Relationship between Mn-Induced Contractile Inhibition and Cyclic AMP Content in the Longitudinal Muscle of Estrogen-Treated Rat Uterus

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Summary: In longitudinal myometrial strips of estrogen-treated rats, nifedipine ($0.03 \mu\text{M}$) induced the gradual inhibition of phasic contractions evoked by repetitive electrical stimulations, with inhibition reaching a plateau level ($43.4 \pm 2.6\%$ of the control group, $n=6$) within 20 min. In contrast, 0.5 mM Mn induced a rapid and transient suppression of the phasic contractions ($58.3 \pm 8.9\%$ of the control group, $n=10$, at 4 min) followed by sustained inhibition of the contractions, the level of which was slightly lower than that of the control group ($84.2 \pm 5.0\%$, $n=10$, at 20 min). The Mn also induced a rapid and transient increase in cellular cAMP level, which

reached a peak ($201.2 \pm 20.8\%$ of the control group, $n=5$) at 2 min and decreased to the pre-stimulation level ($=100\%$) within 15 min. Pre-treatment with 1 mM isobutylmethylxanthine (IBMX), a phosphodiesterase inhibitor, potentiated the effect of Mn on the cAMP level. The transient effects of Mn, but not of nifedipine, on the contractions and cAMP levels (and their potentiation by IBMX) do not support the simple action of Mn as a Ca antagonist, but are compatible with the intracellular dual action of Mn on adenylate cyclase. [Japanese Journal of Physiology, 47, 143–146, 1997]

Key words: Mn, cAMP, rat myometrium.

Mn has been generally regarded as a Ca antagonist to suppress action potential and the contraction of various types of smooth muscles, including the longitudinal muscle of estrogen-treated rat uterus [1, 2]. The inhibition of myometrial contraction is also induced by dibutyl cAMP, forskolin, isoprenaline, and relaxin through an increase in cellular cAMP content [2, 3]. Mn is also known to activate adenylate cyclase in membrane-disrupted myometrium [4]. In this study, we found that phasic contraction and electrical activity of the longitudinal muscle of estrogen-treated rat uterus, as induced by repetitive electrical stimulation, were initially depressed and subsequently recovered gradually during the application of 0.5 mM Mn for 45 min. The changes in cAMP content caused by 0.5 mM Mn were sequentially examined in the absence and presence of 1 mM IBMX, a potent phosphodiesterase inhibitor.

About 2 weeks after being subjected to ovariec-

tomies, estradiol- 17β -benzoate was applied to female Wistar rats (250–300 g) by subcutaneous injection ($40 \mu\text{g/d}$) for 3 d. Longitudinal muscle strips were dissected from the middle portion of the uterus and equilibrated with Krebs solution at 36°C for 1 h before the experiment began. Electric pulses (0.5 s duration) with a supramaximal intensity were applied every 90 s by platinum electrodes that were placed near both ends of the strips. Phasic contractions generated by repetitive electrical stimulation were measured by a force transducer. The Krebs solution had the following composition (mM): NaCl 123.1, NaHCO_3 15.5, KCl 4.7, KH_2PO_4 1.2, MgCl_2 1.2, CaCl_2 2.5, and glucose 11.5. The solution was bubbled with a gas mixture of 95% O_2 and 5% CO_2 , and the resulting pH was 7.4 at 36°C . Forskolin, nifedipine, IBMX, and cAMP were obtained from Sigma (USA). Mn was applied as MnCl_2 .

In Fig. 1a, phasic contractions were progressively depressed by $0.03 \mu\text{M}$ nifedipine, which is an organic

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blocker for L type Ca channel. Thereafter, nifedipine-induced depression reached a plateau level within 20 min, which was $43.4 \pm 2.6\%$ (mean $\pm$ SEM, $n=6$) of the control group. Figure 1b shows that contractile depression was caused immediately by the application of 0.5 mM Mn and reached a maximum ($58.3 \pm 8.9\%$ of control group, $n=10$) in 4 min. Thereafter, the phasic contractions recovered gradually to $84.2 \pm 5.0\%$ ($n=10$) in 20 min, even in the continuous presence of Mn.

The electrical activity of the muscle cells was recorded intracellularly with a glass microelectrode filled with 3 M KCl ($30\text{--}50\text{ M}\Omega$ in resistance). The muscle strip was mounted in a partition chamber [5]. Mechanical activity was recorded simultaneously. Action potential generated by electrical stimulation consisted of spikes and a plateau component (Fig. 1c, left). The application of 0.5 mM Mn for 3 min decreased the amplitude of the initial spike, the duration of the plateau component, and the frequency of repetitive spikes on the plateau component. Consequently, the duration and amplitude of contractions became smaller (Fig. 1c, middle). Thereafter, the duration of action potential and contraction recovered, but the am-

plitude of the initial spike continued to decrease even after the prolonged application (15 min) of 0.5 mM Mn (Fig. 1c, right).

The cellular content of cAMP was measured by high-performance liquid chromatography (HPLC). After 1 h incubation with Krebs solution, tissues were exposed to 0.5 mM Mn for 1, 2, 5, 15, and 45 min. In another series of experiments, 1 mM IBMX was applied 30 min before and during the application of 0.5 mM Mn. Cellular cAMP was extracted by a conventional method with a minor modification [2, 6]. A HPLC system from Hitachi, Ltd. (Tokyo, Japan) was used, and consisted of a L-6000 pump, a L-4000 UV detector, a Rheodyne 7125 injector, and a D-2500 Chromato-Integrator. The column used was a Wakosil II 3C18HG 4.6×150 mm (Wako Pure Chemical Industries, Ltd.). The mobile phase for HPLC was a mixture of 100% methanol and 20 mM KH_2PO_4 (1:9, v/v), which was degassed under reduced pressure before use. The flow rate was 1.0 ml/min, and the injection volume of the samples was 10 μl . Peaks were monitored at a wavelength (λ) of 254 nm. Room temperature was maintained at 25°C. Validation of the cAMP in the tissue extracts was carried out by the comparison of peak retention times, co-chromatography, and comparison of peak-height ratios (280 nm/254 nm) with a reference standard cAMP. The detection limit was 0.3125 pmol per injection.

