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Magnesium ions and oxytocin sensitivity of the male mouse anococcygeus

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The sensitivity of the mouse anococcygeus to contraction by oxytocin was shown to be Mg^{2+}-dependent. Decreasing $[Mg^{2+}]_0$ from the optimal concentration of 1 to 0 mM caused a 20-fold parallel rightward displacement of the oxytocin dose-response curve. Contractions to oxytocin-related peptides (Arg-vasotocin, Arg-vasopressin and Lys-vasopressin) were also Mg^{2+}-dependent, but those to other drugs (carbachol, methoxamine and thyrotrophin releasing hormone) were not. The onset of the potentiating effect of Mg^{2+} was rapid, full potentiation occurring within 70 s. 1-Deaminopenicillamine 8-ornithine-vasotocin produced competitive antagonism of responses to oxytocin, but was more potent in the absence (pA_2 = 8·01) than in the presence of Mg^{2+} (1 mM; pA_2 = 7·52). Thus, physiological concentrations of $[Mg^{2+}]_0$ enhanced oxytocin agonist potency but decreased oxytocin antagonist potency; possible mechanisms are discussed.

The interaction between magnesium ions (Mg^{2+}) and neurohypophyseal peptides has been recognized for many years (van Dyke & Hastings 1928; Fraser 1939; Stewart 1949; Munsick 1968). In the case of oxytocin, most studies have been carried out on the isolated uterus, and have given inconsistent results (Munsick 1968). Some studies have shown enhancement of uterine contractions to oxytocin in the presence of low concentrations (≤2 mM) of Mg^{2+} (Stewart 1949), others have reported enhancement followed by inhibition (Krejčí et al 1967), while others have shown no significant effect on oxytocin itself, but potentiation of some of its analogues (Bentley 1965). It is likely that these discrepancies arise from the use of the uterus as the test tissue, since its sensitivity to oxytocin varies markedly during the oestrus cycle, and the pronounced rhythmic contractions shown by the isolated preparation are often offset by modifying the ionic composition of the bathing medium, usually by reducing Ca^{2+} (Munsick 1968). Thus, it would be useful to study the interaction between Mg^{2+} and oxytocin on other preparations lacking such problems. Recently, it has been shown that low concentrations of oxytocin contract anococcygeus muscles isolated from male mice (Gibson et al 1984), the sensitivity of the preparation being similar to that of the isolated uterus (Botting & Gibson 1985). The anococcygeus muscles, in-vitro, show no rhythmic contractions, and as yet no hormone-dependent changes in oxytocin sensitivity have been detected. Therefore, in the present paper, the male mouse anococcygeus has been used to study the relationship between Mg^{2+} and tissue sensitivity to oxytocin.

METHODS

Male mice (LACA strain; 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 by the ventral bar, in 1 ml glass organ baths. The bathing medium was Krebs-bicarbonate buffer; the 'normal' composition of this was: (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. The medium was maintained at 37 °C and was 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 with the appropriate bathing medium (see later) for 60 min before beginning the experiment.

Agonist peptides and drugs 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 of agonist 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, Ariëns & van Rossum 1957) and slopes were calculated by regression analysis of the straight line portion of the dose-response curve (between 20–80% of the maximum response). When agonist potency was being examined, only one dose-response curve was obtained from each preparation.

The oxytocin antagonist was added to the Krebs bathing medium at the appropriate concentration

and was in contact with the tissue for 30 min before testing its effect on oxytocin sensitivity. Antagonist pA_2 values were calculated by regression analysis of Schild plots (Arunlakshana & Schild 1959), obtained by repeating oxytocin dose-response curves in the presence of increasing concentrations of antagonist. In control experiments, it was found that up to four oxytocin dose-response curves could be repeated, with 30 min intervals, without significant alteration in sensitivity.

The Mg^{2+} concentration of the bathing medium $[Mg^{2+}]_0$ was modified by varying the amount of $MgSO_4$ added to the solution; no compensation was made for the resultant small change in tonicity. In preliminary experiments, it was found that responses to oxytocin were similar in Krebs solution containing 1 mM $MgSO_4$ or 1 mM $MgCl_2$; thus, changes in $[SO_4^{2-}]_0$ did not contribute to the observed effects. Throughout this paper, the concentrations of Mg^{2+} refer to the amount knowingly added to the bathing medium; no account was taken of the trace amounts which might contaminate other solutes.

