α-Thioglycerol sensitive excitatory oxytocin receptors in fowl rectum

S.K. Mishra and V. Raviprakash[1]

Division of Pharmacology and Toxicology, Indian Veterinary Research Institute, Izatnagar-243 122 (India), 1 June 1981

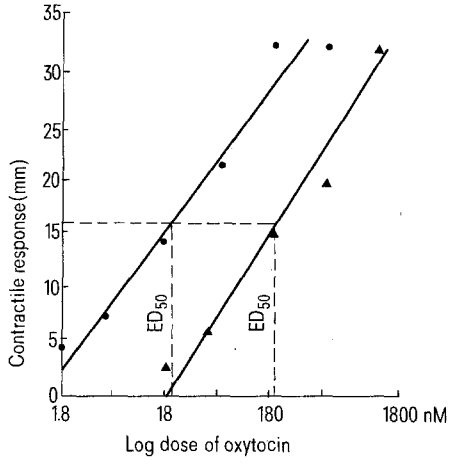
Summary. The selective antagonism of the excitatory responses of oxytocin by α-thioglycerol and its potentiation by Mg^{++} indicate the presence of specific oxytocin receptors in fowl rectum. In addition, the sensitivity of the rectum to very small doses of oxytocin suggests a possible facilitatory role of neurohypophyseal hormones in oviposition.

Despite many studies on the action of neurohypophyseal hormones on uterine smooth muscles[2,3], mammary myoepithelial tissues[4,5], and on vascular smooth muscles[6-8], their effect on gastrointestinal smooth muscle has received little attention. Some work has been done on the reactions of isolated intestines, but the results are complex and variable; they were either shown to have no effect[9], to cause contractions of the longitudinal muscles of the guinea-pig ileum[10] or to inhibit the circular muscles of the rabbit colon[11] and guinea-pig *Taenia coli*[12]. During the course of our investigations on the effects of posterior pituitary hormones on fowl gastrointestinal tissues, we observed that oxytocin stimulated the duodenum and rectum to contract. The present study was carried out with a view to elucidating the mechanism of excitatory action of oxytocin on fowl rectum.

Materials and methods. 1-8-week-old white leghorn birds of either sex were stunned by a blow on the head and bled from the carotid arteries. Rectum pieces 2-3 cm long were dissected out and mounted in an isolated tissue bath of 10 ml volume containing Krebs-Henseleit solution continuously aerated with CO_2 (5%)-O_2 (95%) mixture. The bath temperature was maintained at 32±1 °C. Changes in the mechanical activity of the longitudinal muscles of the rectum were recorded isotonically on a slow-moving kymograph. Preparations were allowed to equilibrate for at least 30 min with a load of 0.5 g before the drugs were added. Agonists were left in contact with the tissue for periods of 1.5-2 min added at an interval of 10 min. Antagonists were added 15 min before the respective agonists were tested. To study the effect of Mg^{++} ions on the oxytocin responses, Krebs-Henseleit solution containing 0 or 2.4 mM of $MgSO_4$ was used. Some experiments were done on cooled preparations; in this case the rectum pieces were kept at 4 °C for 96 h before the experiments were conducted. In cases when the tissues were depolarized, NaCl and $NaHCO_3$ in the Ca^{++} free Krebs-Henseleit solution were replaced by equimolar amounts of K_2SO_4 and $KHCO_3$ respectively. The drugs used were oxytocin (Pitocin, 450-500 IU/mg: Parke-Davis), vasopressin (Pitressin, 100 IU/mg: Parke-Davis), acetylcholine chloride (B.D.H.), histamine dihydrochloride (B.D.H.), 5-hydroxytryptamine creatinine sulphate (5-HT: E. Merck), adenosine triphosphate (ATP: Sigma), α-thioglycerol (Sigma), atropine sulphate (C.H. Boehringer), mepyramine maleate (May & Baker), lysergic acid diethylamide (LSD: Sigma), caffeine (Sigma) and pentolinium (Ansolysen: May & Baker). The concentration of drugs described in this paper is given as the final concentration in a 10 ml bath.

