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Problems Associated with I-125 Oxytocin Binding to Membrane Receptors

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Radioiodinated oxytocin prepared by the lactoperoxidase method exhibited a substantial biologic activity in uterotonic assay of the rat uterus.

^{125}I-oxytocin was bound to the uterine membrane particulate fraction, but the unlabelled oxytocin did not inhibit the binding of ^{125}I oxytocin to the membrane fraction of rat uterus. Cold iodinated oxytocin, however, inhibited the ^{125}I-oxytocin binding to the membrane fraction of rat uterus in proportion to its concentration.

These results suggest that ^{125}I-oxytocin is not a suitable radioligand for oxytocin receptor binding study.

Previous attempts to determine the oxytocin concentration in biologic fluids using the bioassay (Bisset and Walker, 1954; Noddle, 1964; Branda and Ferrier, 1971) and radioimmunoassay (Bashore, 1972; Kumaresan *et al.*, 1974; Vasicka *et al.*, 1978; Dawood *et al.*, 1978a, 1978b) techniques were not particularly successful. The bioassay technique is generally not sensitive enough to measure such a low level of oxytocin as is found in the blood. The radioimmunoassay technique, on the other hand, has many advantages over bioassay. However, its main disadvantage is the possibility that the material measured may not correspond to the biologically active material (Chard, 1977). For these reasons, the technique employing cell receptor (receptor assay) was developed and it is generally believed that the receptor assay can provide an accurate reflection of the biologically effective level of a hormone (Chard, 1977).

Receptor assay was first described for estrogens (Korenman, 1968), and has since been applied to various peptide hormones (Chard, 1977; Roth, 1973). Soloff and his colleagues (Soloff and Swartz, 1973, 1974; Soloff *et al.*, 1973, 1974 and 1977; Soloff, 1975, 1976; Robert *et al.*, 1976), using this technique, have characterized the oxytocin receptors in sow, rat, sheep and human uterus. They observed that ^{3}H-oxytocin is accumulated in membrane fractions of the uterus and mammary gland, and this accumulation is inhibited by synthetic analogues of oxytocin in direct proportion to their uterotonic potencies. Other investigators (Bockaert *et al.*, 1970, 1972; Crankshaw *et al.*, 1978; Nissenson *et al.*, 1978) have also attempted to characterize the oxytocin receptors in various tissues using ^{3}H-oxytocin as a ligand.

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However, ^{125}I-oxytocin has not been successfully utilized in oxytocin-receptor study despite the fact that ^{125}I-oxytocin is more easily prepared in the laboratory. If ^{125}I-oxytocin exhibits biological activity, this compound may be useful as a ligand for oxytocin receptor assay. But the chemical nature of this compound has not been fully characterized and its biological activity has been subject to much controversy (Morgat *et al.*, 1970; Thompson et al., 1972; Marbach and Rudinger, 1974; Flouret *et al.*, 1977).

We, therefore, undertook the present study to investigate the characteristics of ^{125}I-oxytocin binding to various tissues and its biological significance.

MATERIALS AND METHODS

Experimental tissues

Uterus, liver, skeletal muscle (abdominal muscle), lung, kidney and heart were obtained from a rat which had received daily injections of estradiol 17β (10 μg/100 gm of body weight) and progesterone (150 μg/100 gm of body weight) for 7 days. Sow uterus was obtained from a local slaughterhouse and kept in ice-cold Krebs-Hensenleit Ringer (KH Ringer) solution (115 mM NaCl, 4.5 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, 22 mM $NaHCO_3$, and 5 mM glucose, pH 7.4) until it was stored at -50°C in the laboratory.

Iodination of oxytocin

Oxytocin was iodinated using the lactoperoxidase method (Miyachi *et al.*, 1972) as described by Dawood *et al.* (1978 b).

Measurement of biological activity of radioiodinated oxytocin

Biological activities of radioiodinated oxytocin fractions were measured by uterotonic assay. After treatment of estradiol 17-β and progesterone for 7 days, a rat was sacrified by a blow on the head, uterine horns were quickly excised, dissected free from fat and cut open longitudinally to obtain a rectangular strip. The tissue strip was mounted vertically with one end fixed and the other end attatched to a Grass FTO3C force transducer and incubated in a 30°C bath containing 10 ml of KH Ringer solution (pH 7.4) gassed with a 95% O_2—5% CO_2 mixture. Spontaneous contractions were not seen under these conditions.

The isometric tensions induced by the addition of standard oxytocin or radioiodinated oxytocin were recorded on a Grass model 7 polygraph.

^{125}I-oxytocin binding to tissue slices

Uterus, liver, lung, kidney, skeletal muscle (abdominal muscle) and heart were obtained from the rat which had been treated with estradiol 17-β and progesterone. After the animal was sacrified by a blow on the head, these tissues were quickly removed and placed in an ice cold KH Ringer solution (pH 7.4) gassed with a 95% O_2—5% CO_2 mixture. Thin slices of each tissue were prepared using a Stadie-Riggs microtome. The slices were blotted on a filter paper, weighed and placed into a test tube containing an appropriate incubation medium. For competitive binding between labelled and unlabelled oxytocin, the incubation medium consisted of 1 ml of KH Ringer solution, 10 μl of radioiodinated oxytocin (20,000 cpm) and 500 ng of unlabelled oxytocin in the experimental series and unlabelled oxytocin was eliminated in the control series. Incubation was carried out in a shaker at 20 °C for 1 hour. After incubation was comple-

ted, slices were washed three times in the same KH Ringer solution and placed in a test tube for radioactivity determination in a Logic model 221 gamma counter.

