Br. J. Pharmac. (1980), **69**, 379–382

EFFECTS OF OXYTOCIN ON THE ISOLATED VAS DEFERENS OF THE RAT

JUAN V. BENEIT, AGUSTIN HIDALGO & JUAN L. TAMARGO

Department of Pharmacology, Universidad Complutense, Madrid 3, Spain

1 The effect of oxytocin was studied on the isolated vas deferens of the rat.

2 Oxytocin (25 to 200 mu/ml) reversibly reduced the contractile response induced by noradrenaline, dopamine and acetylcholine.

3 Oxytocin (400 mu/ml) abolished the response to potassium.

4 The contractions evoked in the vas deferens by field stimulation were also reduced by oxytocin (50 to 200 mu/ml). The inhibitory effect of the drug was related to the external calcium concentration.

5 It is suggested that oxytocin depresses the contractile responses in the vas deferens at least partly by reducing the availability of calcium from an extracellular source.

Introduction

The role of oxytocin in male animals is not clearly understood. It has been found that oxytocin increases the volume and the concentration and number of spermatozoa per ejaculate in rams (Knight & Lidsay, 1970), rabbits (Kihlström & Melin, 1963) and bulls (Bereznev, 1963) and that it increases the rate of transport of spermatozoa through the ductus deferens in rams (Ewy, Bielanski & Zapletal, 1963). All these studies suggest that the effects of oxytocin could well be due to a stimulating action of the hormone on the contractile elements of the male genital ducts and the accessory glands during emission.

Although oxytocin may influence several types of smooth muscle, the responses of the various tissues are highly divergent. Thus, oxytocin reduces the amplitude of contraction of muscle from the stomach, taenia coli and ureter, whereas in the uterus it increases the frequency and amplitude of contraction (Milenov, 1976). The present study was undertaken to investigate the effects of oxytocin on the contractile response of the vas deferens of the rat to different agonists, noradrenaline (NA), dopamine, acetylcholine (ACh) and potassium, or to field stimulation.

Methods

Experiments were carried out on the vas deferens of Sprague-Dawley rats (220 to 270 g). The animals were killed by a blow on the head and bled out. The vasa were excised, stripped of external material and suspended in 10 ml organ baths containing modified Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2 and glucose 11.0. The solution was maintained at 37°C and gassed with 95% O_2 and 5% CO_2. Contractions were measured isometrically with a Grass FT03 force-displacement transducer and recorded on a Grass polygraph. Preparations were allowed to equilibrate for 1 h under an initial load of 1 g before they were exposed to drugs. Following this equilibration period a full dose-response curve was obtained by a stepwise increase in concentration of the agonist. Each concentration was applied for 30 s and then washed out thoroughly before the next higher concentration was added. There were 8 min intervals between successive concentrations of the agonist. Peak tension development after each concentration was used as the response in the construction of the dose-response curve. This procedure was repeated at 30 min intervals until two successive curves were obtained in which the responses to successive addition of the agonist were almost identical in height. Then another 1 h equilibration period ensued. During the last 10 min of this period, oxytocin was added to the organ bath and remained in the bathing solution throughout the construction of the dose-response curve. Only one agonist was used in each experiment. In some experiments the effect of oxytocin on the response of the vas deferens to 85 mM potassium chloride was examined. Twenty-five minutes elapsed between successive doses of 85 mM potassium. Field stimulation of the vasa was performed with progressively longer trains of pulses