The following drugs were used: carbachol (BDH); methoxamine hydrochloride (Wellcome); oxytocin (preservative-free Syntocinon, Sandoz); thyrotrophin releasing hormone (Sigma); Arg-vasopressin (Sigma); Lys-vasopressin (Sigma); Arg-vasotocin (Sigma); 1-deaminopenicillamine 8-ornithine-vasotocin (gift from M. Manning, Toledo, Ohio). All doses refer to final bath concentrations.

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

RESULTS

The effect of $[Mg^{2+}]_0$ on responses to oxytocin

In normal Krebs solution, containing 1 mM Mg^{2+}, oxytocin (0·1–10 nM) produced dose-related contractions of the mouse anococcygeus muscle (Fig. 1), the sensitivity being similar to that reported previously (Botting & Gibson 1985). Increasing $[Mg^{2+}]_0$ to 2 mM, or reducing it to 0·75 mM, did not alter the parameters of the dose-response curve (Fig. 1). However, reduction below 0·75 mM caused a progressive decrease in the pD_2 value, with no change in maximum response or slope of the dose-response curve. The difference in pD_2 value on changing from 1 to 0 mM $[Mg^{2+}]_0$ represents a 20-fold parallel rightward displacement of the dose-response curve.

These results showed clearly that the presence of Mg^{2+} enhanced the oxytocin sensitivity of the mouse anococcygeus. To determine the rate of onset of this potentiation, a different experimental protocol was adopted (Fig. 2). Addition of Mg^{2+} (0·1–1·0 mM) to

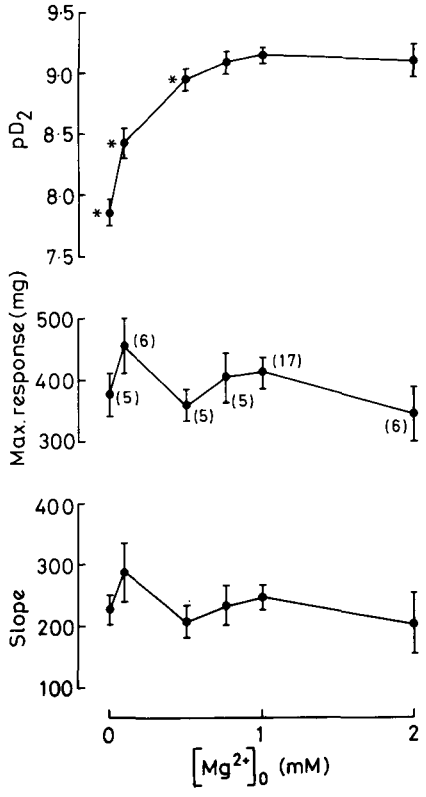

FIG. 1. The influence of the magnesium ion concentration of the Krebs solution $[Mg^{2+}]_0$ on pD_2 values, maximum responses, and slopes of dose-reponse curves of oxytocin-induced contractions of the mouse anococcygeus. Points represent mean ± s.e. Numbers in parentheses represent the number of muscle preparations studied at each $[Mg^{2+}]_0$. *$P < 0·05$, value significantly different from that of 1 mM Mg^{2+} (normal Krebs).

muscles bathing in Mg^{2+}-free Krebs solution had no effect on tone. However, if an initial, small contraction was induced by oxytocin (2–5 nM) subsequent addition of Mg^{2+} caused a further rise in tension (Fig. 2). The onset of this potentiation was rapid; the time taken to reach the new equilibrium tension was 69 ± 5 s (n = 6). Using this protocol it was possible to construct a dose-response curve for the further contractions produced by the cation (Fig. 2); the curve was similar to that obtained in the previous experiments, the optimal $[Mg^{2+}]_0$ being around 1 mM (compare Figs 1 and 2).