Membrane preparations of uterus, skeletal muscle and liver

Uteri from rat and sow were dissected free of fat, rinsed of blood, minced and homogenized in five volumes of buffer solution (0.25 M sucrose, 1 mM $MgCl_2$, 5 mM tris, pH 7.4) using a Virtis homogenizer at 4°C. All subsequent procedures were carried out at 4°C. After filtration through a single layer of gauze, the homogenate was centrifuged at 8,000 xg for 10 min. in a Sorvall centrifuge (model RC 2-B, rotor SM24) and the pellet was discarded. Potassium chloride was added to the supernatant until the final concentration of KCl became 0.6 M and it was stored overnight in a cold room for extraction of actomyosin. The resulting supernatant was centrifuged at 20,000 xg for 10 min. in a Sorvall centrifuge. The 20,000 xg pellet was washed twice with a cold tris buffer solution (0.05 M tris, 5 mM $MgCl_2$, pH 7.4) and then resuspended in the same buffer. The 20,000 xg supernatant was centrifuged at 105,000 xg for 1 hour in a Beckmann ultracentrifuge (model L 2-65 B, rotor 65). The pellet obtained was washed twice in a cold tris buffer solution and then resuspended in the same solution. These 20,000 xg and 105,000 xg pellet suspensions were stored at −50°C until being used.

Membrane fractions of skeletal muscle and liver were prepared by the same procedure as described above, but the step of KCl adding was ommitted in the liver membrane preparation.

The protein concentration of the suspension was determined by the method of Lowry *et al.* (1951). Since Na-K-ATPase is one of the marker enzymes of the plasma membrane (Emmelot *et al.*, 1964), the Na-K-ATPase activities of various preparations were determined by a modified method of Towle and Copenhaver (1970).

Binding of ^{125}I-oxytocin to 20,000 xg and 105,000 xg pellets

The frozen suspension was thawed slowly and rehomogenized in a glass-glass homogenizer at 4°C. In general, incubation mixtures consisted of tris buffer (0.05 M tris, 5 mM $MgCl_2$, pH 7.4), varying amounts of membrane particulates and ^{125}I-oxytocin with or without unlabelled oxytocin.

The total volume of incubation mixture was adjusted to 0.3~0.4 ml.

The conditions of the assay are detailed in the appropriate section of RESULTS. Incubations were carried out at 20°C. In most studies, the period of incubation was 1 hour. At the end of incubation, reaction was terminated by diluting the incubation medium, i.e., by the addition of 1ml of ice-cold tris buffer solution to each tube, and the tubes were centrifuged at 4°C for 10 min. (for 20,000 xg pellet) or 30 min. (for 105,000 xg pellet) at 20,000 xg. The resulting pellets were washed three times with the same buffer solution. Radioactivity in each pellet was determined in a Logic gamma counter (model 221).

RESULTS

Radioiodination of oxytocin

The percentage of utilization of ^{125}I and the specific activity of radioiodinated oxytocin were calculated by the method of Saxena *et al.* (1968). Fortyfive to fiftyfive percent of the ^{125}I was labelled to oxytocin. The specific

activity of the radioiodinated oxytocin ranged from 225 to 275 mCi/mg. Aggregates of radioisotope labelled(but, probably damaged) oxytocin was found in elution fractions 8—10 from the Sephadex G—25 column. The concentrated labelled oxytocin was found in fractions 12—16(Fig. 1). The oxytocin concentrations of the radioiodinated oxytocin fractions from the Sephadex column were measured by uterotonic assay, and found to be 14～40 ng per 100 μl of elute(Fig. 1). The percentage of the oxytocin labelled with ^{125}I was 75～85%.

Binding of ^{125}I-oxytocin to various tissues, in vitro

To determine if ^{125}I-oxytocin is recognized by oxytocin target tissues, ^{125}I-oxytocin was incubated with various tissues in the absence and presence of unlabelled oxytocin(500 ng/assay tube). After 1 hour of incubation, the radioactivity in the tissue was counted. As shown in Fig. 2, the radioactivity in the uterine tissue was greater than those in other tissues. In all tissues, however, the radioactivity was not lowered by the addition of unlabelled oxytocin, indicating that the nature of binding of the ^{125}I-oxytocin may be different from that of unlabelled oxytocin or it may be bound to site(s) other than the oxytocin binding site(s). The greater binding in uterine tissue than in other tissues indicates that there exist some tissue specificity in the ^{125}I-oxytocin binding.

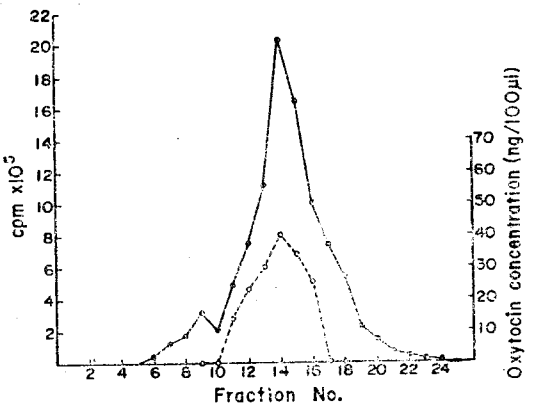

Fig. 1: Separation of ^{125}I-oxytocin on the Sephadex G-25 gel filtration.
After radioiodination of oxytocin, the remaining free ^{125}I was absorbed by addition of inorganic iodine resin and chromatographed on the Sephadex G-25 column which was prewashed with 0.05 M tris-HCl buffer solution(pH 8.0) containing 0.5% of bovine serum albumin (BSA). The column was eluted with this buffer solution and elute fractions of 0.5 ml each were collected. Solid symbols(●-●), represent radioactivity of these fractions. First peak(fraction 8-10) may correspond to aggregated ^{125}I-oxytocin fraction. Open symbols(○-○), represent ^{125}I-oxytocin concentrations of elutes measured by uterotonic assay.

