any noticeable change in binding activity.

The uterus from a pregnant sow was obtained from a local slaughterhouse and transported to the laboratory immersed in Tyrode's solution and then resuspended in 35 volume. The suspension was stored at -70° for several weeks without any noticeable change in binding activity.

Counts per min | Percentage of change
--- | ---
Control | 1285 ± 166
Plus 5 µg oxytocin | 1374 ± 74
Plus 5 µg [4-proline]oxytocin | 1307 ± 150

Percentages of change in binding activity by 64, 73, and 85%, respectively, by the presence of nonradioactive oxytocin (Fig. 1A). [4-proline]Oxytocin, a relatively inactive analog (14), did not reduce the binding of [3H]oxytocin as much as oxytocin (Table I). The radioactivity associated with the 1,000, 20,000, and 105,000 g pellets was reduced significantly (p < 1%) by 64, 73, and 85%, respectively, by the presence of nonradioactive oxytocin in the incubation medium (Table I). The reduction of radioactivity in the cytosol fraction (12%) was not significant. The radioactivity in the particulate fraction was not reduced by the presence of nonradioactive oxytocin in the incubation medium (Table I).

Table I

Distribution of radioactivity from [3H]oxytocin in subcellular fractions of rat uterus

<table>
<thead>
<tr>
<th>Incubation medium</th>
<th>1000 × g pellet</th>
<th>20000 × g pellet</th>
<th>105000 × g pellet</th>
<th>1050000 × g supernatant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Counts per min</td>
<td>Percentage of change</td>
<td>Counts per min</td>
<td>Percentage of change</td>
<td>Counts per min</td>
</tr>
<tr>
<td>Control</td>
<td>1285 ± 166</td>
<td></td>
<td></td>
<td>1614 ± 102</td>
</tr>
<tr>
<td>Plus 5 µg oxytocin</td>
<td>488 ± 65</td>
<td>-64</td>
<td>369 ± 96</td>
<td>-73</td>
</tr>
<tr>
<td>Plus 5 µg [4-proline]oxytocin</td>
<td>1118 ± 64</td>
<td>-13</td>
<td>1307 ± 150</td>
<td>-5</td>
</tr>
</tbody>
</table>

The ability of oxytocin and the synthetic analogs to compete with [3H]oxytocin for uterine binding sites was determined by six-point parallel line assays as described by Finney (12). Where relative potencies and 95% confidence limits are expressed, the analysis of variance showed the assays to be statistically valid.

Protein Determination—Protein determinations of 20,000 × g pellets dissolved in 1 ml NaOH (7) were made according to the method of Lowry et al. (13) with bovine serum albumin as the standard.

RESULTS

Subcellular Distribution of Radioactivity from [3H]Oxytocin in Rat Uterus—About 77% of the total radioactivity present in rat uterine segments after incubation with [3H]oxytocin was in the three particulate fractions obtained by differential centrifugation (Table I). The radioactivity associated with the 1,000, 20,000, and 105,000 g pellets was reduced significantly (p < 1%) by 64, 73, and 85%, respectively, by the presence of nonradioactive oxytocin in the incubation medium (Table I). The reduction of radioactivity in the cytosol fraction (12%) was not significant. The [H] associated with the pellets was taken up specifically because the presence in the incubation medium of [4-proline]oxytocin, a relatively inactive analog (14), did not reduce the binding of [3H]oxytocin as much as oxytocin (Table I).

Binding of [3H]Oxytocin to 20,000 × g Particles from Rat Uterus—The binding of [3H]oxytocin to rat uterine particles was studied under incubation conditions found to be optimal for oxytocin binding to mammary particles (7). The binding of [3H]oxytocin to the 1,000 to 20,000 × g particulate fraction from rat uterus was decreased in proportion to the log of increasing amounts of nonradioactive oxytocin (Fig. 1A). [4-proline]Oxytocin did not compete with [3H]oxytocin for the binding sites.
The specificity of [3H]oxytocin binding to uterine 20,000 × g particles. Each tube contained 1 mg of particulate protein, 9,000 dpm of [3H]oxytocin (about 150 pg), and increasing amounts of nonradioactive peptide in 250 μl of Tris buffer (0.05 M, pH 7.6, containing 5 mM Mg2+ and 0.1% gelatin). Incubation was carried out for 1 hour at 20° and was terminated by centrifugation at 20,000 × g for 10 min at 4°. The pellet was combusted to yield 3H2O. Each point is the mean of triplicates.