The effect of $[Mg^{2+}]_0$ on responses to oxytocin-related agonists

Arg-vasotocin (0·4–20 nM), Arg-vasopressin (1–100 nM), and Lys-vasopressin (10–400 nM) also caused contraction of the mouse anococcygeus. Like oxytocin, the pD_2 values of these peptides, but not

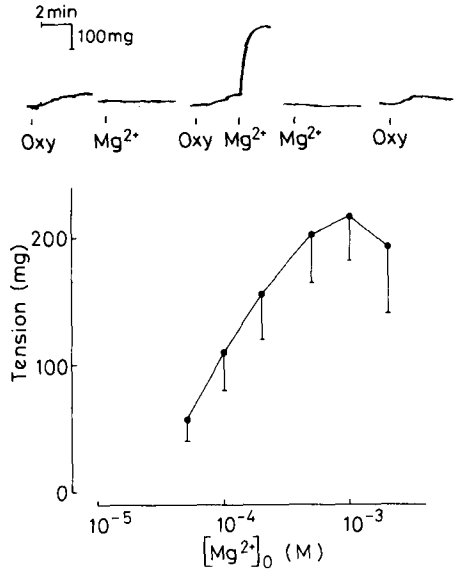

FIG. 2. Upper panel: Contractions of a mouse anococcygeus muscle preparation to oxytocin (Oxy; 5 nM) and Mg^{2+} (1 mM). The muscle was bathed in Mg^{2+}-free Krebs solution. Mg^{2+} by itself produced no change in tone. However, in the presence of a small, initial contraction induced by oxytocin, Mg^{2+} produced a further, rapid rise in tension. Lower panel: Dose-response curve of the further contractions produced by Mg^{2+} (n = 6) in the presence of a small, initial contraction induced by oxytocin (protocol similar to that shown in the middle trace of the upper panel). Oxytocin (2–5 nM) was added to give an initial contraction of 50–100 mg, and the cation was added when the oxytocin response had reached a plateau. Points represent mean ± s.e.

the maximum responses or slopes of the dose-response curves, were greater in normal than in Mg^{2+}-free Krebs solution (Table 1).

The effect of $[Mg^{2+}]_0$ on responses to other drugs

Dose-response curves of contractions to carbachol, methoxamine, and thyrotrophin releasing hormone were similar in normal or Mg^{2+}-free Krebs solution (Table 2).

Table 1. Parameters of the dose-response curve to some oxytocin-related peptides in normal and Mg^{2+}-free Krebs solution.

	$[Mg^{2+}]_0$ mM	n	pD_2	Maximum response (mg)	Slope
Arg-vasotocin	0	5	7·82 ± 0·05*	404 ± 34	245 ± 15
	1	11	8·68 ± 0·05	403 ± 35	314 ± 36
Arg-vasopressin	0	5	7·43 ± 0·03*	346 ± 27	261 ± 42
	1	8	8·46 ± 0·09	395 ± 20	221 ± 24
Lys-vasopressin	0	5	6·80 ± 0·08*	320 ± 22	231 ± 20
	1	7	7·52 ± 0·05	404 ± 42	279 ± 47

n = Number of muscle preparations studied in each group. Values given represent mean ± s.e.
* $P < 0·05$ value significantly different from that of 1 mM Mg^{2+}.

Table 2. Parameters of the dose-response curve to non-oxytocin-like drugs in normal and Mg^{2+}-free Krebs solution.

	$[Mg^{2+}]_0$ mM	n	pD_2	Maximum response (mg)	Slope
Methoxamine	0	5	6·87 ± 0·02	620 ± 36	440 ± 36
	1	5	6·79 ± 0·08	668 ± 24	440 ± 27
Carbachol	0	6	5·62 ± 0·09	345 ± 39	235 ± 39
	1	6	5·72 ± 0·07	387 ± 31	284 ± 35
Thyrotrophin releasing hormone	0	5	5·50 ± 0·15	172 ± 16	161 ± 19
	1	5	5·58 ± 0·23	192 ± 6	226 ± 32

n = Number of muscle preparations studied in each group. Values represent mean ± s.e.

The effect of $[Mg^{2+}]_0$ on the potency of an oxytocin antagonist

1-Deaminopenicillamine 8-ornithine-vasotocin (dP-Orn^8-VT) antagonized responses to oxytocin in both normal and Mg^{2+}-free Krebs solution. In both cases the antagonism was competitive (slope of Schild plot not significantly different from unity), but the pA_2 value, and hence antagonist potency, was greater in Mg^{2+}-free Krebs solution (Table 3).

Table 3. pA_2 values and slopes of Schild plots of dP-Orn^8-VT against oxytocin in normal and Mg^{2+}-free Krebs.

$[Mg^{2+}]_0$ mM	pA_2[a]	Slope[b]	n[c]
0	8·01 ± 0·14*	1·02 (0·88–1·14)	5
1	7·52 ± 0·06	1·04 (0·90–1·14)	6

[a] Values are mean ± s.e.
[b] Values are mean (95% confidence limits).
[c] Number of muscles studied in each group.
* $P < 0·05$ significantly different from that of 1 mM Mg^{2+}.

