OXYTOCIN RECEPTORS IN RAT OVIDUCT*

Melvyn S. Soloff

Department of Biochemistry
Medical College of Ohio
Toledo, Ohio 43614

Received July 21,1975

Summary: Particles from rat oviduct homogenates sedimenting between 1,000 x g for 10 min and 48,000 x g for 30 min bound [^{3}H]oxytocin *in vitro*. The apparent K_d for oxytocin binding to high affinity sites in particles prepared from estrogen-treated rats was 1.8 x 10^{-9} M. About 215 fmoles of oxytocin were bound per mg of particulate protein. Oviducal preparations from untreated rats had about 25% the affinity for oxytocin of preparations from estrogen-treated rats. Oxytocin analogues were bound to oviducal particles in the same rank order as their uterotonic potencies: (desamino)oxytocin > (4-threonine)oxytocin > oxytocin > (8-lysine)vasopressin >> desaminotocinol. No oxytocin binding could be shown with the particulate fractions from rat ovary. The binding of oxytocin to the oviduct and uterus are similar in affinity, number of binding sites, ligand specificity, and the increase in response to estrogen treatment.

Contractions of the oviduct induced by ovarian steroids and prostaglandins are thought to play a role in the movement of ova through the oviduct (1-14). Oxytocin, which also stimulates oviducal contractions (15), is localized in smooth muscle cells of the rat oviduct, as determined by autoradiography with [^{3}H]oxytocin (16). The specific localization of radioactivity, however, is nondetectable when the oviduct is incubated with [^{3}H]oxytocin of low specific activity (16). These results indicate that the rat oviduct possesses high affinity sites for oxytocin. The present studies characterize oxytocin binding in the rat oviduct under experimental conditions giving optimal binding in particulate fractions from the rat mammary gland (17), rat uterus (18), and sow myometrium (18).

MATERIALS AND METHODS

Peptides. (Tyrosyl-^{3}H)oxytocin, 31 Ci per mmole (19) was synthesized by Schwarz-Mann, and was reported to have a radiochemical purity of greater than 99% upon high voltage electrophoresis. The [^{3}H]oxytocin had a potency

*This research was supported by Grant HD 8406 from the National Institutes of Health.

of 452 International Units per mg in the isolated rat uterus assay (20). Synthetic oxytocin, (8-lysine)vasopressin, (desamino)oxytocin (Sandopart) and desaminotocinol were gifts from Sandoz, Ltd., Basel. (4-Threonine)oxytocin was a gift from Dr. Maurice Manning of this department.

Preparation of oviducal particulate fractions. Oviducts were taken from Sprague-Dawley rats (Spartan Research Laboratories, Haslett, MI.), 175-200 g. Further preparative procedures were performed at 4°. The oviducts from 24 rats were homogenized in 9 volumes of Tyrode's solution, pH 7.6, with a conical all-glass homogenizer. The homogenate was blended with a Polytron PT 10 ST (Brinkmann Instruments) for 15 sec at the maximum setting. In some experiments the particulate fractions sedimenting at 1,000 x g for 10 min, 20,000 x g for 10 min, and 165,000 x g for 30 min were prepared. In other experiments the homogenate was centrifuged at 1,000 x g for 10 min, the pellet was discarded and the resulting supernatant was centrifuged at 165,000 x g for 30 min. The resulting pellets were resuspended in Tyrode's solution and stored at -70°.

Binding assay. The frozen samples were thawed rapidly and centrifuged at the appropriate $\omega^2 t$ for the separate particulate fractions, or at 48,000 x g for 30 min for the 1,000 to 165,000 x g particles. The pellets were resuspended in 0.05 M Tris-maleate buffer, pH 7.6, containing 5 mM $MgCl_2$ and 0.1% gelatin (hereafter referred to as Tris buffer), recentrifuged and homogenized in Tris buffer to give 3 to 7 mg of protein per ml. Protein concentrations were determined by the method of Lowry et al. (21), with bovine serum albumin as the standard.

Each assay tube contained 0.6 to 1.4 mg of particulate protein, about 9,000 dpm of [^{3}H]oxytocin (ca. 150 pg) and the appropriate amount of non-radioactive oxytocin or oxytocin analogue in a total volume of 250 µl of Tris buffer. Incubation was carried out for 1 hr at 22°, and was terminated by centrifugation of the tubes under the same conditions used to prepare the particles. Determination of the bound and unbound radioactivity was carried out as described previously (17). The data were analyzed as described by Scatchard (22). Linear regressions were determined by the method of least squares.