Bindings of ^{125}I-oxytocin to 20,000 xg and 105,000 xg particles from rat uterus, liver and skeletal muscle

Since the ^{125}I-oxytocin binding to uterus, liver and skeletal muscle were greater than those to the other tissues(Fig. 2), these tissues were chosen in the study of ^{125}I-oxytocin binding to the membrane receptor. The rece-

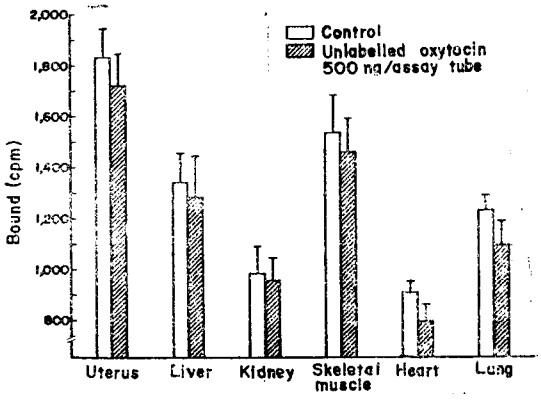

Fig. 2: Uptake of ^{125}I-oxytocin, in vitro.
Tissue slices obtained from female rats, which received estrogens and progesterone for 7 days, were incubated in 1 ml of KH Ringer solution containing ^{125}I-oxytocin with or without 500 ng of unlabelled oxytocin per assay tube. After the end of incubation, each slice was washed three times with KH Ringer solution and radioactivity was counted.
All values represent mean radioactivity bound to 50 mg of wet tissue±S.E. in 5 experiments.

ptors of peptide hormones are reported to be located in the cell membranes(Roth, 1973). Previous studies of oxytocin binding to the receptors in uterus and mammary gland used 20,000 xg and 105,000 xg particulate fractions (Soloff and Swartz, 1973, 1974; Soloff *et al.*, 1973, 1974, 1977; Soloff 1975, 1976; Robert *et al.*, 1976). Soloff and Swartz(1973, 1974) reported that ^{3}H-oxytocin binding to the 105,000 xg supernatant(cytosol) of the mammary gland and uterus from rat and sheep were not reduced by the addition of the unlabelled oxytocin. Nissenson *et al.* (1978) reported that in the 95,000 xg pellet from rat uterus specific ^{3}H-oxytocin binding was 3~5 times greater than in the 10,000 xg pellet. They also found that the 95,000 xg pellet contained fragments of plamsa membrane with minimal mitochondrial contamination in electron microscopy. We, therefore, used the 20,000 xg and 105,000 xg particulate fractions for the study of oxytocin binding to membrane receptors.

After the 20,000 xg and 105,000 xg particulate fractions of rat uterus were prepared, the activity of Na-K-ATPase, a membrane marker enzyme, was measured in each fraction. As summerized in Table 1, Na-K-ATPase activity in the 105,000 xg particulate fraction was significantly greater($P<0.001$) than that in the 20,000 xg fraction in the uterus. Similarily, the binding of ^{125}I-oxytocin was significantly greater($P<0.001$) in the 105,000 xg fraction than in the 20,000 xg fraction of rat uterus. In both fractions, however, the bind-

Table 1. Na-K-ATPase activities in particulate membrane fractions of rat uterus

	Total ATPase	Mg ATPase	Na-K-ATPase
	(μM Pi/mg protein·hr)		
20,000 xg pellet	0.8223±0.0631	0.7991±0.0608	0.0232±0.0078
105,000 xg pellet	0.6381±0.0782*	0.5882±0.0828*	0.0499±0.0035*

Mean±S.E. of 9 experiments. *$P<0.001$

Table 2. Comparison of ^{125}I-oxytocin binding to membrane fractions of rat uterus, skeletal muscle and liver

		Absence of unlabelled oxytocin	Presence of unlabelled oxytocin(1μg/tube)
Uterus*	20,000 xg	1265±116	1114±74
	105,000 xg	1846±142***	1732±164***
Skeletal muscle**	20,000 xg	429	423
	105,000 xg	451	453
Liver**	20,000 xg	472	478
	105,000 xg	710	683

* Membrane particulates, 0.75 mg of protein, was incubated for 1 hour at 20°C in 0.3 ml of tris buffer(0.05 M tris, 5 mM $MgCl_2$, pH 7.4) containing 33,000 cpm of ^{125}I-oxytocin with or without 1μg of unlabelled oxytocin. Each value is the mean cpm(±S.E.) of 5 experiments.

** Each tube contained 1.38 mg of particulate protein in 0.3 ml of tris buffer with 28,000 cpm of ^{125}I-oxytocin in presence or absence of 1μg of unlabelled oxytocin. Each value is the mean cpm of 3 experiments.

*** Significantly greater than the value at 20,000 xg fraction.($P<0.001$ in unpaired t-test)

ing of ^{125}I-oxytocin was not apparently altered by the addition of unlabelled oxytocin (Table 2). Table 2 also shows the binding of ^{125}I-labelled oxytocin to the membrane fractions of liver and skeletal muscle. Again, the binding of ^{125}I-oxytocin was greater in 105,000 xg fraction than in the 20,000 xg fraction and it was not affected by the addition of unlabelled oxytocin.