Scatchard analysis (15) of oxytocin binding to rat uterine particles gave an apparent Kd of 1.8 × 10⁻⁹ M. The binding capacity was 1.8 × 10⁻¹³ moles of oxytocin bound per mg of particulate protein.

Binding of [3H]Oxytocin to 20,000 × g Particles from Sow Uterus—Further studies on the rat uterus were impractical because of the large number of animals needed. Therefore, the 20,000 × g particulate fraction from the uterus of the pregnant sow was tested for [3H]oxytocin-binding activity. As shown in Fig. 1B, the binding of [3H]oxytocin was reduced in proportion to the log concentration of increasing amounts of nonradioactive oxytocin. Binding was not affected by [4-proline]oxytocin even at a concentration 21 times greater than that of [3H]oxytocin.

Binding Specificity and Affinity—As shown in Fig. 1B, [4-threonine]oxytocin, [8-valine]oxytocin, and [8-lysine]vasopressin reduced the binding of [3H]oxytocin to sow uterine 20,000 × g particles in proportion to the log of their concentrations. The regressions were parallel, indicating a common set of binding sites. The potency of each analog relative to that of oxytocin was: [4-threonine]oxytocin, 0.96 (0.82 → 1.12, 95% confidence limit); [8-valine]oxytocin, 1.09 (0.94 → 1.26); and [8-lysine]vasopressin, 0.17 (0.13 → 0.24).

Scatchard analysis of oxytocin binding to the 20,000 × g particles of pig myometrium also was examined. This preparation had the same capacity for oxytocin as the 20,000 × g particles, but its affinity for oxytocin was about 3 times greater, Kd = 4.7 ± 0.3 (S.E.) × 10⁻⁹ M (n = 4). The 20,000 × g particles had the same binding specificity as the 20,000 × g particles. Further studies on the 105,000 × g fraction were not carried out.

Effect of Incubation Time and Temperature on Binding—The binding of [3H]oxytocin to 20,000 × g particles from pig myometrium was dependent upon time and temperature (Fig. 3). At 37°, equilibrium was reached by 20 min. At 20°, the amount of [3H]oxytocin bound was about 2.5 times greater at equilibrium, which occurred by 40 min. At 4°, binding had not reached equilibrium by 80 min. All further incubations were carried out at 20° for 1 hour.

Effect of pH and Divalent Cations on Binding—Binding to sow uterine particles was greatest between pH 7.4 and 7.8. Maximal binding in 50 mM Tris buffer was obtained with about 5 mM Mn²⁺ (Fig. 4). The Kₘ for the Mn²⁺ enhancement of oxytocin binding was 4.0 ± 0.6 (S.E.) × 10⁻⁴ M, as determined by the method of Wilkinson (16). Co²⁺ at 1 and 5 mM was as effective
as Mn\textsuperscript{2+}. Mg\textsuperscript{2+} and Zn\textsuperscript{2+} were about 66 and 30%, respectively, as effective as Mn\textsuperscript{2+} in the enhancement of [\textsuperscript{3H}]oxytocin binding. Concentrations of Co\textsuperscript{2+} and Zn\textsuperscript{2+} greater than 5 mM reduced the pH of the Tris buffer and, therefore, were not examined. Ca\textsuperscript{2+}, 1 and 5 mM, did not affect binding (Fig. 4). Binding was absent in the presence of 1 mM EDTA.

**Nature of Binding Substance**—Binding was significantly (p < 5%) depressed in increasing order by phospholipase C, trypsin, phospholipase A, and protease, but not by phospholipase D or neuraminidase (Table II). The inhibitory activity of the relatively crude preparation of phospholipase A may not be due to the catalytic activity of the enzyme itself because boiling it for 20 min gave the same results. Also, the addition of a 2-fold excess of its substrate, lecithin, did not alter the inhibition. Lecithin alone did not affect binding (Table II).

Binding also was inhibited by N-ethylmaleimide, p-hydroxymercuribenzoate, and thioglycollate, but not by iodoacetamide, butanedione, or sodium fluoride (Table III).

