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UTERINE RECEPTORS FOR OXYTOCIN: CORRELATION BETWEEN ANTAGONIST POTENCY AND RECEPTOR BINDING

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1 The apparent dissociation constants (K_d) of four competitive antagonists of oxytocin were estimated from their ability to compete with [^{3}H]-oxytocin for binding sites in particulate fractions from rat uterine homogenates.

2 These apparent K_d values were not significantly different from the K_d values calculated from the published potency of each compound as an antagonist of oxytocin-induced uterine contractions.

3 These results support the conclusion that the binding sites for oxytocin are part of the receptor complex. Furthermore, 'spare receptors' for oxytocin do not appear to be present in significant quantities, and the relative potency of each antagonist appears to depend upon its affinity for the receptor site rather than its intrinsic activity.

4 The antagonists used in these studies were [*N*-acetyl, 2-*O*-methyltyrosine]oxytocin, [1-(β-mercapto-β,β-diethylpropionic acid)]oxytocin, [1-(β-mercapto-β,β-pentamethylenepropionic acid)]-oxytocin, and [1-(deaminopenicillamine), 4-threonine]oxytocin.

Introduction

Oxytocin-stimulated uterine contractions can be inhibited competitively by several synthetic analogues of oxytocin. These antagonists are either partial agonists, eliciting uterine contractions below the maximum response produced by oxytocin, or devoid of oxytocic activity. It seems apparent that the antagonists compete with oxytocin for uterine receptor sites. Antagonist activity has been explained by a low intrinsic activity (Ariëns, van Rossum & Simonis, 1956) or efficacy (Stephenson, 1956), or by a slow rate of dissociation of the partial agonist from the receptor site (Paton, 1961).

In view of the demonstration of specific binding sites for [^{3}H]-oxytocin in the uterus of several species (Soloff, Swartz, Morrison & Saffran, 1973; Soloff & Swartz, 1974; Soloff, Swartz & Steinberg, 1974; Soloff, 1975a) it is possible to estimate the binding affinities of the oxytocin antagonists directly and to correlate the values with antagonist activity. Four of the most potent oxytocin antagonists have been examined in the present studies.

Methods

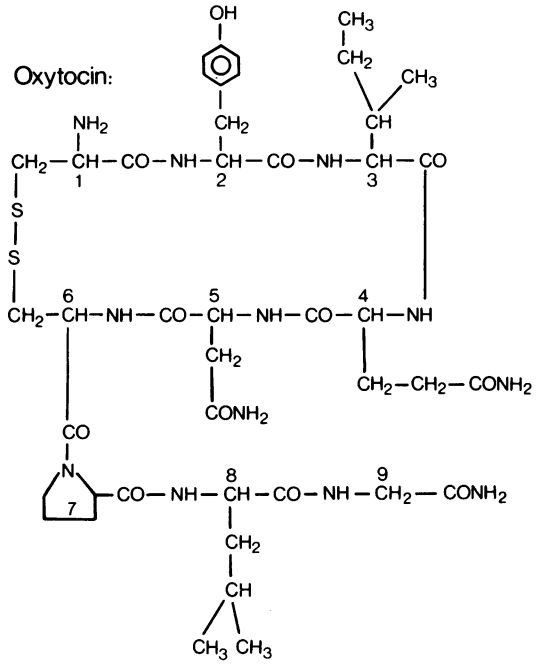
Binding assay

Uteri were removed from rats (CFE, Carworth, 175–200 g) which were injected subcutaneously with 5 μg of diethylstilbestrol dipropionate in 0.2 ml cotton-seed oil on each of the two days before they were killed. The particulate fraction sedimenting between 1,000 *g* for 10 min and 165,000 *g* for 30 min was prepared and assayed for [^{3}H]-oxytocin binding activity (Soloff, 1975a).

