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Effects of magnesium chloride on the contractile response of uterus to several agonists in Ca-free solution

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The effects of $MgCl_2$ on the oestrogen-dominated rat uterus have been examined. Tissues were preincubated in a Ca^{2+}- and Mg^{2+}-free medium containing 3 mM EDTA. Most experiments were subsequently performed in a similar medium containing either no EDTA or EDTA (1 mM). When $MgCl_2$ was added cumulatively (1–32 mM) no contractile responses were obtained in Ca,Mg-free medium or in Ca,Mg-free high K^+ solution. When 2 mM $CaCl_2$ was added, a sustained contraction was obtained. Subsequent addition of cumulative concentrations of $MgCl_2$ caused concentration-dependent relaxation. Oxytocin, 2 μM, produced a small and sustained contraction in a Ca,Mg-free medium. Addition of $MgCl_2$, 2 mM, increased this contraction. In a Ca,Mg-free medium vanadate (8×10^{-5} M) did not evoke spasm of uterine smooth muscle. After addition of $MgCl_2$ in cumulative amounts (1–32 mM) in the presence of vanadate, a concentration-dependent contraction was obtained. The present work shows that in Ca-free solution, maintained contractions induced by oxytocin and vanadate are augmented by Mg^{2+}.

Many drugs can initiate contraction in a manner independent of membrane potential change. This was first demonstrated by Schild (1964) who showed that smooth muscles completely depolarized by high-K^+ solutions could be made to contract further on application of some agonists (Bolton 1979). This contractile response could be due either to drug-receptor interaction directly opening channels, with a consequent increase in Ca^{2+} entry, or to the release of Ca^{2+} from internal stores.

The existence of this internal Ca^{2+} store that can be mobilized by excitatory drugs has already been proposed for the uterus (Edman & Schild 1962; Sakai et al 1982; Lalanne et al 1984) and the release of internal Ca^{2+} has also been demonstrated by stimulating myometrial preparations in Ca^{2+}-free solution with different agonists: oxytocin (Sakai et al 1982), PGE_1 (Villar et al 1985), acetylcholine, PGE_2 and vanadate (Mironneau et al 1984).

The underlying mechanisms for the maintained contractions produced by stimulant substances in Ca-free solution have not been well analysed, essentially because measurement of the changes in ionized Ca concentration inside smooth muscle cells remains difficult (Neering & Morgan 1980).

Smooth muscle contraction elicited by an agonist in Ca-free solution generally requires the presence of Mg^{2+} in the extracellular medium. This fact suggests that the Mg ion may play a major role in stimulus-contraction coupling and, therefore, the purpose of our experiments was to examine the role of extracellular Mg^{2+} in the response of uterine smooth muscle to several drugs acting through different mechanisms: KCl (opening of voltage-dependent channels), oxytocin (opening receptor-activated channels) and vanadate (inhibiting Ca^{2+}, Mg^{2+}-ATPase; Mironneau et al 1984).

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METHODS

Preparation of uterine horns

Female Wistar rats, 150–200 g, were given oestradiol benzoate (5 mg mL^{-1} i.p.); 24 h later they were killed by a blow on the head and exsanguinated. One uterine horn was removed and mounted in a 30 mL organ bath chamber filled with Ringer Locke solution bubbled with a mixture of 95% O_2 and 5% CO_2 at 31 °C.

Experimental procedure

The uterine horn was equilibrated for 1 h in Ringer Locke solution under a resting tension of 0·5 g. Then the solution was replaced by Ca,Mg-free solution containing 3 mM EDTA and incubation was continued for 50 min. Subsequently, the solution was replaced by Ca,Mg-free solution containing 1 mM EDTA and the uterus was incubated for 20 to 30 min to see if the EDTA concentration modified the contractile response.

When the agonist addition was made, the contact time was approximately 5 min. Mechanical responses of the myometrium were recorded isometrically on a recorder (680 HP) with an amplifier (8805C HP) and a force displacement transducer (Gould Statham UC2).

