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The mouse anococcygeus muscle as a preparation for the bioassay of oxytocin

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Oxytocin (0·1–10 nM) caused reproducible, dose-related contractions of the male mouse anococcygeus. Desensitization was not a major problem. The muscle was shown to be a useful oxytocin bioassay preparation, having a good index of precision (0·04).

For the estimation of oxytocin concentrations in plasma and tissue extracts, the use of bioassay has largely been superseded by immunoassay. However, bioassay is still used routinely for the quantification of synthetic oxytocin. The British Pharmacopoeia (1980) lists three official oxytocin bioassay preparations; fowl blood pressure, rat uterus, and milk ejection in lactating rats. Each of these methods has problems; fowl blood pressure shows a regular wave-like pattern and, therefore, timing of injections has an important influence on the accuracy of the bioassay (Sturmer 1968), while the uterus and milk ejection assays require female animals in particular hormonal states. We report here that the anococcygeus muscle of the male mouse may provide a useful, and cheap, alternative preparation for the bioassay of oxytocin. It has recently been shown that the muscle contracts in the presence of low concentrations of oxytocin (Gibson et al 1984), being more sensitive to this peptide than to vasopressin.

Methods

Male mice (LACA strain from A. Tuck, Essex, UK; 25–35 g) were stunned and bled from the neck. Both anococcygeus muscles were dissected and set up in series, joined at the point of unification on the ventral rectum (Gibson & Wedmore 1981), in 1 ml glass organ baths containing Krebs-bicarbonate solution (mM: NaCl, 118·1; KCl, 4·7; $MgSO_4$, 1·0; KH_2PO_4, 1·2; $NaHCO_3$, 25·0; $CaCl_2$, 2·5; glucose, 11·1), which was gassed continuously with 95% O_2 : 5% CO_2 and maintained at 37 °C. 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 left for 60 min before beginning the assay. Synthetic oxytocin (Syntocinon–preservative free, Sandoz; Batch 67060; 450 U mg^{-1}) was used throughout.

Results and discussion

Oxytocin (0·1–10 nM) produced dose-related contractions of the mouse anococcygeus muscle (Fig. 1), which were unaffected by phentolamine (1 μM), atropine (100 nM), or tetrodotoxin (1 μM). Repeated submaximal doses gave reproducible responses (Fig. 2). Oxytocin-induced contractions reached equilibrium within 2–3 min; if the oxytocin was left in contact with the tissue the response slowly waned, although following washout subsequent responses were near normal (Fig. 2). Thus, desensitization of the tissue to oxytocin was not a major problem.

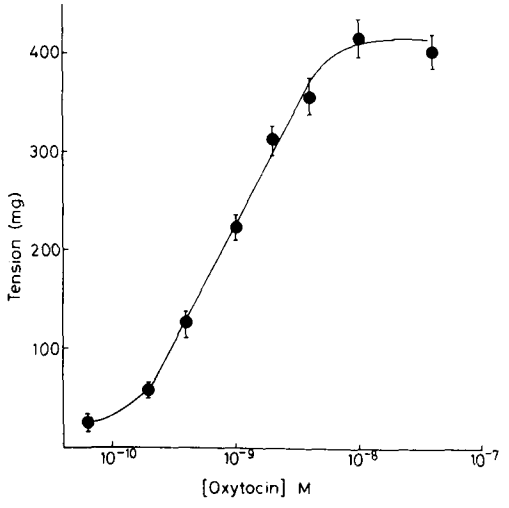

FIG. 1. Dose-response curve of oxytocin-induced contractions of the mouse anococcygeus muscle. Each point is the mean of 17 muscle preparations. Vertical bars represent standard errors.

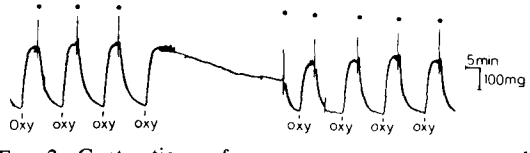

FIG. 2. Contractions of a mouse anococcygeus muscle preparation to repeated submaximal doses of oxytocin (Oxy : 2 nM). ●, denotes washout.