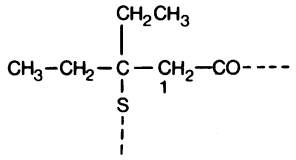
Peptides

[Tyrosyl-^{3}H]-oxytocin, 31 Ci per mmol (Morgat, Hung, Cardinaud, Fromageot, Bockaert, Imbert & Morel, 1970) was synthesized by Schwarz-Mann and was reported to have full biological activity (452 iu per mg) in the rat isolated uterus assay (Stürmer, 1968; Fitzpatrick & Bently, 1968). More than 90% of the radioactivity migrated with authentic oxytocin upon thin layer chromatography (Soloff & Swartz, 1973).

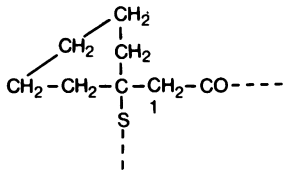
Synthetic oxytocin (Syntocinon) was a gift from Sandoz, Ltd., Basel. [*N*-acetyl, 2-*O*-methyltyrosine]oxytocin was a gift from Drs K. Jošt and J.H. Cort, Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Science, Prague. [1-(β-Mercapto-β,β-diethylpropionic acid)]-oxytocin and [1-(β-mercapto-β,β-pentamethylene-propionic acid)]oxytocin were gifts from Dr V. du Vigneaud, Department of Chemistry, Cornell University. [1-(Deaminopenicillamine), 4-threonine]oxytocin was a gift from Dr M. Manning of this Department. [3-Proline]oxytocin was a gift from



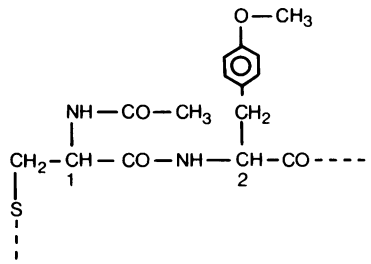
[1-(ß-Mercapto-ß,ß-diethylpropionic acid)] oxytocin:



[1-(ß-Mercapto-ß,ß-pentamethylenepropionic acid)] oxytocin:



[*N*-acetyl, 2-*O*-methyltyrosine] oxytocin:



[1-(Deaminopenicillamine),4-threonine]oxytocin:

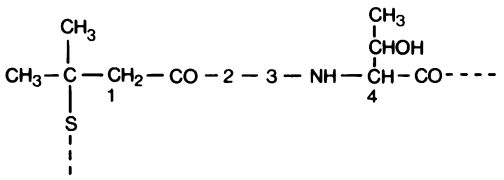


Figure 1 Structure of oxytocin and antagonists, with numbers indicating the position of the individual amino acid residues.

Dr R. Walter, Department of Physiology, University of Illinois Medical Center. Kallidin (lysyl-bradykinin) was purchased from Schwarz-Mann.

The structure of oxytocin and the antagonists [*N*-acetyl-2-*O*-methyl-tyrosine]oxytocin, [1-(*β*-mercapto-*β*,*β*-diethylpropionic acid)]oxytocin, [1-(*β*-mercapto-*β*,*β*, pentamethylenepropionic acid)]oxytocin and [1-(deaminopenicillamine), 4-threonine]oxytocin are shown in Figure 1.

Results

The amount of [^{3}H]-oxytocin bound to uterine particles was reduced in proportion to the log concentration of increasing amounts of nonradioactive oxytocin and the oxytocin antagonists (Figure 2). The regressions were parallel, indicating a common set of binding sites for the peptides. Kallidin, a potent uterotonic peptide which is structurally unrelated to oxytocin (Stewart, 1972), did not compete with [^{3}H]-oxytocin for binding sites at a molar ratio of greater than 1200 (Figure 2). [3-Proline]oxytocin, a virtually inert analogue of oxytocin (Roy, Havran, Schwartz & Walter, 1975), also did not compete for oxytocin binding sites in the dose range studied (Figure 2).