Solutions. Physiological solutions had the following composition: Ringer Locke solution (mM): NaCl 154, KCl 5·63, $CaCl_2$ 2·16, $MgCl_2$ 2·10, $NaHCO_3$ 5·95 and glucose 5·55. Ca,Mg-free or Ca-free solutions, had the same composition except that $CaCl_2$ and $MgCl_2$ or $CaCl_2$ were, respectively, omitted and EDTA (3 or 1 mM) was added. High K^+ (56 mM) solution was obtained by substituting NaCl for KCl in equimolecular amounts in the Ca,Mg-free solution. The high K^+ solution did not contain EDTA.

Drugs used. Oxytocin (Syntocinon) was obtained from Sandoz. Sodium orthovanadate was purchased from Sigma. All other reagents were of analytical grade.

Statistical analysis. Statistical significance was evaluated with Student's *t*-test on unpaired basis and $P = 0·05$ was taken as the upper limit of significance.

RESULTS

In the oestrogen-dominated rat uterus, no contractile responses were obtained when $MgCl_2$ (1–32 mM) was added cumulatively to Ca,Mg-free solution containing EDTA 1 mM (n = 6).

A second experiment was designed to assess whether Mg^{2+} could enter K^+-depolarized uterine muscle cells and evoke contraction or whether it could inhibit the influx of Ca through voltage-sensitive channels. In this experiment the uterus, preincubated in Ca,Mg-free solution containing 1 mM EDTA was bathed for 20 min in high K^+ solution and cumulative amounts of $MgCl_2$ (1–32 mM) were introduced into the bath. Tension development did not occur (Fig. 1). After $MgCl_2$ washout, $CaCl_2$ (2 mM) was added. A sustained contraction was obtained and, subsequent addition of cumulative concentrations of $MgCl_2$ (1–32 mM) evoked concentration-dependent relaxation (Fig. 1) (6 preparations).

Another set of experiments was carried out to evaluate the influx of Mg^{2+} through receptor-operated channels. Two uterine horns from the same animal were incubated in a Ca-free solution, one of them with and the other without Mg^{2+}.

The uterine horn incubated in a Ca-free solution with Mg^{2+}, containing 3 mM EDTA, was stimulated by addition of a submaximal dose of oxytocin (10^{-2} unit mL^{-1}; Sakai et al 1981). The first spasm was evoked 5 min after incubating the uterine horn in Ca-free solution and maximum tension of 747 ± 109 mg was achieved (Table 1).

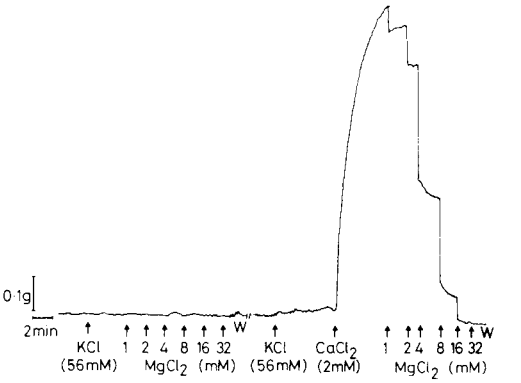

FIG. 1. No contraction was obtained after application of $MgCl_2$ in cumulative amounts in uterus depolarized by KCl 56 mM in Ca, Mg- and EDTA-free solution. Contraction was induced by addition of $CaCl_2$ (2 mM) and subsequent addition of cumulative amounts of $MgCl_2$ evoked concentration-dependent relaxation. W = wash.

Table 1. Maximum tension (mg) achieved by addition of oxytocin in presence (+) or absence (−) of Mg^{2+} in a Ca-free medium.

Incubation time (min)	Control Mg (+)	Mg (−)	
5	747 ± 109	364 ± 38	$P < 0·02$
50	187 ± 20	106 ± 11	$P < 0·01$
70	192 ± 19	47 ± 3	$P < 0·0005$

Mean ± s.e., n = 5.

After incubating the uterus for 50 min in the same solution, a sustained spasm was obtained by a second addition of oxytocin (10^{-2} unit mL^{-1}) but its magnitude was smaller. The tissue relaxed completely on washing out the agonist, and a subsequent addition of oxytocin made after incubating the uterus for 20 min in Ca-free solution containing 1 mM EDTA produced a similar spasm: 192 ± 19 mg (Fig. 2A).