**Characterization of Radioactivity from [\textsuperscript{3H}]Oxytocin after Incubation with Uterine 20,000 \times g Particles**—Fifty per cent of the radioactivity bound to the 20,000 \times g pellet was extracted with 0.1 ml of 0.1 \texttimes HCl. Upon thin layer chromatography of the extract, a single peak of radioactivity corresponding to the position of carrier oxytocin was found (data not shown). The radioactivity in the medium after 1 hour of incubation also appeared as a single oxytocin peak upon thin layer chromatography. These results imply that [\textsuperscript{3H}]oxytocin was not altered during the incubation. However, the specific binding activity remaining in the incubation medium after the 1-hour incubation period was reduced 40% upon a second incubation with fresh 20,000 \times g particles (Table IV). The specific binding activity refers to the difference in binding between samples containing 0 and 32 ng of nonradioactive oxytocin. The reduction in binding occurring after the second incubation ranged from 20 to 40% in five experiments.

These results indicate that a portion of the oxytocin in the

**TABLE II**

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of Oxytocin Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12,350 \pm 260</td>
</tr>
<tr>
<td>0.1 mg oxytocin</td>
<td>12,800 \pm 260</td>
</tr>
<tr>
<td>32 ng oxytocin</td>
<td>12,500 \pm 230</td>
</tr>
<tr>
<td>No competitor</td>
<td>6,300 \pm 130</td>
</tr>
<tr>
<td>32 ng oxytocin + fresh uterine</td>
<td>6,500 \pm 20</td>
</tr>
<tr>
<td>Particles</td>
<td>2,700 \pm 60</td>
</tr>
</tbody>
</table>

**TABLE III**

<table>
<thead>
<tr>
<th>Compound</th>
<th>% of Oxytocin Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12,350 \pm 260</td>
</tr>
<tr>
<td>0.1 mg oxytocin</td>
<td>12,800 \pm 260</td>
</tr>
<tr>
<td>32 ng oxytocin</td>
<td>12,500 \pm 230</td>
</tr>
<tr>
<td>No competitor</td>
<td>6,300 \pm 130</td>
</tr>
<tr>
<td>32 ng oxytocin + fresh uterine</td>
<td>6,500 \pm 20</td>
</tr>
<tr>
<td>Particles</td>
<td>2,700 \pm 60</td>
</tr>
</tbody>
</table>

**TABLE IV**

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Disintegrations per min bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubation with medium containing [\textsuperscript{3H}]oxytocin</td>
<td>12,500 \pm 230</td>
</tr>
<tr>
<td>32 ng oxytocin</td>
<td>6,300 \pm 130</td>
</tr>
<tr>
<td>Reincubation of above medium + fresh uterine particles</td>
<td>6,500 \pm 20</td>
</tr>
<tr>
<td>No competitor</td>
<td>2,700 \pm 60</td>
</tr>
<tr>
<td>32 ng oxytocin</td>
<td>12,350 \pm 270</td>
</tr>
<tr>
<td>Reincubation of medium containing no [\textsuperscript{3H}]oxytocin initially + [\textsuperscript{3H}]oxytocin + fresh uterine particles</td>
<td>6,600 \pm 210</td>
</tr>
</tbody>
</table>
medium was altered upon incubation. Alternatively, some of the receptor sites might have been inactivated by factors released into medium during the incubation period. To determine whether the loss in binding activity was due to factors in the medium, 20,000 × g particles were incubated for 1 hour in the absence of [3H]oxytocin. The particles then were removed by centrifugation. [3H]Oxytocin was added to the supernatant and the mixture was incubated with fresh 20,000 × g particles. As shown in Table IV, the binding of [3H]oxytocin to uterine particles after the second incubation was the same as after an incubation with fresh medium. Therefore, the factors responsible for the reduction of oxytocin binding were not in the incubation medium and must be associated with the particles themselves.

**Table V**

<table>
<thead>
<tr>
<th>Nucleoside triphosphate</th>
<th>Disintegrations per min bound</th>
<th>Disintegrations per min bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1990 ± 46</td>
<td>655 ± 10</td>
</tr>
<tr>
<td>Control + 32 ng oxytocin</td>
<td>1040 ± 52</td>
<td>660 ± 9</td>
</tr>
<tr>
<td>ATP</td>
<td>1485 ± 21</td>
<td>605 ± 22</td>
</tr>
<tr>
<td>ATP + 32 ng oxytocin</td>
<td>1410 ± 76</td>
<td>720 ± 11</td>
</tr>
<tr>
<td>GTP</td>
<td>1610 ± 23</td>
<td>655 ± 10</td>
</tr>
<tr>
<td>GTP + 32 ng oxytocin</td>
<td>1410 ± 76</td>
<td>720 ± 11</td>
</tr>
<tr>
<td>CTP</td>
<td>1610 ± 23</td>
<td>655 ± 10</td>
</tr>
<tr>
<td>CTP + 32 ng oxytocin</td>
<td>1610 ± 23</td>
<td>655 ± 10</td>
</tr>
</tbody>
</table>