0007–1188/80/070379-04$01.00

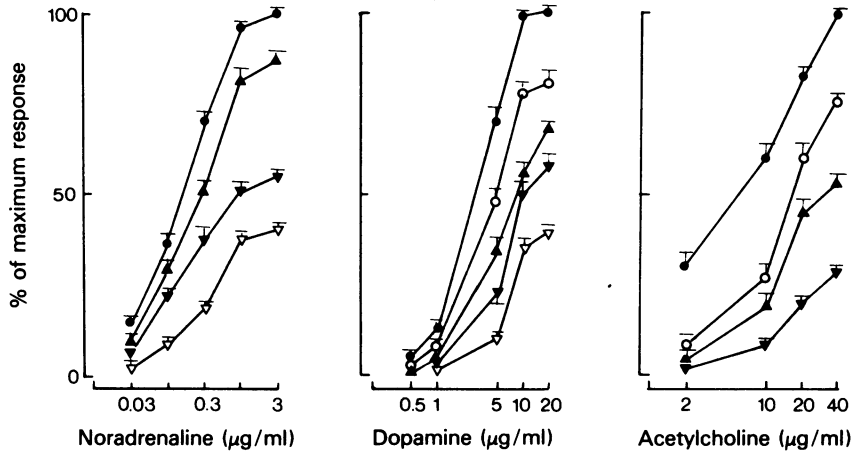

Figure 1 Effect of oxytocin on the dose-response curves to noradrenaline, dopamine and acetylcholine on the rat vas deferens. Each point represents the mean of eight experiments; vertical bars represent the s.e. means. Ordinate scale: % of the maximum response; abscissa scale: concentration of the drugs. Controls (●); after oxytocin, (○) 25 mu/ml; (▲) 50 mu/ml; (▼) 100 mu/ml; (▽) 200 mu/ml.

(supramaximal voltage, 0.1 ms, 10 Hz) delivered at 1 min intervals. The drugs used were: noradrenaline bitartrate (Winthrop), dopamine hydrochloride (Sigma), oxytocin (Syntocinon, Sandoz), acetylcholine chloride (Sigma) and potassium chloride (Merck). All concentrations refer to the salts.

The results are expressed as mean ± s.e. mean. The concentration of oxytocin required for 50% inhibition of the maximal tissue response to the agonists (ID_{50}) was based on the geometrical means. Statistical significance was determined by Student's *t* test for paired data.

Results

None of the oxytocin concentrations used (from 1 to 1000 mu/ml) produced any change in the resting tension of the isolated vas deferens.

Effects of oxytocin on responses to different agonists

After incubation of the preparation with oxytocin (25 to 200 mu/ml) the contractile responses to all doses of NA and dopamine were significantly reduced, resulting in a progressive shift of the dose-response curve for both agonists to the right and a marked depression in the maximum response (Figure 1a and b). Application of higher concentrations of oxytocin (400 to 1000 mu/ml) did not significantly increase the inhibition produced by 200 mu/ml. The ID_{50} of oxytocin against NA and dopamine was 130.1 ± 11.5 mu/ml and 140.3 ± 13.3 mu/ml, respectively. The effects of oxytocin were rapidly reversed by replacing the media with drug-free Krebs solution. Rhythmic contractions of the isolated vas deferens occurred in the presence of the higher concentrations of NA and dopamine which were necessary to obtain near maximal contractions. Oxytocin, at concentrations higher than 50 mu/ml gradually decreased the frequency and height of these contractions until the tissue became quiescent.

Figure 1c shows the effect of oxytocin (25 to 100 mu/ml) on the contractile response of the vas deferens to ACh (1 to 40 μg/ml). The antagonism of the responses to ACh occurred with concentrations of oxytocin higher than 25 mu/ml. Accompanying the shift of the dose-response curve for ACh to the right was a progressive decrease of the maximum response. The ID_{50} of oxytocin against ACh was 57,6 ± 5,8 mu/ml.

The contractile response of the vas deferens induced by 85 mM potassium was also reduced by oxytocin (25 to 200 mu/ml). At higher concentrations, 400 mu/ml, oxytocin produced a complete blockade of the contractile response. The ID_{50} of oxytocin against potassium was 85.0 ± 4.5 mu/ml. The effects of oxytocin were rapidly reversed by repeated washing of the preparation.