DISCUSSION

The results of this study clearly show that low concentrations of $[Mg^{2+}]_0$ potentiate oxytocin-induced contractions of the mouse anococcygeus, and therefore support the general contention that the actions of neurohypophyseal peptides on smooth muscle are Mg^{2+}-sensitive (Somlyo et al 1966; Somlyo & Somlyo 1970). The optimal $[Mg^{2+}]_0$ was found to be 1 mM, which is similar to the concentrations normally included in mammalian physiological salines (1·0–1·2 mM). Plasma Mg^{2+} is normally in the range 0·75–1·0 mM (Bowman & Rand 1980). However only about 50–60% of this is in the form of free Mg^{2+}, the remainder being bound to proteins and other macromolecules. Thus, Mg^{2+}-induced changes in oxytocin sensitivity of the mouse anococcygeus occur at concentrations of the free cation likely to be encountered physiologically.

Although there is general agreement, strengthened by the present study, that Mg^{2+} potentiates the contractile actions of neurohypophyseal peptides (Somlyo & Somlyo 1970), the influence of the ion on smooth muscle sensitivity to other drugs is less clear. Altura & Altura (1971) reported that removal of Mg^{2+} from the bathing medium enhanced responses of rabbit aortic strips to acetylcholine (ACh), angiotensin, and K^+, reduced those to adrenaline, and had no effect on those to histamine or 5-hydroxytryptamine (5-HT). Contractions of rabbit mesenteric blood vessels to 5-HT (Goldstein & Zsoter 1978) and of dog coronary arteries to noradrenaline (NA), ACh, 5-HT, angiotensin, and K^+ (Turlapaty & Altura 1980) were enhanced in Mg^{2+}-free medium, whereas those of bovine coronary arteries to NA and ACh were unaffected (Kalsner 1983). Thus, it seems that the influence of Mg^{2+} on drug sensitivity of smooth muscle depends on the tissue examined. In the mouse anococcygeus, 1 mM Mg^{2+} produced a selective potentiation of contractions evoked by oxytocin and related peptides, having no effect on contractions to carbachol, methoxamine, or thyrotrophin releasing hormone. A similar selectivity was reported by Somlyo et al (1966) in dog somatic arteries.

Bentley (1965) suggested that Mg^{2+} enhances neurohypophyseal peptide actions by altering the configuration of the peptide receptor, thereby increasing affinity. The view that the peptide receptor is the locus of the potentiating effect of Mg^{2+} has been supported by other organ bath (Somlyo et al 1966; Schild 1969) and receptor-ligand binding studies (Soloff et al 1977; Pearlmutter & Soloff 1979). However, other workers have suggested that Mg^{2+} might act at a site beyond the receptor to enhance the stimulus-response coupling process (Krejčí et al 1967; Altura 1975). The results of the present study support the view that Mg^{2+} alters the oxytocin-receptor interaction. First, Mg^{2+} potentiated selectively oxytocin and related peptides, having no effect, in the concentrations used, on the contractile effects of three other drugs acting through different receptor systems. Secondly, the pA_2 values of the oxytocin antagonist dP-Orn^8-VT (Sawyer et al 1980) were different in normal and Mg^{2+}-free Krebs solution. Since in both cases the slope of the Schild plot was unity the pA_2 values equal the pK_D values (Arunlakshana & Schild 1959), and therefore the presence or absence of Mg^{2+} altered the affinity constant of the antagonist. However, while Mg^{2+} enhanced the potency of oxytocin agonists it reduced that of the antagonist, as has already been found in the uterus (Sawyer et al 1980). This suggests that in Mg^{2+}-free Krebs solution the oxytocin peptide-receptor configuration favours antagonist action, but in the presence of Mg^{2+} it is altered to favour agonist action.

Finally, the antagonist potency of dP-Orn^8-VT in the mouse anococcygeus is similar to that reported in the uterus (Sawyer et al 1980). In milk ejection assays, dP-Orn^8-VT acts as an oxytocin agonist (Sawyer et al 1980). Thus, it is possible that the mouse anococcygeus may be a useful preparation for screening new oxytocin analogues for antagonist action, being free of some of the problems associated with the isolated uterus (Sawyer et al 1980).

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