The regressions were analyzed as parallel line assays according to Finney (1964). The relative inhibitory potencies and 95% confidence limits of the antagonists, determined in 2 separate experiments for each, are shown in Table 1. Assuming that the analogues act by competition with oxytocin at a

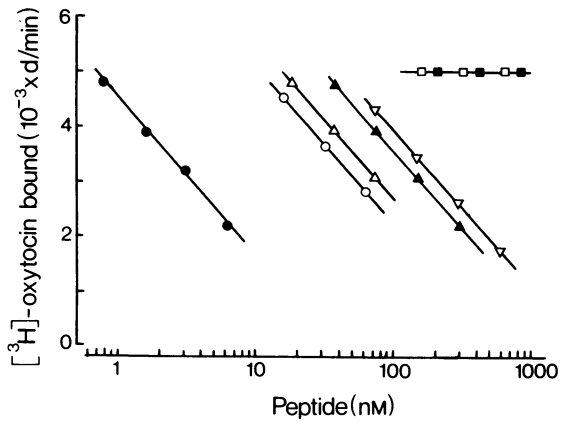


Figure 2 The binding of oxytocin and oxytocin antagonists to uterine particles. Each tube contained about 1 mg of particulate protein, 12,000 d/min of [^{3}H]-oxytocin (about 175 pg), and increasing amounts of nonradioactive peptide in 250 µl of Tris buffer (50 mM, pH 7.6, containing 5 mM $MgCl_2$ and 0.1% gelatin). Incubation was carried out for 1 h at 22°C and terminated by centrifugation of the tubes at 48,000 *g* for 30 minutes. The pellet was combusted to yield ^3H_2O. Each point is the mean of triplicates. Oxytocin (●); [1-(deaminopenicillamine), 4-threonine]oxytocin, (○); [1-(β-mercapto-β,β-pentamethylenepropionic acid]oxytocin, (△); [1-(β-mercapto-β,β-diethylpropionic acid]oxytocin, (▲); [*N*-acetyl, 2-*O*-methyltyrosine]oxytocin, (▽); [3-proline]oxytocin, (□); kallidin (■).

common binding site, the relative inhibitory potency for each compound provides a measure of its relative binding affinity. The relative inhibitory potency of each compound with respect to oxytocin was calculated from the results shown in Figure 2.

The apparent K_d for oxytocin binding, estimated from the linear segment of Scatchard (1949) plots was 1.6 ± 0.04 (s.e. mean) nM in 5 separate experiments (see Figure 3 for a representative plot). This value is comparable to the apparent K_d of 1.8 nM which was found in previous studies (Soloff & Swartz, 1974). The apparent K_d values calculated for each antagonist are given in Table 1.

The published activity of each antagonist (Table 1) is expressed as pA_2, the negative log of the molar concentration of antagonist which reduces the effect of a double dose of oxytocin to that of a single dose (Schild, 1947). The pA_2 value is equal to the negative log of the K_d for the antagonist-receptor interaction, assuming that one antagonist molecule binds to one receptor molecule (Ariëns & van Rossum, 1957). In all cases, the 95% confidence limits for the apparent K_d of each antagonist overlapped the K_d estimated from pA_2 (Table 1).

Discussion

Studies on oxytocin binding sites in the reproductive tract have been carried out on broken cell preparations (Soloff & Swartz, 1974; Soloff, *et al.*, 1974; Soloff, 1975a; Soloff, 1975b) in which there are no known biochemical correlates of oxytocin-receptor interaction. A causal relationship between the binding of oxytocin and the initiation of the contractile response is implicit, however, because several agonists have been shown to bind to uterine particles in approximate proportion to their uterotonic activities (Soloff & Swartz, 1974). The present studies show

Table 1 Comparison of the K_d values derived from antagonist activity and binding assay

	Bioassay		*Binding assay*	
Antagonist	pA_2	K_d (10^{-8} M)	*Relative inhibitory potency (%)*	K_d (10^{-8} M)*
[*N*-acetyl, 2-*O*-methyltyrosine] oxytocin	7.03†	9.33	1.6 (1.3→2.0)⁺ 1.5 (1.3→1.8)	10 (12 →8.0)⁺ 11 (12 →8.9)
[1-(β-Mercapto-β,β-diethylpropionic acid)]oxytocin	7.24‡	5.75	2.5 (1.5→3.7) 2.5 (1.8→3.3)	6.4 (11 →4.3) 6.4 (8.9→4.8)
[1-(β-Mercapto-β,β-pentamethylene-propionic acid)]oxytocin	7.43§	3.71	4.2 (3.6→5.3) 3.4 (2.6→4.5)	3.8 (4.4→3.0) 4.7 (6.2→3.6)
[1-(Deaminopenicillamine), 4-threonine]oxytocin	~7.7‖	~2.0	6.1 (4.2→8.2) 5.9 (4.7→7.1)	2.6 (3.8→2.0) 2.7 (3.4→2.2)

* K_d oxytocin = 1.6 ± 0.04 nM. ⁺95% confidence limits.

