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An oxytocin receptor in anococcygeus muscles isolated from male mice

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1 The nature of the neurohypophyseal peptide receptor in the anococcygeus muscles from male mice was investigated.

2 The rank order of potency of naturally occurring peptides was oxytocin > Arg-vasotocin > Arg-vasopressin > Lys-vasopressin, which is similar to that found in the uterus and mammary gland.

3 Selective agonists on the oxytocin (OT) receptors of the uterus and mammary gland (Thr^4-OT; Gly^7-OT; Thr^4-Gly^7-OT) were also potent agonists in the mouse anococcygeus.

4 Competitive antagonists of uterine responses to oxytocin (dP-TyrMe-Thr^4-OT; dP-TyrMe-OT; dP-Thr^4-OT; dp-Orn^8-OT) were also competitive antagonists of oxytocin-induced contractions of the mouse anococcygeus.

5 It is concluded that the neurohypophyseal peptide receptor of the male mouse anococcygeus is of the oxytocin type; antagonist pA_2 values suggest that this receptor resembles, but may not be identical to, the uterine oxytocin receptor. Possible physiological and pharmacological implications of these observations are discussed.

Introduction

The anococcygeus is a bilaterally-paired smooth muscle tissue which straddles the rectum, and for which a physiological function has yet to be defined (Gillespie, 1981). During an investigation of putative neuropeptide transmitters, it was discovered that mouse anococcygeus muscles, *in vitro*, were very sensitive to contraction by neurohypophyseal peptides (Gibson *et al.*, 1984). Of particular interest was the high potency of oxytocin (OT), which produced contractions in concentrations similar to those effective in established OT bioassay preparations (Botting & Gibson, 1985). Further, the anococcygeus muscles were taken from male mice and, as yet, there are no well-established examples of smooth muscle tissues from male mammals which are sensitive to low (nanomolar) concentrations of OT. Consequently, this new example deserved closer attention. In the initial study (Gibson *et al.*, 1984) it appeared that OT was more potent than Arg-vasotocin (AVT) and Arg-vasopressin (AVP), suggesting that the neurohypophyseal peptide receptor in the mouse anococcygeus might be of the OT type. The object of the present study was to test this possibility.

Methods

Male mice (LACA strain from A. Tuck, Essex, U.K.; 25–35 g) were stunned and bled from the neck. The two anococcygeus muscles from each animal were dissected as described previously (Gibson & Wedmore, 1981) and were set up, in series, joined at the point of unification on the ventral rectum, in 1 ml glass organ baths. The bathing medium was Krebs-bicarbonate buffer (mM: NaCl 118.1, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $NaHCO_3$ 25.0, $CaCl_2$ 2.5 and glucose 11.1) which was maintained at 37°C and gassed continuously with 95% O_2: 5% CO_2. A resting tension of 200–400 mg was placed on the tissue and changes in tension recorded by a Grass FTO3 force-displacement transducer attached to a Lectromed pen recorder. Muscles were equilibrated for 45 min before beginning the experiment.

Agonist peptides were added to the organ baths in volumes not exceeding 50 μl and were left in contact with the tissue for 5 min, or until any consequent rise in tone had reached a peak. Following washout, further doses were not added until muscle tone had returned

to baseline. pD_2 values ($-\log_{10}$ of the molar concentration of agonist producing 50% of the maximum response, Ariens & van Rossum, 1957) were calculated by regression analysis of the straight line portion of the dose-response curve (between 20–80% of the maximum). When agonist potency was under study only one dose-response curve was obtained from each preparation.

Antagonist peptides were added to the reservoir of Krebs solution to give the appropriate concentration, and were in contact with the tissue for 30 min before testing their effect on the OT dose-response curve. Antagonist pA_2 values were calculated by regression analysis of Schild plots (Arunlakshana & Schild, 1959) obtained by repeating OT dose-response curves in the

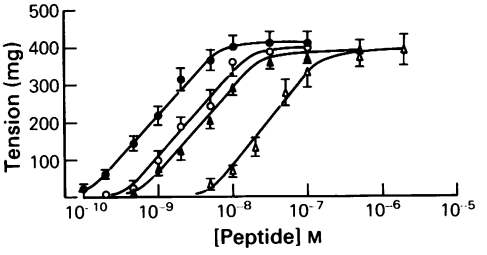

Figure 1 Dose-response curves of contractions of the male mouse anococcygeus muscle in response to oxytocin (●, $n = 11$), Arg-vasotocin (○, $n = 11$), Arg-vasopressin (▲, $n = 9$), and Lys-vasopressin (△, $n = 8$). Each point represents mean with s.e.indicated by vertical lines.