**Discussion**

The sensitivity of the uterine response to oxytocin increases upon administration of the synthetic estrogen diethylstilbestrol (17), and also in pregnancy (18). Accordingly, uterine from estrogen-treated rats and the pregnant sow were used in our study to examine oxytocin binding under conditions where the uterus might have the greatest affinity for the hormone. Our findings show that [3H]oxytocin was bound specifically and with high affinity to subcellular particles from rat and pig uterus. Therefore, the oxytocin binding site likely is a component of the oxytocin receptor system. Oxytocin was bound to the 20,000 × g particles from sow uterus with an apparent Kd of 1.8 × 10⁻⁴ M, which is equivalent to about 800 microns of oxytocin per ml. This concentration of oxytocin is not significantly different from that causing a half-maximal contraction of the isolated rat uterus, 1,000 ± 340 (S.E.) micromers per 2 ml (17). To our knowledge there are no data on the dose of oxytocin giving a half-maximal contraction of the isolated pig uterus. The dissociation constants for oxytocin binding to 20,000 × g particles from sow uterus and rat uterus were virtually the same. The Kd for oxytocin binding to the 105,000 × g particulate fraction from sow uterus, 4.7 × 10⁻¹⁸ M, was about one-third that of the 20,000 × g fraction. These results suggest that the purity of the particulate preparation may affect the apparent Kd.

The binding of oxytocin to sow uterine particles was specific. [4-proline]Oxytocin, which differs from oxytocin by a proline in position 4 instead of a glutamine, did not compete for [3H]oxytocin binding sites in the dose range studied. These results agree with the marginal uterotonic activity of [4-proline]oxytocin on the rat uterus (14). [8-lysine]Vasopressin, which differs from oxytocin by isoleucine → phenylalanine in position 3 and leucine → lysine in position 8, had 17% of the binding activity of oxytocin. [8-lysine]Vasopressin is about 1% as biologically active as oxytocin in the rat uterus (19). Analogs in which threonine replaces glutamine in position 4 of oxytocin (4-threonineoxytocin), or valine replaces isoleucine in position 8 (8-valineoxytocin), were not discernibly different from oxytocin in binding activity. However, [4-threonineoxytocin and [8-valineoxytocin are 200 and 60% as active as oxytocin on the isolated rat uterus, respectively (20, 21). The oxytocin analogs bind to rat mammary particles in the same rank order as their biological activities, milk ejection (7). The comparison between binding and biological activities of the analogs in sow uterus is not possible because the uterotonic activities are not known. The poor agreement found between oxytocin potency in rat uterus and binding potency in sow myometrium may reflect species differences in the specificity of uterine receptors for oxytocin analogs. Indeed, the uterine response to certain oxytocin analogs has been seen to differ in the rat, cat, and human (18).

The oxytocin binders in the uterus and mammary gland are similar in many ways: [3H]oxytocin binding sites in both targets were distributed rather evenly among the 1,000, 20,000, and 105,000 × g particulate fractions obtained by differential centrifugation. There was no binding in the cytosol fraction. [3H]Oxytocin was bound when added directly to the 20,000 × g particles from rat and sow uterus and from rat mammary gland. The affinities of the binders for oxytocin in the uterus and mammary gland were consistent with the concentration of hormone which gives a biological response. The binding to the pig myometrial or rat mammary particles was optimal around pH 7.6, was enhanced by Mn²⁺ = Co²⁺ > Mg²⁺ > Zn²⁺, and was absent in 1 mM EDTA. Ca²⁺ did not affect the binding of oxytocin to uterine or mammary sites. Binding appeared to be optimum at 20° in both tissues. Trpysin depressed binding to both targets, phospholipases, neuraminidase, butanediol, and sodium fluoride essentially did not. Binding of [3H]oxytocin to both myometrial and mammary sites was markedly reduced by 1 mM ATP.