It is, therefore, apparent that the binding of ^{125}I-oxytocin to these membrane fractions is most likely non-specific in nature. This will be discussed later.

Effects of incubation time and pH of the medium on the ^{125}I-oxytocin binding to membrane fractions

The binding of ^{125}I-oxytocin to the 20,000 xg and 105,000 xg fractions from rat uterus

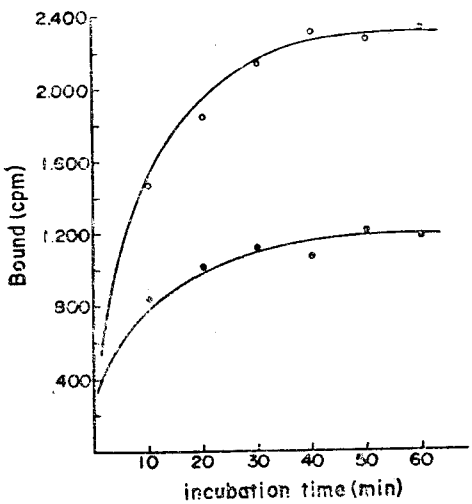

Fig. 3. Effect of incubation time on the binding of ^{125}I-oxytocin to membrane fractions of rat uterus.
Each tube contained 0.62 mg (20,000 xg fraction) or 0.94 mg (105,000 xg) of particulate protein in 0.3 ml of tris buffer (0.05M tris, 5 mM $MgCl_2$, pH 7.4) with 35,000 cpm of ^{125}I-oxytocin. Incubation was terminated by the addition of 1 ml of tris buffer solution and centrifugation. The resulting pellets were washed three times with tris buffer solution and radioactivities were counted. Each point represents the mean of 3 experiments. ●-●, 20,000 xg fraction; ○-○, 105,000 xg fraction.

was dependent upon the incubation time (Fig. 3). Steady state binding was achieved after 40 min. of incubation.

The binding of ^{125}I-oxytocin to sow uterine membrane particulate fractions increased linearly with the medium pH between 6.0~8.5 without showing any optimum pH (Fig 4).

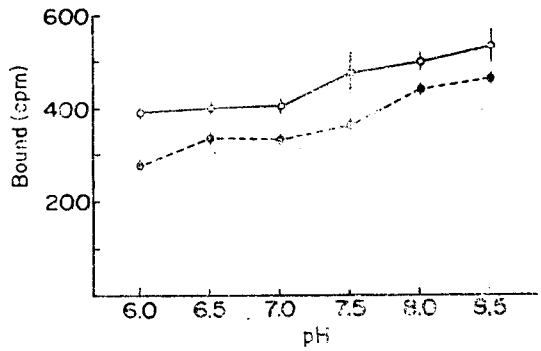

Fig. 4. Effect of medium pH on the binding of ^{125}I-oxytocin to the membrane fractions of sow uterus.
Each tube contained 0.72 mg (20,000 xg fraction) or 0.90 mg (105,000 xg) of paticulate protein in 0.3 ml of tris buffer (0.05 M tris, 5 mM $MgCl_2$, 0.1% gelatin) with 20,000 cpm of ^{125}I-oxytocin. Each point represents the mean±S.E. of 5 experiments. ●···●, 20,000 xg fraction; ○—○, 105,000 xg fraction.

This result is in contrast with that of Soloff and Swartz (1973, 1974) who found a definite pH optimum of 7.6 for the binding of ^{3}H-oxytocin to uterine membrane fractions.

Scatchard analysis of ^{125}I-oxytocin binding to the membrane fractions

Fig. 5 illustrates a typical result of ^{125}I-oxytocin binding to membrane fractions at various ^{125}I-oxytocin concentrations. These data were subjected to Scatchard analysis (Scatchard, 1949) to obtain various kinetic constants. Figs. 6 and 7 represent Scatchard plots of ^{125}I-oxytocin binding to the 20,000 xg and 105,000 xg particulate fractions from rat uterus. As is seen, the curves consist of two

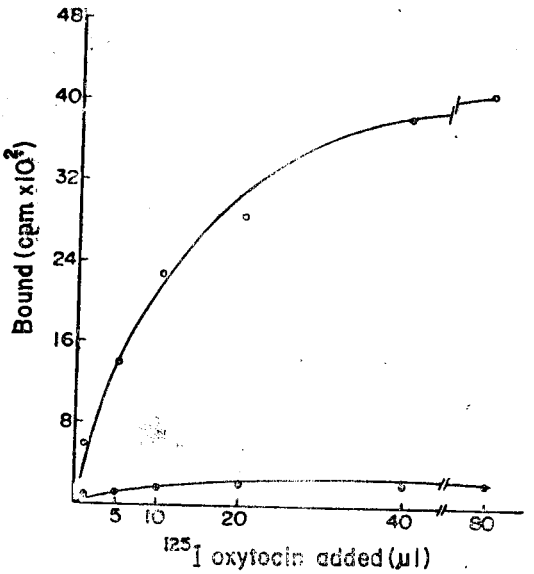

Fig. 5. ^{125}I-oxytocin binding to 105,000 xg fraction of rat uterus plotted as a function of ^{125}I-oxytocin concentrations.