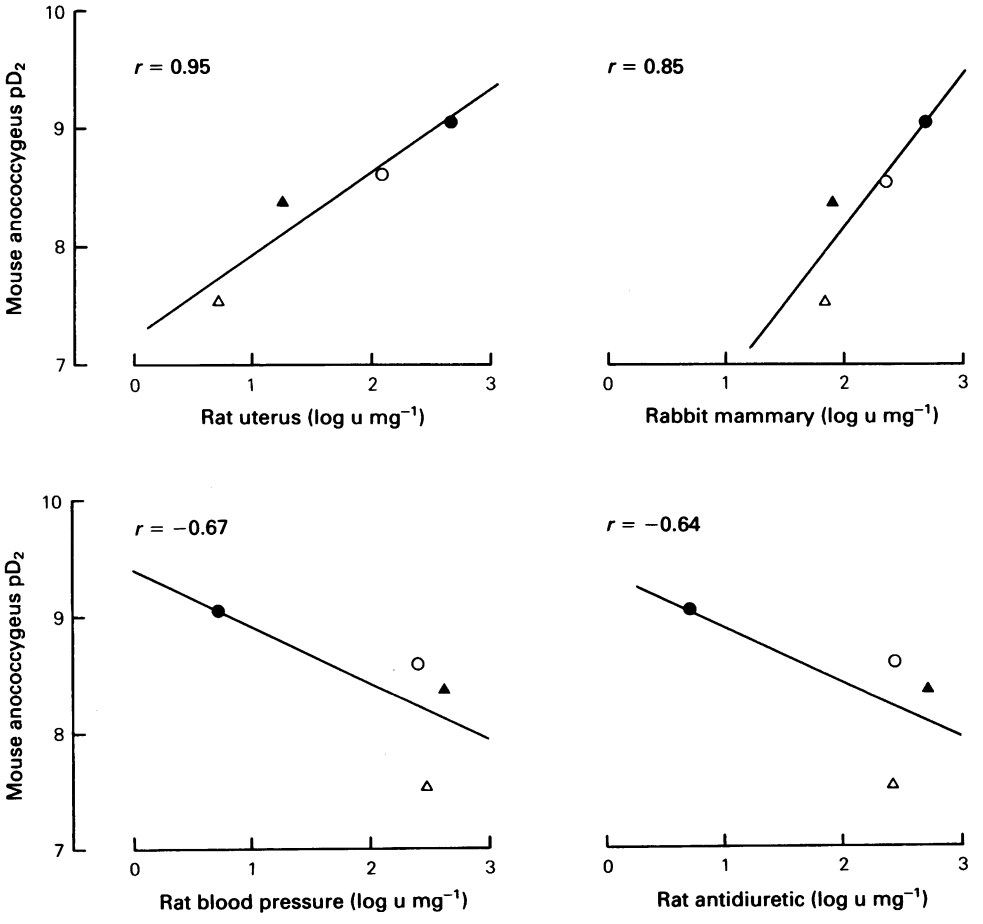

Figure 2 Graphs showing the correlation between the potencies of oxytocin (●), Arg-vasotocin (○), Arg-vasopressin (▲), and Lys-vasopressin (△) in the mouse anococcygeus muscle (measured as the pD_2 values of the curves used in Figure 1) and the four established neurohypophyseal peptide activities in mammals (measured as log u mg^{-1}, taken from the data of Berde & Boissonas, 1968). r represents the correlation coefficient.

presence of increasing concentrations of antagonist. In control experiments, it was found that up to four OT dose-response curves could be repeated, with 30 min intervals, without significant alteration in sensitivity.

Drugs obtained from commercial sources were: oxytocin (OT; preservative-free Syntocinon, Sandoz); Arg-vasopressin (AVP; Sigma); Lys-vasopressin (LVP: Sigma); Arg-vasotocin (AVT; Sigma).

Other drugs were kindly donated by Prof. M. Manning, Toledo, Ohio, U.S.A. These were: Thr^4-oxytocin (TOT); Gly^7-oxytocin (GOT); Thr^4-Gly^7-oxytocin (TGOT); 1-deaminopenicillamine-Thr^4-oxytocin (dPTOT); 1-deaminopenicillamine-Orn^8-oxytocin (dPOOT); 1-deaminopenicillamine-2-methyltyrosine-oxytocin (dPTyrMeOT); 1-deaminopenicillamine-2-methyltyrosine-Thr^4-oxytocin (dPTyrMeTOT).