RESULTS

[^{3}H]Oxytocin binding was studied with the 1,000, 20,000 and 165,000 x g particulate fractions from rat oviducal homogenates. The apparent K_d values, as determined by Scatchard analyses, were 1.7×10^{-8} M, 7.7×10^{-9} M and 4.8×10^{-9} M for the 1,000, 20,000 and 165,000 x g fractions, respectively.

Particles sedimenting between 1,000 and 48,000 x g were used in subsequent studies to obtain the maximum amount of binding material which could be assayed without need of an ultracentrifuge, and to remove the bulk of lower affinity sites. Scatchard analyses indicated either two classes of binding sites or negative cooperativity (23) within the range of oxytocin concentrations examined, 0.53 to 3.2 nM. The apparent K_d for oxytocin binding

to the high affinity site was 1.8 x 10^{-9} M (Fig. 1). The concentration of binding sites as estimated from the x-intercept of the Scatchard plot was 5.5 x 10^{-10} M, corresponding to 215 fmoles of oxytocin bound per mg of particulate protein (Fig. 1). Ovarian particles did not bind oxytocin (Fig. 1). Binding by the low affinity oviducal sites appeared to asymptotically approach binding by the ovarian sites. The apparent K_d of oxytocin binding to the high affinity sites, if estimated by subtracting binding by the low affinity sites from the experimental curve in Fig. 1 (24), was about 5.4 x 10^{-10} M; about 62.5 fmoles of oxytocin were bound per mg of particulate protein. These studies were performed with oviducts taken from intact rats injected s.c. with 5 µg of diethyl stilbestrol (DES) in 0.2 ml of cotton-seed oil for 2 days. When oviducts were taken from groups of 24 untreated, randomly cycling rats the apparent K_d for the

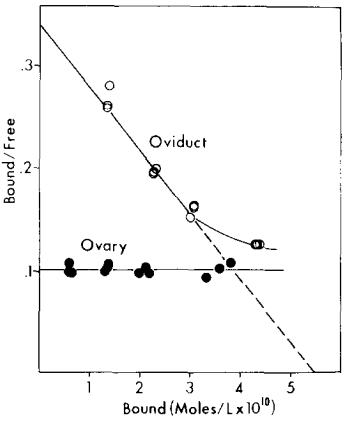

Fig. 1. Comparison of the binding of [^{3}H]oxytocin by oviducal and ovarian 1,000 to 48,000 x g particles. The oviducts and ovaries were taken from rats treated with estrogen for 2 days. Each tube contained either 0.64 mg of oviducal protein or 1.4 mg of ovarian protein, 9,000 dpm of [^{3}H]oxytocin (ca. 150 pg) and 0, 200, 400 or 800 pg of nonradioactive oxytocin in a total volume of 250 µl Tris buffer. Incubation was terminated after 1 hr at 22° by centrifugation of the tubes. The radioactivity was determined in the resulting pellet (bound) and supernatant (free). The slope for the higher affinity sites was estimated as shown by the broken line.

high affinity[1] oxytocin binding sites was 7.5 ± 1.9 (S.E.) x 10^{-9} M (n=3).

The binding of oxytocin to oviducal particles was specific for the ligand. The amount of [^{3}H]oxytocin bound was reduced in proportion to the log concentration of increasing amounts of nonradioactive (desamino)oxytocin, (4-threonine)-oxytocin, oxytocin and (8-lysine)vasopressin (Fig. 2). The regressions were parallel indicating a common set of binding sites for these peptides. The relative binding potencies and 95% confidence limits of these analogues are shown in Table I. Desaminotocinol, the alcohol of the (desamino)oxytocin ring, did not compete for [^{3}H]oxytocin binding sites in the dose range studied (Fig. 2)

Less than 20% of the oxytocin-binding activity of the oviducal 1,000 to 48,000 x g particles was lost during the 1 hr incubation period. The reduction in binding activity was assessed by incubating particles with [^{3}H]oxytocin which had been exposed for 1 hr to another sample of oviducal particles (17,18). There were no differences seen between the chromatographic distributions (17,18)