† Krejčí, Kupková, Barth & Jošt (1973); ‡ Vavrek, Ferger, Allen, Rich, Blomquist & Du Vigneaud (1972); § Nestor, Ferger & Du Vigneaud (1975); ‖ Manning, Lowbridge & Sawyer (1975).

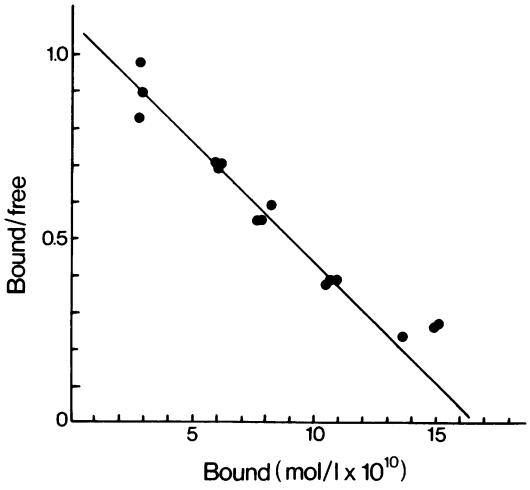


Figure 3 Scatchard analysis of oxytocin binding to uterine particles.

that the binding of the oxytocin antagonists as well was proportional to their potency as inhibitors of oxytocin-induced contractions by the isolated uterus. [3-Proline]oxytocin, a relatively inert analogue of oxytocin (Roy *et al.*, 1975) and kallidin, a potent uterotonic peptide (Stewart, 1972), but structurally unrelated to oxytocin, did not have any demonstrable affinity for oxytocin binding sites. These results, therefore, clearly indicate that the binding sites are components of the oxytocin receptor. The assumption that the same number of sites is available to oxytocin and the antagonists appears to be valid.

In addition to being an oxytocin antagonist, [*N*-acetyl, 2-*O*-methyl-tyrosine]oxytocin is a partial agonist (Krejčí, Kupková, Barth & Jošt, 1973). The diethyl- and pentamethylene-propionic acid antagonists, however, appear to be devoid of oxytocic activity (Vavrek, Ferger, Allen, Rich, Blomquist & Du Vigneaud, 1972; Nestor, Ferger & Du Vigneaud, 1975). Partial agonists/competitive antagonists are postulated to be capable of full receptor occupancy, but they either dissociate very slowly from the receptor (Paton, 1961) or they lack some of the structural characteristics necessary for the initiation of the stimulus (Ariëns *et al.*, 1956; Stephenson, 1956). In the present studies the affinity of oxytocin receptors for the analogues has been estimated directly. The concurrence of the binding and antagonistic activities of the analogues demonstrates that the potency of each antagonist was a function of its affinity for the receptor site rather than its intrinsic activity.