The results of eight bioassay experiments, performed on separate tissues, are given in Table 1. Oxytocin solutions of 'unknown' concentration were assayed against standard solutions using a four-point design (Schild 1942). Dose-ratios of 2 and 3 were used. Contact time was 3 min and a dose-frequency of 8 min was found most appropriate. Analyses of variance were performed

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Table 1. Results of eight oxytocin bioassays using the mouse anococcygeus muscle.

Actual ratio of potency	Estimated ratio of potency	Fiducial limits (95%)	Index of precision (S/b; Holton 1948)
1·0	1·07	1·02–1·11	0·016
1·0	1·00	0·83–1·17	0·06
1·0	1·01	0·83–1·20	0·08
1·1	1·17	1·00–1·35	0·05
1·2	1·20	1·02–1·42	0·06
1·2	1·20	1·11–1·30	0·03
1·2	1·17	1·13–1·20	0·028
1·3	1·32	1·24–1·41	0·023

to obtain fiducial limits of the estimates of potency ratios. Over the eight assays, the mean ± standard error of the index of precision was 0·043 ± 0·008.

In some other experiments, the tissue was superfused with Krebs solution at a rate of 3·5 ml min^{-1}. Although such preparations gave dose-related contractions to oxytocin, the sensitivity was less than in the static organ bath.

In conclusion, the mouse anococcygeus muscle provides a useful tissue for the bioassay of synthetic oxytocin. Its sensitivity is comparable with that of the rat uterus (Follett & Bentley 1964) and the rat mammary myoepithelium (Polacek et al 1967); the stability of the mouse anococcygeus, and the fact that it is taken from male animals with no apparent dependence on hormonal state, suggests that it may have some advantages over the latter two preparations as an inexpensive bioassay of synthetic oxytocin. Further, the preparation has the advantage that a wide range of doses can be accommodated on the linear part of the dose-response curve. The relatively long dose-interval (8 min) means that two or more preparations can easily be managed concurrently.

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GABA inhibits excitatory neurotransmission in rat pelvic ganglia

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Intravenous GABA inhibited, in a bicuculline-sensitive manner, contractions of the urinary bladder induced by preganglionic nerve stimulation in urethane anaesthetized rats, while it had no significant effect on contractions produced by postganglionic nerve stimulation. In addition, intravenous GABA strongly inhibited DMPP-induced bladder contractions; this effect was also prevented by bicuculline. These experiments suggest that GABA inhibits excitatory neurotransmission in rat pelvic ganglia through a mechanism involving, at least in part, the activation of postsynaptic GABA A receptors.

In recent years much attention has been devoted to determine the potential effects of γ-aminobutyric acid (GABA) on the excitatory neurotransmission to the urinary bladder in various animal species (Sillen 1980; Maggi et al 1983, 1984; Taniyama et al 1983; Santicioli et al 1984; Kusunoki et al 1984).

We have previously reported that intravenous GABA, in a bicuculline-sensitive manner, transiently inhibits the micturition reflex in urethane anaesthetized rats (Maggi et al 1983). In adult rats, GABA appears to be devoid of significant effects on postganglionic excitatory neurotransmission (Maggi et al 1983, 1984). Since little intravenous GABA crosses the blood brain barrier (Kuriyama & Sze 1971), we hypothesized that, in our experimental conditions, GABA would impair neurotransmission at pelvic ganglia level (Maggi et al 1983). Interestingly, Taniyama et al (1983) described, in the guinea-pig isolated bladder, a bicuculline-sensitive inhibitory effect of GABA on the cholinergic component of the postganglionic excitatory neurotransmission. Unlike guinea-pigs, the rat bladder is almost devoid of intramural ganglion cells (Elmer 1978; Kusunoki et al 1984), so we thought that the same biological effect (i.e. a bicuculline-sensitive reduced excitability of postganglionic neurons) had been observed under different experimental conditions (in-vivo but not in-vitro in rats, in-vitro in guinea pigs), because of species-related anatomical differences (Maggi et al 1984).

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