Each tube contained 0.94 mg of intact or heat denatured proteins, tris buffer (0.05 M tris, 5 mM $MgCl_2$, pH 7.4) and ^{125}I-oxytocin. The total volume of final incubation mixture was adjusted to 0.4 ml. ○, intact membrane; ●, denatured membrane. Each point represents the mean of 3 experiments.

components indicating that the membrane fraction contains two different binding sites with high and low affinities. In the heat

Table 3. **Dissociation constant (Kd) of ^{125}I-oxytocin to high and low affinity binding sites in the intact membrane and to the denatured membrane fractions of rat uterus**

		High affinity site	Low affinity site
20,000 xg Fraction	Intact	4.8	11.9
	Denatured	3.0	
105,000 xg Fraction	Intact	5.8	13.3
	Denatured	6.7	

(nM)

Each value is estimated from the slopes of binding line shown in Figs. 6 and 7, and is the mean of 3 experiments.

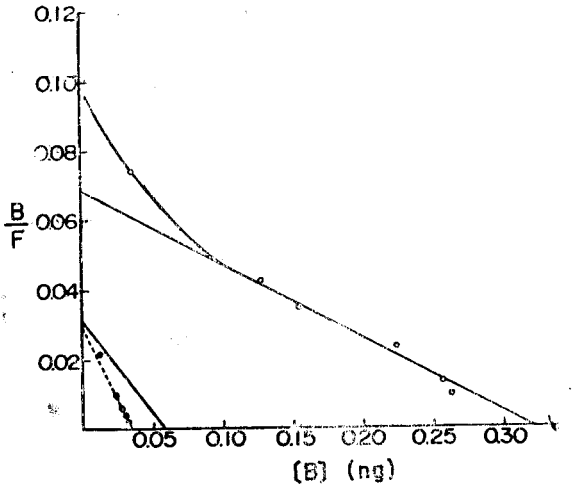

Fig. 6. Scatchard plot of ^{125}I-oxytocin binding to 20,000 xg fraction of rat uterus.

Each tube contained 0.62 mg of intact or heat denatured particulate protein, tris buffer (0.05 M tris, 5 mM $MgCl_2$, pH 7.4) and an appropriate ^{125}I-oxytocin. The total volume of final incubation mixture was adjusted to 0.4 ml. At the end of incubation, tubes were centrifuged and radioactivities were determined from pellet (B) and supernatant (F). The line of high affinity binding was obtained by taking the difference between the total binding curve and extrapolated line of low affinity binding. Each point represents the mean of 3 experiments. ○, intact membrane; ●, denatured membrane.

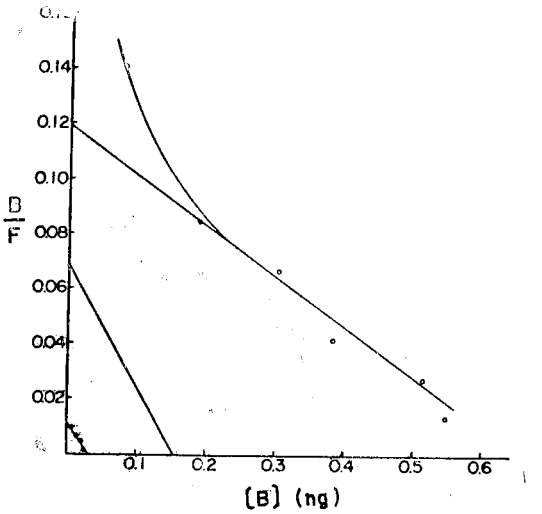

Fig. 7. Scatchard plot of ^{125}I-oxytocin binding to 105,000 xg fraction of rat uterus. Values are based on the data in Fig. 5.

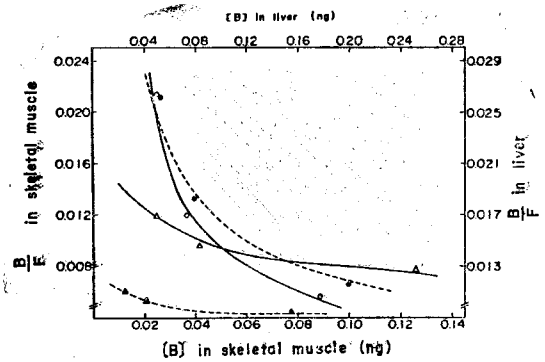

Fig. 8. Scatchard plots of ^{125}I-oxytocin binding to 20,000 xg fractions of rat skeletal muscle and liver.

The conditions of incubation other than protein concentration were the the same as in Figs. 5 and 6. The amounts of particulate protein in the tubes were 1.3 mg and 0.7 mg for liver and skeletal muscle, respectively. However, because of the small number of data points, the kinetic constant was not calculated. The binding curves show at least 2 kinds of binding sites. ○, intact membrane of liver; ●, denatured membrane of liver; △, intact membrane of skeletal muscle; ▲, denated membrane of skeletal muscle. Each point represents the mean of 2 experiments.

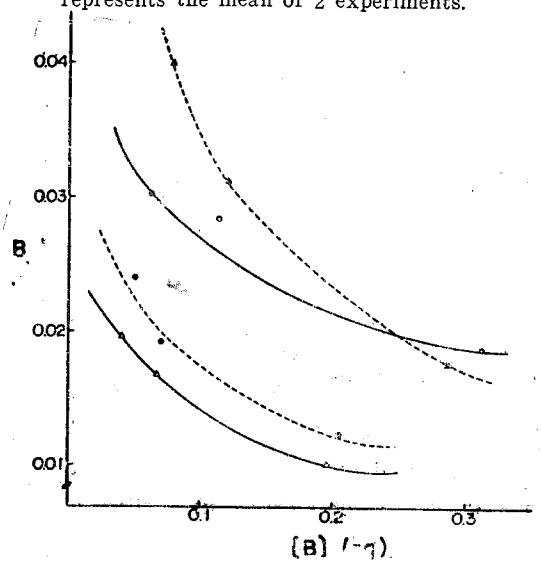

Fig. 9. Scatchard plots of ^{125}I-oxytocin binding to 105,000 xg fractions of rat skeletal muscle and liver.