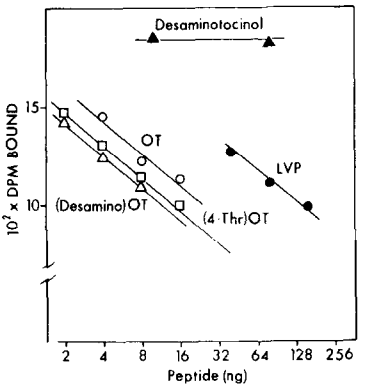

Fig. 2. The specificity of oxytocin binding to oviducal particles. The 1,000 to 48,000 x g particulate fraction from nonestrogen treated rat oviducts was assayed as described in the legend to Fig. 1. Each point is the mean of triplicates. Desaminotocinol (▲—▲); (desamino)-oxytocin (Δ—Δ); (8-lysine)vasopressin (●—●); oxytocin (o—o); (4-threonine)-oxytocin (□—□).

[1]The apparent K_d values for high affinity sites were determined from the linear portion of Scatchard plots and not by subtracting low affinity sites.

Table 1. Summary of the relationships between the relative binding potencies in rat oviduct and the uterotonic potencies of oxytocin analogues

Peptide	Relative Potency		
	Binding	Uterotonic	(ref.)
Oxytocin	1	1	
Desaminoxytocin	2.1 (1.6 → 2.7)[a]	1.8	(33)
(4-Thr)Oxytocin	1.7 (1.3 → 2.2)	2.0	(34)
(8-Lys)Vasopressin	0.19 (.14 → .28)	0.01	(35)
Desaminotocinol	n.d.[b]	~0[c]	

[a]95% Confidence Limits
[b]Not detectable under the experimental conditions (<0.04)
[c]Guttmann, S., Personal Communication

of radioactivity in the [^{3}H]oxytocin standard, in the medium after 1 hr of incubation, and extracted from oviducal particles with 5 mM EDTA in 50 mM Tris-maleate, pH 7.6. The R_f of the radioactive peak was identical to that of authentic oxytocin.

DISCUSSION

These experiments demonstrate that oxytocin is specifically bound to oviducal particles with high affinity. The apparent K_d of the oviduct from estrogen treated rats, 1.8×10^{-9} M, was the same as that determined with rat uteri (18)[1]. This value also is comparable to that found in the myometrium of the pregnant sow, 1.5×10^{-9} M (18) and human, ca. 2×10^{-9} M (25)[1]. The number of binding sites per mg of particulate protein from the oviduct, 215

fmoles, also was comparable to the number reported in rat, sow and human uteri. The broad distribution of binding activity among the three particulate fractions of the oviduct also has been observed with oxytocin receptors in the mammary gland (17) and uterus (18).

Treatment of rats with 5 μg of DES for 2 days increased the affinity of oviducal binding sites for oxytocin by 4 fold. A similar increase has been noted with uterine particles 24 hr after a single injection of DES into ovariectomized rats (26). This increased binding of oxytocin may explain why the uterine sensitivity to oxytocin is greater in proestrus and estrus, when endogenous estrogen levels are elevated (27-30), and after estrogen treatment (31,32).

The affinity of the oviducal particles for the oxytocin analogues corresponded to the uterotonic potencies of all the analogues except (8-lysine)vasopressin (Table I). (8-Lysine)vasopressin, which has only 1% the activity of oxytocin in eliciting uterine contractions, was bound with 19% the affinity of oxytocin (Table I). Rat uterine particles also bind (8-lysine)vasopressin with 19% the affinity of oxytocin (25). The reasons for the differences between the binding and biological potencies of (8-lysine)-vasopressin are not clear.

Oxytocin has been reported to increase the contractile activity of isolated ovaries from rats and guinea pigs in natural estrus (36). The present studies, however, have not demonstrated oxytocin receptors in ovarian particles under conditions where binding was found with oviducal particles. The difference in oxytocin binding activity between the ovary and oviduct illustrates the tissue specificity of oxytocin binding sites. The oviducal sites for oxytocin were similar to uterine sites with respect to their apparent K_d, number per mg of protein, ligand specificity, and increased affinity due to estrogen treatment.

ACKNOWLEDGEMENTS

I thank Ted Swartz for technical assistance, Dr. Maurice Manning of this department for the (4-threonine)oxytocin, Sandoz, Ltd., Basel, for the

oxytocin, (desamino)oxytocin, desaminotocinol and (8-lysine)vasopressin, Dr. Lee Faber for reviewing the manuscript, Ramesh Parekh for the statistical analysis and Cindy Stanley for the typescript.