In the experiment shown in Fig. 2B, the other uterine horn was exposed to Ca,Mg-free solution containing EDTA (3 mM). When oxytocin was added 5 min after incubating the uterine horn in Ca,Mg-free solution, a single and transient spasm was obtained and the maximum tension showed a signifi-

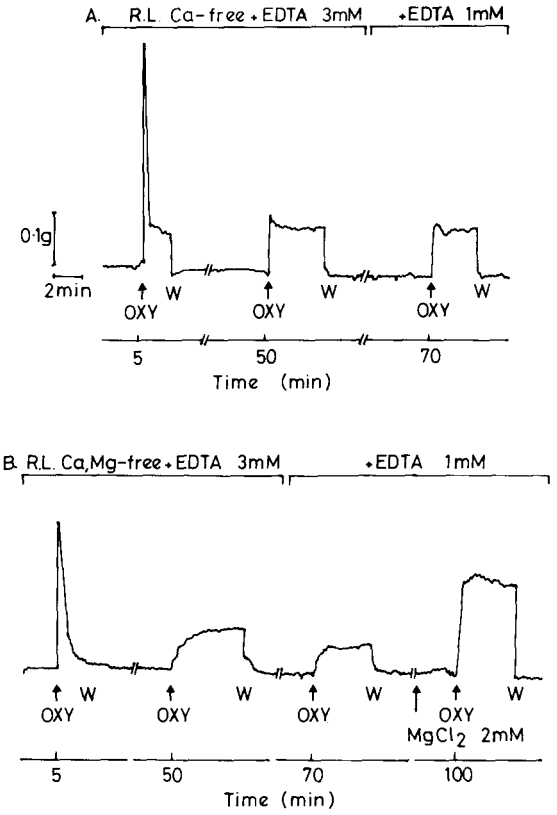

FIG. 2. A. Ca-free contractions of oestrogen-dominated rat uterus induced by oxytocin (OXY) 10^{-2} unit mL^{-1} at 5 and 50 min after incubating in Ca-free EDTA (3 mM) solution. After incubation for 20 min in the same solution but containing EDTA 1 mM, a further addition of oxytocin induces similar contraction. R.L. = Ringer Locke, W = wash. B. Ca,Mg-free contractions of the other uterine horn induced by oxytocin (OXY) 10^{-2} unit mL^{-1} at 5 and 50 min after incubating in Ca,Mg-free EDTA 3 mM solution. Subsequent incubation for 20 min in Ca,Mg-free EDTA 1 mM solution and addition of oxytocin induces a smaller contraction. R.L. = Ringer Locke, W = wash.

cant decrease relative to the control (Table 1). After the uterus had been incubated for 50 min in the same solution, addition of oxytocin 10^{-2} unit mL^{-1} promoted a sustained spasm, but the magnitude was smaller than the control response in the uterine horn incubated in Ca-free solution (Table 1). Subsequent incubation for 20 min in Ca,Mg-free solution containing EDTA 1 mM and addition of oxytocin induced a maintained spasm but its amplitude was small: 47 ± 3 mg.

When $MgCl_2$ 2 mM was added and 5 min later oxytocin was also added, a maintained spasm was elicited and its magnitude was noticeably greater than the preceding oxytocin response obtained in Ca,Mg-free medium: 182 ± 23 mg (Table 1).

To ascertain whether oxytocin responses tended to become smaller with repetitive oxytocin challenge in Ca,Mg-free medium, a new set of experiments was designed and oxytocin was added only once, after incubating the uterine horn for 50 min in Ca,Mg-free solution containing 3 mM EDTA and for 20 min in Ca,Mg-free solution containing 1 mM EDTA. The spasmogenic response obtained: 53 ± 9 mg, n = 5, was similar to that observed in the former experiments in Ca,Mg-free solution (47 ± 3 mg). When $MgCl_2$ was added cumulatively (1–32 mM) a concentration-dependent enhancement of the spasm was obtained (Fig. 3).