The incubation conditions other than the protein content were the same as in Figs. 5 and 6. The protein content was 1.51 mg and 1.34 mg for liver and skeletal muscle, respectively. As shown in Fig. 8, the curves show that the binding site consists of at least two different types. Symbols are the same as in Fig. 8. Each point represents the mean of 2 experiments.

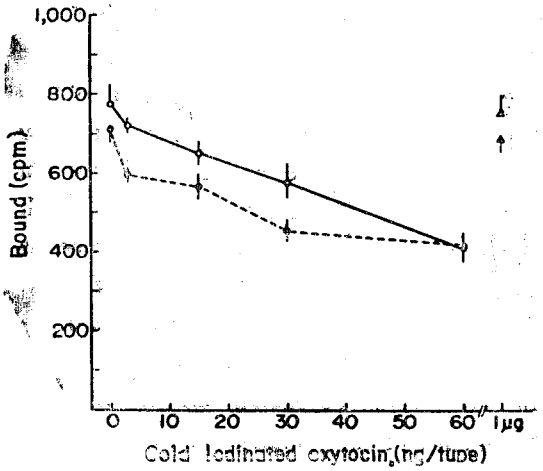

Fig. 10. Inhibition of ^{125}I-oxytocin binding to 20,000 xg and 105,000 xg fractions by cold iodinated oxytocin.

Each tube contained 0.42 mg (20,000 xg fraction) or 0.47 mg (105,000 xg fraction) of particulate protein, 32,000 cpm of ^{125}I-oxytocin, a varying amount of cold iodinated oxytocin and tris buffer (0.05 M tris, 5 mM $MgCl_2$, pH 7.4) solution. The total volume of final incubation mixture was adjusted to 0.4 ml. Solid symbols represent the binding to 20,000 xg fraction, and open symbols to the 105,000 xg fraction. Each point represents the mean±S.E. of 5 experiments. For the purpose of comparison, the binding of ^{125}I-oxytocin in the presence of unlabelled oxytocin was included (solid (20,000 xg) and open (105,000 xg) triangles).

denatured particulate fractions of rat uterus, the concentration of radioactivity was found to be markedly decreased (Fig. 5). A Scatchard plot of these data from the denatured particulate fractions revealed a single straight line (Figs. 6 and 7, solid circles), indicating a single population at the binding site. That is, one binding site was eliminated by heat treatment. At the same time, the binding capacity of the remaining component, estimated from the x-interecept of the line in the scatchard plot, was markedly reduced by heat treatment. Dissociation constant (Kd) of the high and low affinity sites in the intact

membrane and that of denatured membrane fractions are summarized in Table 3.

Figs. 8 and 9 are Scatchard plots of the ^{125}I-oxytocin binding to membrane fractions of rat skeletal muscle and liver. Again, the curves consist of at least two components. In contrast with the uterine membrane fractions, the heat treatment in these membrane fractions did not alter the shape of the curve, although it shifted the curve slightly upward or downward depending on the fractions. This shift of the binding curve presumably resulted from conformational changes of binding sites. Therefore, it is speculated that the binding of ^{125}I-oxytocin to the membrane fractions of skeletal muscle and liver may be nonspecific in nature. Whereas, the binding site in the uterine membrane eliminated by heat treatment is probably specific for oxytocin. However, whether the specific site is the one with high or low affinity is not clear, at present.

Effect of cold iodinated oxytocin on binding of ^{125}I-oxytocin to uterine membrane fractions

Because unlabelled oxytocin did not inhibit the binding of ^{125}I-oxytocin to the membrane fractions of rat uterus, it would be interesting to examine whether cold iodinated oxytocin inhibits the binding of ^{125}I-oxytocin to the cell membrane of rat uterus. Cold iodinated oxytocin was prepared in the same way as described for ^{125}I-oxytocin using NaI instead of the ^{125}I. A small amount of ^{125}I-oxytocin was used as a marker for column chromatography. As illustrated in Fig. 10, the binding of ^{125}I-oxytocin to the 20,000 xg and 105,000 xg particulate fractions from rat uterus were decreased in proportion to the amount of cold iodinated oxytocin added to the medium.

DISCUSSION

For a radioreceptorassay of a hormone, the radioactive hormone must have high specific activity and at the same time retain biologic activity. If a radioiodinated hormone exhibits biologic activity, such a compound might be suitable as a ligand for the hormone-receptor assay. Iodinated hormones, such as ACTH (Lefkowitz *et al.*, 1970), angiotensin (Lin *et al.*, 1970), luteinizing hormone (Catt *et al.*, 1972), human chorionic gonadotropin (Dufau *et al.*, 1972) and insulin (Freychet *et al.*, 1971; Cuatrecasas, 1971) show substantial biological activity. However, the biological actions of iodinated oxytocin are not clearly established. While Morgat *et al.* (quoted from Flouret *et al.*, 1977) reported that the monoiodo-oxytocin is inactive in the avian depressor assay, Marbach and Rudinger (1974) observed that, although the monoiodo-oxytocin has no uterotonic activity, it inhibits the response of the isolated rat uterus to oxytocin and suggested that radioiodinated oxytocin is suitable for the study of uterine receptor binding. Thompson *et al.*, (1972) reported that monoiodo-oxytocin retained stimulatory activity in a toad bladder cyclase assay and glucose oxidation in fat cells. In the above study, ^{125}I-oxytocin showed a specific binding to fat cells. Morover, in the presence of unlabelled oxytocin, lysine and arginine-vasopressin, ^{125}I-oxytocin was displaced from fat cells in proportion to the concentration of unlabelled hormone. But they failed in numerous experiments to detect specific binding of ^{125}I-oxytocin to many other tissues, such as uterus, kidney medulla and toad bladder. They therefore speculated that such a failure is probably due to a high level of hormone inactivating and