Effects of oxytocin on the response to field stimulation

The effects of oxytocin (25 to 1000 mu/ml) on the response of the vas deferens to field stimulation were

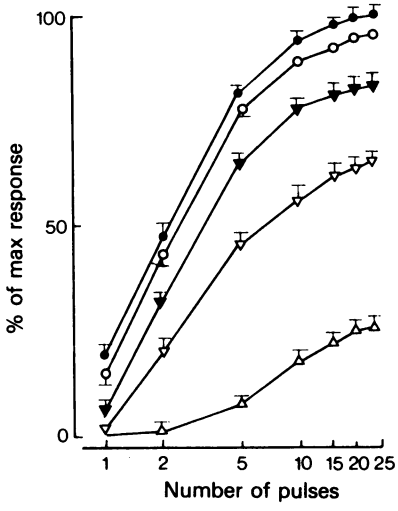

Figure 2 Effect of oxytocin on the contractile responses elicited in the rat vas deferens by field stimulation. Ordinate scale: % of the maximum response; abscissa scale: number of the pulses of the train. Controls (●); after oxytocin, (○) 25 mu/ml; (▼) 100 mu/ml; (▽) 200 mu/ml; (△) 1000 mu/ml. Each point represents the mean of eight experiments; vertical bars represent the s.e. means.

studied in eight experiments. As illustrated in Figure 2, the contractile response was significantly reduced although not abolished by all concentrations of oxytocin used. When the drug was washed out, normal responses to field stimulation gradually reappeared.

In another group of experiments, preparations were stimulated with pulses of supramaximal voltage at a frequency of 10 Hz and a pulse width of 0.1 ms for 5 s every 1 min. Oxytocin (50 to 200 mu/ml) produced a concentration-dependent decrease in the contractile responses to field stimulation and this effect was related to the external calcium concentration in the organ bath. Figure 3 illustrates a typical experiment. An increase in the external calcium concentration from 2.5 mM (Figure 3c) to 3.0 and 4.0 mM (Figure 3a and b, respectively) partial antagonized the inhibitory effects of oxytocin. In contrast, when the external calcium concentration was decreased to 1.0 and 0.5 mM (Figure 3d and e), the same concentration of oxytocin produced a more pronounced inhibition of the twitch height. Verapamil (5×10^{-6} M to 1×10^{-5} M) also reduced the contractile responses to field stimulation and significantly ($P < 0.01$) potentiated the inhibitory effects of oxytocin.

Discussion

Oxytocin markedly reduced the contractile response

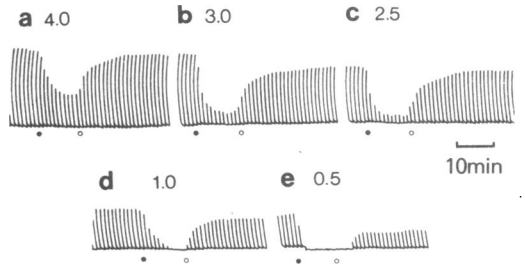

Figure 3 Effect of oxytocin on the contractile responses of the rat vas deferens elicited by 5 s trains of pulses applied at 10 Hz. Between the dots, oxytocin, 200 mu/ml, for 10 min. At the top of each panel numbers indicate the external calcium concentration (mM).

of the rat vas deferens to NA, dopamine and ACh, and abolished that to potassium. In addition, the response to field stimulation, which released the naturally occurring neurohumoral transmitter in the vas deferens was depressed by oxytocin. It has been proposed that nerve endings release both NA and dopamine in the isolated vas deferens of the rat (Simon & Van Maanen, 1976) and since oxytocin depressed the responses to exogenously administered NA and dopamine as well as those elicited by field stimulation, these results may provide evidence for a blocking effect of the drug on sympathetic transmission. However, the fact that even high doses of oxytocin produced on incomplete block of the responses to exogenous catecholamines or to field stimulation, plus confirmation that the ID_{50} of oxytocin against ACh or potassium was lower than that against NA and dopamine, would suggest that the antagonism is due to nonspecific action rather than to a specific α-adrenoceptor or dopamine receptor antagonism. The reduction by oxytocin of ACh-induced responses could be an expression of a muscarinic receptor blocking action of the drug and this possibility has not been excluded in these experiments. A consistent feature of the antagonism exhibited by oxytocin was a depression of the maximum response to the three agonists. This suggests that the antagonism is either of the nonequilibrium competitive or noncompetitive type in contrast to the equilibrium type of competitive antagonism. The fact that the antagonism is exhibited toward drugs acting through different receptors as well as toward ionic stimulation (potassium) indicates that the antagonism is noncompetitive rather than nonequilibrium competitive. Non-competitive spasmolytics do not interact with the agonist site as do competitive agents, but interfere at some common step of the contractile process beyond the receptor. This common step in the excitation-contraction process involves calcium ions (Hurtwitz & Suria, 1971).