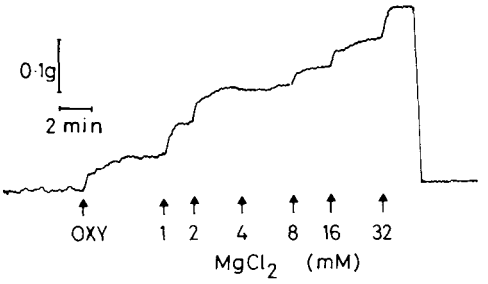

FIG. 3. Contraction induced by oxytocin (OXY) in uterus incubated for 50 min in Ca,Mg-free EDTA 3 mM solution and for 20 min in Ca,Mg-free EDTA 1 mM solution. Addition of cumulative amounts of $MgCl_2$ increases the spasmogenic response in a concentration-dependent manner.

Vanadate has been described as a potent inhibitor of both Na^+,K^+-ATPase (Grover et al 1980) and Ca^{2+},Mg^{2+}-ATPase (Di Polo et al 1979; Varecka & Carafoli 1982). During incubation of uterine preparations with Ca-free solution containing 1 mM EDTA, a submaximal dose of vanadate 8×10^{-5} M produced sustained spasm (Fig. 4A) which was completely offset by washing out vanadate. The maximum tension was 352 ± 49 mg (mean ± s.e., n = 5).

In simultaneous experiments, the other uterine horn was incubated in Ca,Mg-free solution containing EDTA 3 mM for 50 min and a similar solution containing EDTA 1 mM for 20 min. Fig. 4B shows the lack of effects of vanadate 8×10^{-5} M in these conditions. When $MgCl_2$ (1–32 mM) was added cumulatively, spasmogenic responses were obtained in a concentration-dependent manner in the presence of vanadate (n = 6). The maximum tension achieved was 410 ± 54 mg (mean ± s.e.).

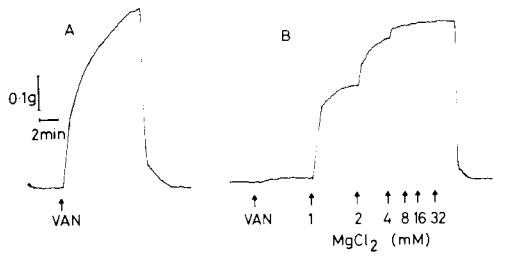

FIG. 4. A. Contraction induced by vanadate 8×10^{-5} M (VAN) in uterus incubated for 50 min in Ca-free solution containing EDTA 3 mM and for 20 min in Ca-free solution containing EDTA 1 mM. B. In the other uterine horn, no contraction was obtained after application of vanadate 8×10^{-5} M (VAN) when the uterus was incubated in a similar solution without Mg^{2+}. Addition of cumulative amounts of $MgCl_2$ in presence of vanadate evoked spasm in a concentration-dependent manner.

DISCUSSION

Our aim was to investigate the involvement of Mg^{2+} in contractile responses of rat uterus incubated in Ca,Mg-free solution. Cumulative concentrations of $MgCl_2$ were without effect when added to the oestrogen-dominated rat uterus incubated in Ca,Mg-free solution. In similar conditions, when the uterus was exposed to a high concentration of KCl (56 mM) to depolarize cell membranes and open voltage-sensitive Ca^{2+} channels, addition of cumulative concentrations of $MgCl_2$ did not promote tension development. This suggests that Mg^{2+} either does not penetrate the voltage-dependent Ca^{2+} channels or it does penetrate into the cell but does not have the capacity to activate the contractile machinery or to release stored Ca^{2+}.

However, when $CaCl_2$ was used to promote a sustained contraction, addition of cumulative amounts of $MgCl_2$ induced a concentration-dependent relaxation that may be due to Mg^{2+} blockade of voltage-dependent Ca^{2+} channels.

The contraction induced by oxytocin in the myometrium involves activation of specific receptors located in the plasma membrane (Bolton 1979) and is extremely resistant to Ca^{2+} removal (Sakai et al 1981; Ashoori et al 1985; Villar et al 1986). Furthermore, the tissue clearly loses Ca^{2+} in Ca-free medium under the same conditions in which the oxytocin response remains more or less constant (Ashoori et al 1985). It may be that the contraction is caused by a mechanism in which Ca^{2+} is not involved, as proposed by Casteels et al (1981).