REFERENCES

1. Seckinger, D.L. and Synder, F.F. (1926) Bull. Johns Hopkins Hosp. 39, 371-378.
2. Greenwald, G.S. (1961) Fertil. Steril. 12, 80-95.
3. Greenwald, G.S. (1963) Fertil. Steril. 14, 666-674.
4. Harper, M.J.K. (1964) J. Endocrinol. 30, 1-19.
5. Harper, M.J.K. (1965) Endocrinology 77, 114-123.
6. Harper, M.J.K. (1965) J. Endocrinol. 31, 217-226.
7. Harper, M.J.K. (1966) Endocrinology 78, 568-574.
8. Chang, M.C. and Harper, M.J.K. (1966) Endocrinology 78, 860-872.
9. Boling, J.L. and Blandau, R.J. (1971) Biol. Reprod. 4, 174-184.
10. Spilman, C.H. (1974) Prostaglandins 7, 465-472.
11. Spilman, C.H. (1974) Fertil. Steril. 25, 935-939.
12. Maia, H. and Coutinho, E.M. (1968) Amer. J. Obstet. Gynecol. 102, 1043-1047.
13. Salomy, M. and Harper, M.J.K. (1971) Biol. Reprod. 4, 185-194.
14. Sica-Blanco, Y., Cibils, L.A., Remedio, M.R., Rozada, H. and Gil, B.E. (1971) Am. J. Obstet. Gynecol. 111, 91-97.
15. Rorie, D.K. and Newton, M. (1965) Fertil. Steril. 16, 27-32.
16. Soloff, M.S., Rees, H.D., Sar, M. and Stumpf, W.E. (1975) Endocrinology 96, 1475-1477.
17. Soloff, M.S. and Swartz, T.L. (1973) J. Biol. Chem. 248, 6471-6478.
18. Soloff, M.S. and Swartz, T.L. (1974) J. Biol. Chem. 249, 1376-1381
19. Morgat, J.L., Hung, L.T., Cardinaud, R., Fromageot, P., Bockaert, J., Imbert, M. and Morel, F. (1970) J. Labeled Compd. 6, 276-284.
20. Munsick, R.A. (1960) Endocrinology 66, 451-457.
21. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.
22. Scatchard, G. (1949) Annu. N.Y. Acad. Sci. 51, 660-672.
23. DeMeyts, P., Roth, J., Neville, D.M., Jr., Gavin, J.R., III, and Lesniak, M.A. (1973) Biochem. Biophys. Res. Commun. 55, 154-161.
24. Rosenthal, H.E. (1967) Analyt. Biochem. 20, 525-532.
25. Soloff, M.S., Swartz, T.L. and Steinberg, A.H. (1974) J. Clin. Endocrinol. Metab. 38, 1052-1056.
26. Soloff, M.S. (1975) Biochem. Biophys. Res. Commun. 65, 205-212.
27. Flatters, M. (1954) Arch. Exp. Pathol. Pharmakol. 221, 171-176.
28. Schneider, W. and Stumpf, C.L. (1953) Arch. Int. Pharmacodyn. Ther. 94, 406-415.
29. Guerne, J.M. and Stutinsky, F. (1961) J. Physiol. Paris 53, 357-358.
30. Chan, W.Y., O'Connell, M. and Pomeroy, S.R. (1963) Endocrinology 72, 279-282.
31. Follett, B.K. and Bentley, P.J. (1964) J. Endocrinol. 29, 277-282.
32. Fitzpatrick, R.J. and Bentley, P.J. (1968) in Neurohypophysial Hormones and Similar Polypeptides, Handbook of Experimental Pharmacology (Berde, B., ed) Vol. 23, pp. 190-285, Springer-Verlag, New York.
33. Chan, W.Y. and du Vigneaud, V. (1962) Endocrinology 71, 977-982.
34. Manning, M., Coy, E. and Sawyer, W.H. (1969) Biochemistry 9, 3925-3930.
35. Berde, B. and Boisonnas, R.A. (1968) in Neurohypophysial Hormones and Similar Polypeptides, Handbook of Experimental Pharmacology (Berde, B., ed) Vol. 23, pp. 802-870, Springer-Verlag, New York.
36. Gimeno, M.F., Borda, L.S., Rettori, V., Borda, E. and Gimeno, A.L. (1973) Acta Physiol. Lat. Am. 23, 363-370.