degrading activities residing in these tissuses. Recently, Flouret *et al.* (1977) reported that freshly prepared monoiodo-oxytocin had no specific agonistic effect for the activity of renal medullary cyclase system and for uterine contraction. However, the monoiodo-oxytocin stored for several months in cold (either as a solid form in a desicator or as a forzen solution) stimulated the contractility of rat uterus and the activity of the renal medullary cyclase. They, however, stated that the monoiodo-oxytocin is not suitable for the receptor assay as it has very low affinity (100 times lower than that of ^{3}H-oxytocin) to uterine and renal tissues.

There are two binding sites (high and low affinity) of ^{125}I-oxytocin in the rat uterine membrane fraction and one binding site was eliminated after heat denaturation in this membrane fraction (Figs. 6 and 7). But none of binding sites of ^{125}I-oxytocin in membrane fractions of skeletal muscle and liver was eliminated by heat (Figs. 8 and 9). The apparent Kd(s) of high affinity binding site of rat uterine membrane fractions (4.8 nM for 20,000 xg and 5.8 nM for 105,000 xg fraction) are comparable to those measured with ^{3}H-oxytocin. Soloff and Swartz (1974) reported that the Kd for ^{3}H-oxytocin binding was 1.8nM in 20,000 xg particulate fraction from rat uterus, 1.5 nM in 20,000 xg particulate fraction from sow uterus and 2.0~2.8 nM in human myometrium (Soloff *et al.*, 1974). Nissenson *et al.* (1978) reported that the Kd for ^{3}H-oxytocin binding was 4.1 nM in rabbit uterine membrane fraction. However Kds of low affinity binding sites (11.9 nM for 20,000 xg and 13.3 nM for 105,000 xg fraction; see Table 3) are much higher than those of reported. The differences of Kd between our study and those of others may be due to the differences in animal species, to pretreatment of the animals with various agents or to the species of ligands used in assay.

From the reasons discussed above (i.e., Kds and heat treatment), it is, therefore, speculated that the binding of ^{125}I-oxytocin to the high affinity binding site in uterine membrane is, at least in part, specific. However, the number of specific binding sites is relatively much smaller than that of non-specific binding sites (compare the x-intercept of the 2 different solid lines in Figs. 6 and 7), such that the magnitude of overall binding in *in vitro* analysis may be generally determined by the non-specific binding.

In the present study, we have demonstrated that ^{125}I-oxytocin had definite biological activity in the uterotonic assay. However, the binding of ^{125}I-oxytocin to rat uterine tissue slice and membrane fraction was not inhibited by the addition of unlabelled oxytocin (see Fig. 2 and Table 2) indicating that the nature of binding is probably changed.

There are possibilities that the binding site of ^{125}I-oxytocin is completely different from that of unlabelled oxytocin or that conformational changes in receptor sites after binding of ^{125}I-oxytocin might occur. Yet another possibility is conformational change in the oxytocin molecule itself in the process of radioiodination, since other investigators (Flouret *et al.*, 1977) have found that after storage of iodinated oxytocin, specific competition between labelled and unlabelled oxytocin was resumed.

An interesting point to note is that the binding of ^{125}I-oxytocin to the uterine membrane fraction is inhibited by cold iodinated oxytocin, but not by unlabelled oxytocin (Fig. 10). This result strongly suggests that radioiodination of oxytocin may alter its binding char-

acteristics.

It is, therefore, apparent that ^{125}I-oxytocin is not a suitable radioligand for oxytocin-receptor binding study.

REFRNCEES

Bashore RA: *Studies concerning a radioimmunoassay for oxytocin. Am J Obstet Gynecol 113: 488-496, 1972*

Bisset GW, Walker JM: *Assay of oxytocin in blood. J Physiol 126:588-595, 1954*

Bockaert J, Imbert M, Jard S, et al: *^{3}H-oxytocin binding sites in the isolated frog skin epithelium.: Relation to the physiological response. Molec Pharmacol 8:230-240, 1972*

Bockaert J, Jard S, Morel F, et al: *Uptake of oxytocin-^{3}H by frog skin and bladder epithelial cells. Am J Physiol 219:1514-1521, 1970*

Branda LA, Ferrier BM: *Degradation of oxytocin by human placental tissue. Am J Obstet Gynecol 109:943-947, 1971*

Catt KJ, Dufau ML, Tsuruhara T: *Radioligand-receptor assay of luteinizing hormone and chorionic gonadotropin. J Clin Endocrinol Metab 34:123-132, 1972*

Chard T: *Radioimmunoassay and radioreceptor assay. In; Endocrinology of pregnancy. 2 nd ed, Edited by Fuchs F and Klopper A., Hagerstown, Harper and Row Inc. 1977. pp. 41-62*