On the other hand, the uterine and mammary binding preparations are different. For example, N-ethylmaleimide, 1 mM, reduced the binding of oxytocin to sow myometrial particles by 34%, whereas 5 times the concentration did not affect the binding to mammary particles. Myometrial particles inactivated to 40% of the [3H]oxytocin in the incubation medium, as far as binding activity is concerned, but mammary particles did not inactivate [3H]oxytocin. The nature of the inactivating substance is unknown. The material was not present in the incubation medium, and was associated with the uterine particles. The binding of radioactivity in reused incubation medium was independent of the magnesium concentration between 5 and 20 mM. The inactivating material can be one of several uterine enzymes which fit into the broad category of oxytocinases (22, 23).

Mg²⁺ and Mn²⁺ to an even greater degree, potentiate the action of oxytocin analogs on the isolated rat uterus (24). Bentley has proposed that the metal ions act by increasing the affinity of uterine receptors for oxytocin (24). Our results support this...
hypothesis, insofar as Mn$^{+}$ and Mg$^{+}$ enhanced the binding of [H]$\text{oxytocin}$ to uterine particles. The mechanism by which the metal ions affect the receptor's affinity for oxytocin are not known. The presence of Ca$^{+}$ in the medium bathing an isolated uterus preparation is essential for oxytocin's action (25-27). Yet, our results showed that Ca$^{+}$ did not affect the binding of [H]$\text{oxytocin}$ to its uterine receptor. Thus, Ca$^{+}$ may be involved in molecular events which take place after oxytocin-receptor interaction. Indeed, Csapo has postulated that oxytocin acts by facilitating the movement of Ca$^{+}$ across the cell membrane of uterine smooth muscle cells (28). The Ca$^{+}$, once inside the cell, may elicit contraction by interacting directly with a regulatory protein such as troponin (29). The same conclusions have been drawn for the effect of Ca$^{+}$ on oxytocin's action on the mammmary gland (7).

Oxytocin affects the electrophysiological status of uterine smooth muscle (25-27). Therefore, the uterine receptor sites for oxytocin would appear to reside on the myometrial cell membrane. Our observation of the particulate nature of oxytocin binding sites in the rat and sow uterus is not in opposition to the postulated cell membrane locus of the oxytocin receptor. However, knowledge of the precise subcellular location of the receptor must await its purification from the uterine particles. Our results indicate that the receptor is a protein, at least in part, because of its susceptibility to trypsin. Trypsin must reduce binding by affecting the receptor site because oxytocin is not a substrate for the enzyme (30). Ponsome reduced binding markedly, but its action may have been upon oxytocin itself.

The inhibitory activity of N-$\text{ethylmaleimide}$ on oxytocin binding supports the observation that the contraction of the isolated rat uterus induced by oxytocin is abolished with 1 mM N-$\text{ethylmaleimide}$ (31). Thioglycollate also inhibited the binding activity, as well as abolishing the uterotropic activity of oxytocin (32). Thioglycollate is thought to inactivate oxytocin by reducing the disulfide bond between the half-cystines at positions 1 and 6, and also to act directly upon the myometrial receptor site (32). The inhibition of binding and biological activities by thioglycollate, N-$\text{ethylmaleimide}$, and $\text{p-hydroxymercuribenzoate}$ suggests that both $\text{-SH}$ and $\text{-SH}$ groups are involved in the topography of the binding site. The reasons for the lack of effect of the $\text{-SH}$ reagent iodoacetamide are not clear. Sulfhydryl groups located in hydrophobic regions of the uterine particles may not be accessible to certain $\text{-SH}$ reagents like iodoacetamide, at the concentrations used in this study. Alternatively, divalent cations may bind in the $\text{-SH}$ region and prevent access of $\text{-SH}$ reagent. Phospholipids, which appear to affect the interaction of insulin (33) and glucagon (34-36) with their respective receptors on liver membranes, do not appear to be involved with the oxytocin binding sites of either mammmary gland (7) or uterus (this study).

The inhibitory effect of ATP on [H]$\text{oxytocin}$ binding is not understood. Similar results were found in our studies on oxytocin receptors in the mammmary gland (7). The mechanism of the ATP effect in both mammmary gland and sow uterus is under investigation.

**Acknowledgments**—We thank Ms. Martha Morrison for her assistance; Dr. Murray Safran for support; Dr. Maurice Manning of our department and Sandoz, Ltd. for oxytocin and its synthetic analogs; Mr. Ramesh Parekh for the statistical analyses; and Ms. Cindy Stanley for the typescript.

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