A number of target cells appear to possess 'spare receptors' for drugs. For example, the guinea-pig ileum can be induced to contract maximally by drugs presumably when only a small proportion of the receptor sites are occupied (Stephenson, 1956; Nickerson, 1956; Ariëns, van Rossum & Koopman, 1960). Similar results were obtained with vascular smooth muscle (Furchgott, 1964). The concept of 'spare receptors' has received additional support from experiments in which the concentration of hormone giving a half-maximal response was found to be substantially less than the concentration of radioactive hormone binding to half of the receptor sites, the apparent K_d. Thus, ^{125}I-labelled insulin stimulated half-maximal lipogenesis from glucose in fat cells when about 2% of the receptors were occupied (Gammeltoft & Gliemann, 1973). Comparable results were found with human chorionic gonadotropin-stimulated production of cyclic adenosine 3,5'-monophosphate (AMP) and testosterone by rat testes (Catt & Dufau, 1973), glucagon-stimulated adenylate cyclase activity in rat liver plasma membranes (Birnbaumer & Pohl, 1973), glucagon-stimulated adenylate cyclase in a solubilized preparation from cat heart (Levey, Fletcher, Klein, Ruiz & Schenk, 1974), and ACTH-stimulated cyclic AMP production in adrenal cortical extracts (Lefkowitz, Roth, Pricer & Pastan, 1970). In contrast to these results, the present experiments indicate that there were no detectable spare receptors for the oxytocin antagonists because the apparent K_d of each antagonist estimated by the inhibition of oxytocin-induced uterine contractions was indistinguishable from the apparent K_d estimated by the ability to compete with [^{3}H]-oxytocin for uterine binding sites. Similar findings were reported for the binding of opiates to receptor sites in the guinea-pig intestine (Creese & Snyder, 1975).

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References

ARIËNS, E.J. & VAN ROSSUM, J.M. (1957). pD_x, pA_x and pD_x^1 values in the analysis of pharmacodynamics. *Archs int. Pharmacodyn. Thér.*, **110**, 275–299.

ARIËNS, E.J., VAN ROSSUM, J.M. & KOOPMAN, P.C. (1960). Receptor reserve and threshold phenomena. I. Theory and experiment with autonomic drugs tested on isolated organs. *Archs int. Pharmacodyn. Thér.*, **127**, 459–477.

ARIËNS, E.J., VAN ROSSUM, J.M. & SIMONIS, A.M. (1956). A theoretical basis of molecular pharmacology. *Arzneimittel-Forsch.*, **6**, 282–293.

BIRNBAUMER, L. & POHL, S.L. (1973). Relation of glucagon-specific binding sites to glucagon-dependent stimulation of adenylyl cyclase activity in plasma membranes of rat liver. *J. biol. Chem.*, **248**, 2056–2061.

CATT, K.J. & DUFAU, M.L. (1973). Spare gonadotrophin receptors in rat testis. *Nature, Lond.*, **244**, 219–221.

CREESE, I. & SNYDER, S.H. (1975). Receptor binding and pharmacological activity of opiates in the guinea-pig intestine. *J. Pharmac. exp. Ther.*, **194**, 205–219.

FINNEY, D.J. (1964). *Statistical Method in Biological Assay*. London: Charles Griffin.

FITZPATRICK, R.J. & BENTLEY, P.J. (1968). The assay of neurohypophysial hormones in blood and other body fluids. In *Neurohypophysial Hormones and Similar Polypeptides, Handb. exp. Pharmac.* Vol. 23, ed. Berde, B. pp. 190–285. Berlin and Heidelberg: Springer-Verlag.

FURCHGOTT, R.F. (1964). Receptor mechanisms. *A. Rev. Pharmac.*, **4**, 21–50.

GAMMELTOFT, S. & GLIEMANN, J. (1973). Binding and degradation of ^{125}I-labelled insulin by isolated rat fat cells. *Biochim. biophys. Acta*, **320**, 16–32.

KREJČÍ, I., KUPKOVÁ, B., BARTH, T. & JOŠT, K. (1973). N-Acetyl-2-O-methyl-tyrosine-oxytocin: a specific antagonist of oxytocin. *Physiologia Bohemoslov.*, **22**, 315–322.

LEFKOWITZ, R.J., ROTH, J., PRICER, W. & PASTAN, I. (1970). ACTH-receptors in the adrenal: specific binding of ACTH-^{125}I and its relation to adenyl cyclase. *Proc. natn. Acad. Sci. U.S.A.*, **65**, 745–752.

LEVEY, G.S., FLETCHER, M.A., KLEIN, I., RUIZ, E. & SCHENK, A. (1974). Characterization of ^{125}I-glucagon binding in a solubilized preparation of cat myocardial adenylate cyclase. *J. biol. Chem.*, **249**, 2665–2673.