Comparison between different experimental groups was by Student's t test.

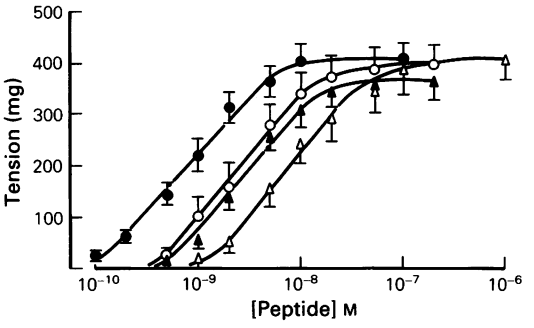

Figure 3 Dose-response curves of contractions of the male mouse anococcygeus muscle in response to oxytocin (●, $n = 11$), Thr^4-oxytocin (○, $n = 9$), Thr^4-Gly^7-oxytocin (▲, $n = 7$), and Gly^7-oxytocin (△, $n = 6$). Each point represents mean with s.e. indicated by vertical line.

Results

Naturally occurring peptides

The naturally occurring neurohypophyseal peptides OT, AVT, AVP, and LVP all produced dose-related contractions of the mouse anococcygeus; the slopes of the dose-response curves and the maximum responses were similar for the four peptides (Figure 1). pD_2 values were calculated from these curves and plotted against the published potencies (in log u mg^{-1}) of the peptides on the four main neurohypophyseal peptide target tissues in mammals (Figure 2). There was a positive correlation between their activities in the mouse anococcygeus and those in the uterus and mammary gland, although only in the case of the uterus was the correlation significant ($P < 0.05$). There was no positive correlation between the mouse anococcygeus and the kidney or the vascular system.

Selective oxytocin receptor agonists

In recent years, a number of neurohypophyseal peptide analogues, selective for OT receptors, have been developed (Manning & Sawyer, 1981). Since the results with the naturally occurring peptides indicated the presence of an OT receptor, three of these selective OT receptor agonists were tested on the mouse anococcygeus; TOT (ratio of oxytocic activity, O/antidiuretic activity, A = 513; ratio of O/vasopressor activity, P = 2146); GOT (O/A = 16,600; O/P = 9,300); and TGOT (O/A = 8,300; O/P = 16,600). All three produced dose-related contractions of the mouse anococcygeus muscle, in nanomolar concentrations; again the slopes of the dose-response curves and the maximum responses were similar to those for OT (Figure 3).

Oxytocin receptor antagonists

Four compounds, previously shown to be competitive antagonists of uterine responses to OT were studied; dPTyrMeTOT, dPTyrMeOT, dPTOT, and dPOOT (Manning & Sawyer, 1981). All four compounds antagonized OT-induced contractions of the mouse anococcygeus, and in each case the antagonism was competitive, the slopes of the Schild plots not being significantly different from 1 (Table 1).

Table 1 pA_2 values and slopes of Schild plots of some oxytocin antagonists in the male mouse anococcygeus

Antagonist	*pA_2*[a]	*Slope*[b]	n[c]
dPTyrMeTOT	9.53 ± 0.03	0.1 (0.94–1.06)	5
dPTyrMeOT	7.96 ± 0.09	0.96 (0.78–1.14)	5
dPTOT	7.69 ± 0.12	1.15 (0.99–1.31)	5
dPOOT	7.52 ± 0.06	1.04 (0.94–1.14)	6

[a]Values given represent mean ± s.e.
[b]Mean values, 95% confidence limits in parentheses.
[c]Number of muscle preparations studied.
dPTyrMeTOT = 1-deaminopenicillamine-2-methyltryrosine-Thr^4-oxytocin; dPTyrMeOT = 1-deaminopenicillamine -2 -methyltyrosine-oxytocin; dPTOT = 1-deaminopenicillamine-Th^4-oxytocin; dPOOT = 1-deaminopenicillamine-Orn^8-oxytocin.