When the uterus had been incubated in Ca,Mg-free solution, oxytocin promoted tension development and the presence of Mg^{2+} increased the spasm. This contraction obtained after addition of oxytocin in Ca,Mg-free medium decreases as time of incubation increases, and this decrease was not modified by previous contractions. In Ca-free solution containing Mg^{2+}, no decrease in the contractile response was observed and a sustained contraction with similar magnitude was obtained independent of incubation time in Ca-free solution. These results indicate that the loss of effect can be due to Mg^{2+} loss from the cells. However, the reduced response could have been the result of greater Ca^{2+} loss in the Mg^{2+}-free medium or the result of both Ca^{2+} and Mg^{2+} loss.

The fact that oxytocin was able to contract uterine smooth muscle when extracellular Ca^{2+} and Mg^{2+} were absent indicates that this contraction probably was caused by an increase in the intracellular free Ca^{2+} concentration via a direct release of stored Ca^{2+}. The evidence that Mg^{2+} increases the contractile response may suggest that this ion can penetrate into the cell and facilitate the contraction.

Bolton (1979) has suggested that the receptor-operated Ca^{2+} channel has a much greater permeability to ions other than Ca^{2+}, and it is, therefore, possible that Mg^{2+} penetrates into the smooth muscle cell through a channel activated by an oxytocin receptor. It is possible that Mg^{2+} penetrates into the cell through receptor-operated channels and not through voltage-dependent channels since Mg^{2+} did not promote uterine contraction when $MgCl_2$ was added to the K-depolarized uterus.

However, oxytocin is known to inhibit Ca^{2+}-stimulated ATPase in myometrial plasma membrane (Åkerman & Wirkström 1979; Carsten & Miller 1977) and this ATPase is considered to be involved in the regulation of intracellular free Ca^{2+} levels. Therefore, ATPase inhibition by oxytocin could be the reason for the release of stored Ca^{2+} and the maintenance of the contraction. It follows then that vanadate, a potent inhibitor of Ca^{2+},Mg^{2+}-ATPase, should act in the same manner. This did not occur; vanadate did not promote smooth muscle contraction when the uterus had been incubated in Ca,Mg-free solution.

Moreover, the failure of vanadate to induce uterine contraction in Ca,Mg-free solution was counteracted by addition of cumulative amounts of Mg, which indicates that the presence of this ion in the extracellular medium may be essential if vanadate is to act. Since Mg^{2+} does not penetrate into the cell non-specifically, or through voltage-dependent channels, it is likely that vanadate acts by permitting Mg^{2+} entry, but the present data are not sufficient to establish the exact mechanism of this action.

It is interesting that Mg^{2+}, an inorganic blocker of voltage-dependent Ca^{2+} channels, acts in the way reported here, increasing the contractile response mediated by oxytocin receptors and playing an essential role in vanadate-induced contraction in Ca-free medium. The present results provide a basis for assuming that the inhibition of Ca-ATPase by oxytocin increases the contractile response induced by this agonist in Ca-free medium but is not an essential step in the process of stimulus-contraction coupling.

Although these pharmacological analyses must be interpreted with reservation and require confirmation, the oxytocin and vanadate response seems somewhat difficult to explain in terms of the generally accepted mechanism in which Ca^{2+} plays an essential role.

The fact that Mg^{2+} increases uterine spasm elicited by oxytocin and is essential for the spasmogenic response induced by vanadate can be explained either by a direct action of the ion on contractile proteins or by a release of stored Ca^{2+} caused by Mg^{2+}. Alternatively, a combination of the above mechanisms might be occurring.

Further support for an intracellular action of Mg^{2+} comes from experiments showing that tension in skinned smooth muscle fibres can be induced in the absence of Ca^{2+} by high $MgCl_2$ concentrations (6–20 mM) (Ikebe et al 1984) similar to those tested in the present work. This observation suggests a direct action of Mg^{2+} on contractile proteins. However, the role played by Mg^{2+} in the contractile response induced by inhibition of Ca^{2+},Mg^{2+}-ATPase indicates that Mg^{2+} may be essential in this action.

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