MANNING, M., LOWBRIDGE, J. & SAWYER, W.H. (1975). The design of neurohypophysial peptides possessing selectively enhanced and inhibitory properties. In *Peptides: Chemistry, Structure and Biology*. ed. Walter, R. & Meienhofer, J. pp. 737–750. Ann Arbor, Mich.: Ann Arbor Sciences Publishers.

MORGAT, J.L., HUNG, L.T., CARDINAUD, R., FROMAGEOT, P., BOCKAERT, J., IMBERT, M. & MOREL, F. (1970). Peptide hormone interactions at the molecular level: preparation of highly labelled ^{3}H oxytocin. *J. Label. Compounds*, **6**, 276–284.

NESTOR, J.J. Jr., FERGER, M. & DU VIGNEAUD, V. (1975). [1β-Mercapto-β,β-pentamethylenepropionic acid]oxytocin, a potent inhibitor of oxytocin. *J. mednl Chem.*, **18**, 284–252.

NICKERSON, M. (1956). Receptor occupancy and tissue response. *Nature, Lond.*, **178**, 697–698.

PATON, W.D.M. (1961). Theory of drug action based on the rate of drug-receptor combination. *Proc. Roy. Soc. B.*, **154**, 21–69.

ROY, J., HAVRAN, R.T., SCHWARTZ, I.L. & WALTER, R. (1975). Oxytocin analogs with substitutions in positions 3 and 4. *Int. J. Peptide & Protein Res.*, **7**, 171–178.

SCATCHARD, G (1949). The attractions of proteins for small molecules and ions. *Ann. N.Y. Acad. Sci.*, **51**, 660–672.

SCHILD, H.O. (1947). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmac. Chemother.*, **2**, 189–206.

SOLOFF, M.S. (1975a). Uterine receptor for oxytocin: effects of estrogen. *Biochem. biophys. Res. Commun.*, **65**, 205–212.

SOLOFF, M.S. (1975b). Oxytocin receptors in rat oviduct. *Biochem. biophys. Res. Commun.*, **66**, 671–677.

SOLOFF, M.S. & SWARTZ, T.L. (1973). Characterization of a proposed oxytocin receptor in rat mammary gland. *J. biol. Chem.*, **248**, 6471–6478.

SOLOFF, M.S. & SWARTZ, T.L. (1974). Characterization of a proposed oxytocin receptor in the uterus of the rat and sow. *J. biol. Chem.*, **249**, 1376–1381.

SOLOFF, M., SWARTZ, T., MORRISON, M. & SAFFRAN, M. (1973). Oxytocin receptors: oxytocin analogs, but not prostaglandins compete with ^{3}H-oxytocin for uptake by rat uterus. *Endocrinology*, **92**, 104–107.

SOLOFF, M.S., SWARTZ, T.L. & STEINBERG, A.H. (1974). Oxytocin receptors in human uterus. *J. clin. Endocr. Metab.*, **38**, 1052–1056.

STEPHENSON, R.P. (1956). A modification of receptor theory. *Br. J. Pharmac.*, **11**, 379–393.

STEWART, J.M. (1972). Structure-activity relationships among the Kinins. In *Structure-Activity Relationships of Protein and Polypeptide Hormones*. ed. Margoulies, M. & Greenwood, F.C. pp. 23–30. Amsterdam: Excerpta Medica.

STÜRMER, E. (1968). Bioassay procedures for neurohypophysial hormones and similar polypeptides. In *Neurohypophysial Hormones and Similar Polypeptides, Handb. exp. Pharmac.*, Vol. 23, ed. Berde, B. pp. 130–189. Berlin and Heidelberg: Springer-Verlag.

VAVREK, R.J., FERGER, M.F., ALLEN, G.A., RICH, D.H., BLOMQUIST, A.T. & DU VIGNEAUD, V. (1972). Synthesis of three oxytocin analogs related to [1-deaminopenicillamine]oxytocin possessing antioxytocic activity. *J. mednl Chem.*, **15**, 123–126.

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