Discussion

The main finding of this study is that the neurohypophyseal peptide receptor in the male mouse anococcygeus is of the OT type. This conclusion is based on several observations. First, the rank order of potency of the naturally occurring peptides (OT > AVT > AVP > LVP) resembles that found in established OT target tissues, the uterus and the mammary gland. Secondly, synthetic peptide analogues, known to be selective agonists on OT receptors (Manning & Sawyer, 1981) were potent agonists in the mouse anococcygeus. Finally, peptides previously shown to be competitive antagonists of OT in the uterus (Manning & Sawyer, 1981), showed similar activity in the mouse anococcygeus. Thus, the mouse anococcygeus is a rare example of a smooth muscle tissue from male mammals that possesses OT receptors. Although all of the studies reported so far have utilised male mice of the LACA strain, we have found anococcygeus muscles isolated from some other strains similarly sensitive to OT (TO strain, Balb/c strain, and C57/DD510 strain; Crook & Gibson, unpublished observations).

dPOOT is an agonist in milk ejection assays (Manning & Sawyer, 1981), but is an antagonist in both the uterus and the mouse anococcygeus. This suggests that the OT receptor in these latter two tissues may be the same. In theory, it should be possible to determine this by comparing the antagonist pA_2 values in the two tissues. Such a comparison is given in Table 2. However, for several reasons, direct comparison is impracticable. Neurohypophyseal peptide receptor function is known to be influenced by small variants in the Mg^{2+} concentration of the bathing medium; this is true for the uterus (Munsick, 1968) as is indicated in Table 2, and for the mouse anococcygeus (Gibson, 1985). In the anococcygeus, optimal OT sensitivity occurs at Mg^{2+} concentrations of 1 mM. However, in the uterus, because of the rhythmic contractions normally found in the isolated preparation, special bathing solutions are often used with lowered Ca^{2+}, and consequently lowered Mg^{2+}, concentrations. Further, the OT sensitivity of the uterus varies greatly during the oestrous cycle (Munsick, 1968). Nevertheless, as can be seen from Table 2, in three cases (dPTyrMeOT, dPTOT, and dPOOT) the pA_2 values found in the mouse anococcygeus are within the range of those found in the uterus, depending on the external Mg^{2+} concentration. However, dPTyrMeTOT is about 100 times more potent in the mouse anococcygeus than in the uterus. Thus, while the OT receptor of the mouse anococcygeus resembles that of the rat uterus, it cannot be concluded that the two receptors are identical.

The observation that the male mouse anococcygeus possesses OT receptors may have some important physiological and pharmacological implications. The physiological function of the anococcygeus is not known, and the role of OT in male mammals is also unclear. There have been a number of suggestions that OT may be released during sexual excitement and may regulate activity of the male reproductive tract (Niemo & Kormano, 1965; Sharma & Hays, 1973; Stoneham *et al.*, 1985). Recently, Peeters *et al.* (1983) reported that stretching the rectum of bulls greatly elevated plasma OT concentrations (up to 300 pM), and proposed that this was due to stimulation of afferent pelvic nerves located in the side walls of the caudal part of the rectum. The anococcygeus both straddles the caudal rectum and continues, in some species, to form the retractor penis. Further work is required to determine the role of the anococcygeus, and of its OT receptors, in normal genital and gastrointestinal function.

The pharmacological implications are twofold, and both arise from the usefulness of the anococcygeus as an isolated preparation compared with the uterus, with its inherent hormone sensitivity and rhythmic activity. First, the anococcygeus might be of use in the elucidation of the mechanisms by which OT induces smooth muscle contraction, these being largely unknown (Bolton, 1979; Olins & Bremel, 1984), and secondly, it might provide a useful model for screening new peptide analogues for OT receptor agonist or antagonist activity (Sawyer *et al.*, 1980).

Table 2 Comparison of pA_2 values of some oxytocin antagonists in the mouse anococcygeus and the rat uterus

Antagonist	*Mouse anococcygeus*	*Rat uterus* 0 mM Mg^{2+}	*Rat uterus* 0.5 mM Mg^{2+}
dPTyrMeTOT	9.53 ± 0.03	7.64 ± 0.14	7.79 ± 0.09
dPTyrMeOT	7.96 ± 0.09	7.76 ± 0.12	7.80 ± 0.12
dPTOT	7.69 ± 0.12	7.52 ± 0.04	6.23 ± 0.11
dPOOT	7.52 ± 0.06	7.89 ± 0.11	6.97 ± 0.11

Values represent mean ± s.e. Rat uterus values are taken from Manning & Sawyer (1981).
dPTyrMeTOT = 1-deaminopenicillamine-2-methyltyrosine-Thr^4-oxytocin; dPTyrMeOT = 1-deaminopenicillamine -2 -methyltyrosine-oxytocin; dPTOT = 1-deaminopenicillamine-Thr^4-oxytocin; dPOOT = 1-deaminopenicillamine-Orn^8-oxytocin.

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