Results. Oxytocin (1.8-54 nM) produced dose-dependent contractions on fowl rectum (n=52) bathed in normal Krebs-Henseleit solution. The onset of oxytocin contraction was within 15-20 sec, reaching a peak in about 1.5-2 min, and the base tone was re-established as soon as the tissue was washed. Repeated exposures of the tissue to oxytocin did not result in tachyphylaxis. Fowl duodenum was comparatively less sensitive to oxytocin than rectum. Although dose-dependent contractions were evident with vasopressin (15.3-45.9 nM) in both duodenum and rectum, the tissues were comparatively less sensitive to it than to oxytocin. The other spasmogens, namely acetylcholine (0.55-5.5 μM), histamine (0.54-5.4 μM) and ATP (5.7-19 μM) elicited dose-dependent excitatory responses whereas 5-HT (0.78-2.6 μM) produced a biphasic response.

α-thioglycerol (20 mM), a specific in vitro oxytocin receptor blocker[13], abolished the responses of oxytocin and potentiated those of acetylcholine by 35% (n=7) and had no effect on responses to other spasmogens. The antagonism was surmountable and reversible. In the presence of α-thioglycerol the log-dose response curve of oxytocin was shifted to the right in a parallel manner indicating competi-



Log concentration-contractile response curve for oxytocin in the absence (●——●) and presence (▲——▲) of α-thioglycerol (20 mM) on isolated fowl rectal preparations. Each point represents the mean of 7 experiments. Note parallel shift and increased ED 50 value in the presence of α-thioglycerol.

Comparative sensitivity of fowl rectum to oxytocin in the absence and presence of Mg^{++}

Concentration of Mg^{++} (mM)	Number of experiments	Threshold concentration of oxytocin (nM) mean (±SE)	ED 50 (nM) mean (±SE)	Maximal contractile response (mm) mean (±SE)
0	5	8.4±1.26	216±25	30±2
2.4	5	2.5±0.16 $p < 0.01$	23.2±2.4 $p < 0.001$	31±1.5

The paired t-test has been used as a test of significance.

tive antagonism (figure). Atropine (0.14 μM), mepyramine (0.25 μM), LSD (3.1 μM) and caffeine (52 μM) which abolished the responses to acetylcholine, histamine, 5-HT and ATP respectively did not affect those to oxytocin. Pretreatment of the tissue with the ganglion blocker pentolinium (19 μM) failed to alter oxytocin responses.

On cooling at 4 °C for 96 h, the spontaneous activity of the tissue was markedly reduced. Nevertheless, in this preparation, contractile responses to oxytocin were still evident (n = 5).

In Mg^{++}-free Krebs-Henseleit solution, oxytocin (5.4–540 nM) produced dose-related contractions (n = 5) though the sensitivity was less as compared with those in normal Krebs-Henseleit solution. On replacing $MgSO_4$ (2.4 mM) in the Mg^{++} free solution, the sensitivity of fowl rectum to oxytocin was restored (n = 5). The comparative sensitivity of the rectal preparations to oxytocin in the absence and presence of Mg^{++} is presented in the table. On the other hand, the presence or absence of Mg^{++} in Krebs-Henseleit solution did not affect the responses to acetylcholine.

Oxytocin (0.18–0.54 μM), acetylcholine (5.5–16.5 μM) and calcium chloride (2.7–9 mM) exhibited a dose-related excitatory effect on fowl rectum bathed in Ca^{++}-free K^+-rich Krebs-Henseleit solution (n = 5). However, the magnitude of the responses to both oxytocin and acetylcholine was markedly reduced and the maximal response as obtained in normal Krebs-Henseleit solution could not be elicited even on increasing the concentration of the drugs. On depolarization, the autorhythmicity of the tissue was completely inhibited and the phasic nature of the oxytocin contraction was changed to a tonic one. Since the magnitude of responses was very small, it was difficult to study the antagonism with blockers.

Discussion. The selective susceptibility of the excitatory actions of oxytocin to α-thioglycerol in fowl rectum suggests the presence of specific oxytocin receptors in this part of the gastrointestinal tract. The presence of oxytocin receptors is further strengthened by the restoration of the sensitivity to oxytocin by readmission of Mg^{++} to Mg^{++}-free Krebs-Henseleit solution. Thus, the present finding is in agreement with those reports of observations on isolated uterine[14,15] and gastrointestinal smooth muscle preparations[16] wherein it has been suggested that Mg^{++} acts as a co-factor in the interactions of neurohypophyseal hormones with their respective receptors.