Contractions induced in the rat vas deferens by NA, dopamine, ACh and potassium are dependent on the presence of external calcium in the bath (Chang, Lai & Chiueh, 1971). It has been proposed that the responses induced by catecholamines and cholinomimetics (Jurkiewicz, Markus & Picarelli, 1975) depend on two sequential calcium compartments: (a) one which is rapidly exchangeable, containing superficial or loosely bound calcium; (b) a second which is slowly exchangeable, where calcium is tightly bound to the cell membrane or to the cell components (Hudgins & Weiss, 1968). On the other hand, potassium-induced responses depend on the fast exchangeable compartment. According to this hypothesis, oxytocin might interfere with the fast but not with the slowly exchangeable compartment. This explains why oxytocin abolished the potassium-induced responses but only partially inhibited the responses to the other three agonists.

Since the loss of calcium from the tightly bound compartment is a slow process it could not be depleted during the short exposure to oxytocin in our experiments and, therefore, the inhibitory effect of oxytocin on the responses to NA, dopamine, and ACh could not be increased by additional doses of oxytocin. On the contrary, the fast component is rapidly depleted when the external calcium is decreased and rapidly repleted when the external calcium is reintroduced or increased (Jurkiewicz *et al.*, 1975). This could explain the dependence on the external calcium concentration of the oxytocin effects found in these experiments.

References

BEREZNEV, A.P. (1963). Oxytocin, a stimulator in the semen production of bulls. *Vestnic Sel'shokhozyaistvennoi Nauki* (Moscov), **8**, 91–93.

CHANG, K.J., LAI, F.M. & CHIUEH, C.C. (1971). Effects of calcium on smooth muscles and on the adrenergic neuron blocking action of guanethidine. *Archs Int. Pharmacodyn.*, **190**, 34–42.

EWY, Z., BIELANSKI, W. & ZAPLETAL, A. (1963). Influence of oxytocin on spermatozoa transport in the ductus deferens of the ram. *Bull. Acad. pol. Sci. Cl.*, **2**, 11, 145–148.

HUDGINS, P.M. & WEISS, G.B. (1968). Differential effects of calcium removal upon vascular smooth muscle contraction induced by norepinephrine, histamine and potassium. *J. Pharmac. exp. Ther.* **159**, 91–97.

HURTWITZ, L. & SURIA, A. (1971). The link between agonist action and response in smooth muscle. *A. Rev. Pharmac.*, **11**, 303–326.

JURKIEWICZ, A., MARKUS, R.P. & PICARELLI, Z.P. (1975). Effect of full agonists following calcium deprivation in rat vas deferens. *Eur. J. Pharmac.*, **31**, 292–304.

KIHLSTRÖM, J.E. & MELIN, P. (1963). The influence of oxytocin upon some seminal characteristics in the rabbit. *Acta physiol. scand.*, **59**, 363–369.

KNIGHT, T.W. & LINDSAY, D.E. (1970). Short and long-terms effects of oxytocin on quality and quantity of semen from rams. *J. Reprod. Fert.*, **22**, 523–529.

MILENOV, K. (1976) Effect of estradiol, progesterone and oxytocin on smooth muscle activity. In *Physiology of Smooth Muscle.* ed. Bulbring E. & Shuba, M.F. pp. 395–402. New York: Raven Press.

SIMON, A. & VAN MAANEN, E.F. (1976). Dopamine receptors and dopaminergic nerves in the vas deferens of the rat. *Archs Int. Pharmacodyn.* **222**, 4–15.

(Received January 30, 1979.
Revised October 5, 1979.)