Crankshaw DJ, Branda LA, Matlib MA, et al: *Localization of the oxytocin receptor in the plasma membrane of rat myometrium. Eur J Biochem 86:481-486, 1978*

Cuatrecasas P: *Insulin-receptor interactions in adipose tissue cells.: Direct measurement and properties. Proc Natl Acad Sci USA 68:1264-1268, 1971*

Dawood MY, Facog M, Raghavan KS, et al: *Oxytocin in human pregnancy and parturition. Obstet Gynecol 51:138-143, 1978a*

Dawood MY, Raghavan KS, Pociask C: *Radioimmunoassay of oxytocin. J Endocrinol 76:261-270, 1978b*

Dufau ML, Catt KJ, Tsuruhara T: *Biological activity of human chorionic gonadotropin released from testis binding sites. Proc Natl Acad Sci USA 69:2414-2416, 1972*

Emmelot P, Bos CJ, Bendetti EL, et al: *Studies on plasma membranes. I. Chemical composition and enzyme content of plasma membranes isolated from rat liver. Biochim Biophys Acta 90: 126-145, 1964*

Flouret G, Terada S, Yang F, et al: *Iodinated neurohypophyseal hormones as potential ligands for receptor binding and intermediates in synthesis of tritiated hormones. Biochemistry 16: 2119-2124, 1977*

Freychet P, Roth J, Neville DM Jr: *Monoiodoinsulin.: Demonstration of its biological activity and binding to fat cells and liver membrane. Biochem Biophys Res Commun 43:400-408, 1971*

Korenman SG: *Radio-ligand binding assay of specific estrogens using a soluble uterine macromolecule. J Clin Endocrinol Metab 28:127-130, 1968*

Kumaresan P, Anandarangam PB, Dianzon W, et al: *Plasma oxytocin levels during human pregnancy and labor as determind by radioimmunoassay. Am J Obstet Gynecol 119:215-233, 1974*

Lefkowitz RJ, Roth J, Pricer W, et al: *ACTH receptors in the adrenal.: Specific binding of ACTH-^{125}I and its relation to adenyl cyclase. Proc Natl Acad Sci USA 65:745-752, 1970*

Lin SY, Ellis H, Weisblum B, et al: *Preparation and properties of iodinated angiotensins. Biochem Pharmacol 19:651-662, 1970*

Lowry OH, Rosebrough NJ, Farr AL, et al: *Protein measurement with the Folin phenol ragent. J Biol Chem 193:265-275, 1951*

Marbach P, Rudinger J: *(2-Iodotyrosine) oxytocin inhibits the response of the rat uterus to oxytocin. Experientia 30:696, 1974*

Miyachi Y, Vaitukaitis JL, Nieschlag E, et al: *Enzymatic radioiodination of gonadotropin.*

J Clin Endocrinol Metab 34:23-28, 1972

Nissenson R, Flouret G, Hechter O: *Opposing effects of estradiol and progesterone on oxytocin receptors in rabbit uterus. Proc Natl Acad Sci USA 75:2044-2048, 1978*

Noddle BA: *Transfer of oxytocin from maternal to fetal circulation in the ewe. Nature 203:414, 1964*

Robert JS, McCracken JA, Gavagan JE, et al: *Oxytocin-stimulated release of prostaglandin F2α from ovine endometrium in vivo.: Correlation with estrous cycle and oxytocin receptor binding. Endocrinology 99:1107-1114, 1976*

Roth J: *Peptide hormone binding to receptors: A review of direct studies in vitro. Metab Clin Exp 22:1059-1073, 1973*

Saxena BB, Demura H, Gandy HM, et al: *Radioimmunoassay of human follicle stimulating and luteinizing hormones in plasma. J Clin Endocrinol Metab 28:519-534, 1968*

Scatchard G: *The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51:660-672, 1949*

Soloff MS: *Uterine receptor for oxytocin.: Effects of estrogen. Biochem Biophys Res Comm 66:205-212, 1975*

Soloff MS: *Uterine receptors for oxytocin.: Correlation between antagonist potency and receptor binding. Br J Pharmacol 57:381-386, 1976*

Soloff MS, Schroeder BT, Chakraborty J, et al: *Characterization of oxytocin receptors in the uterus and mammary gland. Federation Proc 36:1861-1866, 1977*

Soloff MS, Swartz TL: *Characterization of a proposed oxytocin receptor in rat mammary gland. J Biol Chem 248:6471-6478, 1973*

Soloff MS, Swartz TL: *Charactization of a proposed oxytocin receptor in the uterus of the rat and sow. J Biol Chem 249:1376-1381, 1974*

Soloff MS, Swartz TL, Morrison M, et al: *Oxytocin receptors.: Oxytocin analogs, but not prostaglandins, compete with ^{3}H-oxytocin for uptake by rat uterus. Endocrinolgy 92:104-107, 1973*

Soloff MS, Swartz TL, Steinberg AH: *Oxytocin receptors in human uterus. J Clin Endocrinol Metab 38:1052-1056, 1974*

Thompson EE, Freychet P, Roth J: *Monoiodo-oxytocin.: Demonstration of its biological activity and specific binding to isolated fat cells. Endocrinology 91:1199-1205, 1972*

Towle DW, Copenhaver JH Jr: *Partial purification of a soluble Na-K-dependent ATPase from rabbit kidney. Biochim Biophys Acta 203:124-132, 1970*

Vasicka A, Kumaresan P, Han GS, et al: *Plasma oxytocin in initiation of labor. Am J Obstet Gynecol 130:263-273, 1978*