The persistence of contractions in the depolarized preparations (Ca^{++}-free, K^+-rich) indicated that apart from depolarization, oxytocin possibly produces contraction of the tissues by mobilizing the membrane bound Ca^{++} through its receptor-operated channels. Operation of such a mechanism in K^+-depolarized tissues is evident in cases where spasmogens like acetylcholine, carbachol, histamine and 5-hydroxytryptamine produce contractions through interaction with their specific receptors resulting in opening of the receptor-operated-channels and mobilizing loosely bound Ca^{++} that brings about excitation-contraction coupling[17].

The physiological implications of the sensitivity of the fowl rectal segment to low concentrations of oxytocin are yet to be elucidated. Although the present studies do not provide any experimental evidence with regards to arginine-oxytocin (the predominant posterior pituitary oxytocic principle in birds), it may be postulated that the fowl neurohypophyseal hormone might facilitate oviposition by contracting the rectal segment besides its primary excitatory action on the reproductive smooth muscles. This physiological role is further substantiated by the increased blood concentrations of the neurohypophyseal hormones at the time of oviposition[18].

1 Acknowledgment. The authors wish to express their sincere thanks to Dr M.S. Sastry, Head of the Division and Dr C.M. Singh, Director of the Institute for providing the facilities and Mr Dev Singh for his technical assistance.

2 H.H. Dale and P.P. Laidlaw, J. Pharmac. exp. Ther. *4,* 75 (1912).
3 J.M. Marshall, in: Pharmacology of smooth muscle, p. 143. Ed. E. Bulbring, Pergamon Press, Oxford 1964.
4 C. Mendez-Bauer, H.M. Cabot and R. Caldeyro-Barcia, Science *132,* 299 (1960).
5 R.D. Moore and M.X. Zarrow, Acta endocr. *48,* 186 (1965).
6 M. Pickford, in: Oxytocin, p. 68. Ed. R. Carldeyro-Barcia and H. Heller, Pergamon Press, Oxford 1961.
7 R.A. Woodburg and B.E. Abreu, Am. J. Physiol. *142,* 114 (1944).
8 S. Lloyd and M. Pickford, J. Physiol., Lond. *155,* 161 (1974).
9 T. Sollmann, in: A Mannual of Pharmacology and its Applications to Therapeutics and Toxicology, p. 520. W.B. Saunders Co., London 1957.
10 W.D.M. Paton and M.A. Zar, J. Physiol., Lond. *194,* 13 (1968).
11 B. Levy, J. Pharmac. exp. Ther. *140,* 356 (1963).
12 K. Milenov, in: Physiology of smooth muscle. p. 395. Ed. E. Bulbring and M.F. Shuba. Raven Press, New York 1976.
13 P.J. Martin and H.O. Schild, Br. J. Pharmac. *25,* 418 (1965).
14 P.J. Bentley, J. Endocr. *32,* 215 (1965).
15 H.O. Schild, Br. J. Pharmac. *36,* 329 (1969).
16 C. Woo and A.P. Somlyo, J. Pharmac. exp. Ther. *155,* 357 (1967).
17 T.B. Bolton, Physiol. Rev. *59,* 606 (1979).
18 P.D. Sturkie and Y.C. Lin, J. Endocr. *35,* 325 (1966).

Treatment of pregnant rats with haloperidol delays the onset of sexual maturation in female offspring

R. Bhanot and M. Wilkinson[1]

Departments of Physiology and Biophysics, and of Obstetrics & Gynecology, Faculty of Medicine, Dalhousie University, Halifax (Nova Scotia, B3H 4H7 Canada), 18 June 1981

Summary. Treatment of pregnant rats with haloperidol (1 mg/kg/day) during the last week of gestation induces a significant delay in sexual maturation of female offspring.

Chronic exposure of fetal rats to the dopamine antagonist haloperidol (HP) is reported to modify the subsequent development and function of central dopaminergic neurons. Thus behaviour, brain dopamine levels, dopamine receptors and pituitary prolactin secretion are all affected by maternal treatment with HP[2,3]. These observations are of major clinical importance in view of the widespread use of neuroleptics during pregnancy and by nursing mothers[4,5]. Since dopamine is clearly implicated in reproductive control mechanisms[6,7] via well-described hypothalamic