Figure 2 shows the effect of 0.5 mM Mn on the cAMP content and its enhancement by 1 mM IBMX. The cAMP content was 0.310 ± 0.016 pmol/mg wet

Fig. 1. Effects of 0.03 μM nifedipine (a) and 0.5 mM Mn (b) on phasic contractions evoked by repetitive electrical stimulation to the longitudinal muscle of estrogen-treated rat uterus. Application of the agents is indicated by the solid lines above. The dashed lines indicate the peak levels (=100%) of phasic contractions before the application of the agents. The records in (c) show the effects of 0.5 mM Mn on membrane activities (upper traces) and accompanying contractions (lower traces) before (left panel) and after (3 min in center panel and 15 min in right panel) the application of Mn.

Fig. 2. Time courses of the changes in cAMP content induced by 0.5 mM Mn in the absence (open circles) and presence (closed circles) of 1 mM IBMX. For the IBMX-treated strips, IBMX was applied 30 min before the application of Mn. Data are mean with SEM (shown by vertical bars) of 5 independent measurements. * $p < 0.05$, by unpaired Student's *t*-test, as compared to the control values (0 min).

weight ($n=5$) in tissues equilibrated with control solution, and increased to 0.625 ± 0.072 pmol/mg wet weight ($n=5$) 2 min after the application of 0.5 mM Mn. The cAMP content then began to decrease to nearly the control value within 15 min. Thirty minutes after the treatment with 1 mM IBMX, the basal cAMP content increased to 1.903 ± 0.276 pmol/mg wet weight ($n=5$). When 0.5 mM Mn was applied in the presence of IBMX, the cAMP content increased to 4.009 ± 0.253 pmol/mg wet weight ($n=5$) after 5 min, and thereafter decreased to reach the level before the application of Mn. The increase of cAMP content in the tissue may be caused either by the stimulation of adenylate cyclase or a decrease of cyclic nucleotide phosphodiesterase activity. These results favor the view that the application of Mn increases cAMP content by the activation of adenylate cyclase, because the effect of Mn was most pronounced when the tissues were pretreated with IBMX.

The main finding of this study is the initial depression and gradual recovery of phasic contractions during the application of 0.5 mM Mn (Fig. 1). The contractile depression by Mn was accompanied by a decrease in the duration of the action potential (Fig. 1). It was reported that db cAMP decreases both the duration of the action potential and the amplitude of the corresponding phasic contraction in the longitudinal muscle of rat uterus [7], suggesting that the increase of cAMP content causes the decrease in the duration of the action potential in rat uterus. In this study, the Mn-induced elevation of the cAMP content was transient (Fig. 2). It was shown that Mn permeated through the cell membrane immediately after application to the longitudinal and circular muscles of rat uterus [8]. The Mn concentration in the tissue increased continuously over time while the tissue was exposed to 5 mM Mn for 120 min in the ileal muscle of guinea pig [9]. Therefore, it is likely that Mn accumulated in the cell gradually while the tissues were exposed to 0.5 mM Mn during this experiment. It has also been shown in the membrane preparation of rat erythrocytes that Mn is an essential allosteric effector for adenylate cyclase by acting at a metallic site of the catalytic unit of the enzyme: the enzyme activity reached a maximum at about 2 mM Mn and was rather inhibited by supramaximal concentration (>4 mM) [10]. Using a catalytic unit of adenylate cyclase from the rat uterine smooth muscle, it was demonstrated that the activation of adenylate cyclase by forskolin was potentiated more strongly by 1 mM Mn than 5 mM Mn [4]. Thus, adenylate cyclase is stimulated or inhibited depending on the concentration of Mn.

Forskolin (0.2 μ M), a potent adenylate cyclase acti-

vator, mimicked the transient effect of Mn on phasic contractions in rat uterus (data not shown), thus showing compatibility with the possible involvement of an adenylate cyclase/cAMP system in the Mn action. However, the extent of recovery was smaller in forskolin-induced relaxation than in Mn-induced relaxation ($p<0.05$): at 45 min, $71.9 \pm 7.6\%$ (forskolin, $n=6$) and $85.1 \pm 5.7\%$ (Mn, $n=5$). Forskolin (0.2 μ M) also elevated the cAMP level to $210.0 \pm 44.3\%$ at 15 min ($n=3$), although the time to the peak level varied (15–45 min) (data not shown).

In conclusion, it seems probable that when the longitudinal muscle of estrogen-treated rat uterus is exposed to 0.5 mM Mn-contained Krebs solution, Mn may enter into the cell, activate adenylate cyclase, and depress the phasic contractions, beside its simple action as a Ca antagonist. The Mn concentration in the cell increased during prolonged exposure to 0.5 mM Mn solution over the optimal concentration, and therefore suppressed the adenylate cyclase activity. As a result, the cAMP level decreased and phasic contractions recovered. However, Mn concentrations utilized for membrane-disrupted systems and intact cells appear so different that further investigation is needed for the elucidation of the above arguments.

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