Short Communication

Pretreatment with Porcine Relaxin and Mg Ions Enhances
Inhibitory Effects of Dibutyryl Cyclic AMP in
Longitudinal Muscle of Estrogen-Treated
Rat Myometrium

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Summary Phasic contractions were evoked in Mg-free Krebs solution by electrical stimulation in the longitudinal myometrial strip taken from estrogen-treated rats, and the application of $30\text{ }\mu\text{M}$ dibutyryl cyclic AMP (db cAMP) for 15 min depressed the amplitude of contraction to $81\pm 18\%$ ($\pm\text{SD}$, $n=23$) of the control. The contractile depression caused by db cAMP was not significantly altered by pretreatment with either 1 mM Mg (40 min) or 100 mU porcine relaxin (15 min) alone; however it was markedly enhanced to $22\pm 30\%$ ($n=7$) of the control response by the combined pretreatment with Mg and relaxin. The enhancement lasted for longer than 2 h thereafter in Mg-free Krebs solution. It was discussed that some memory factor was induced by relaxin in the presence of 1 mM Mg to respond strongly to db cAMP.

Key words: Mg, db cAMP, relaxin-induced memory.

The plateau potential generated in circular or longitudinal muscle of estradiol-injected rats is inhibited by relaxin, and the phasic contraction is depressed [1, 2]. The relaxin effect is made stronger by the presence of 0.6 mM Mg, and the contractile depression persisted for longer than 2 h after the removal of relaxin [2]. The present experiment aimed to clarify the persistent effect mentioned above at least in part, taking contractile depression caused by db cAMP as an indicator.

Wistar strain female rats (200–250 g) were ovariectomized, were given a single subcutaneous injection of $40\text{ }\mu\text{g}$ estradiol- 17β -benzoate, and were used after 72 h. Longitudinal muscle strips were dissected from the middle portion of the uterus. The control solution was Mg-free Krebs solution ($36.5\pm 0.5^\circ\text{C}$). Platinum electrodes were placed near the ends of the muscle strips for electrical stimulation, and electric pulses, 0.5 s in duration with supramaximal intensity, were applied every 90 s. Acryl and silicone wares were used at all times. Db cAMP (N^6 , $2'$ - O -

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Fig. 1. Effects of $30\text{ }\mu\text{M}$ db cAMP before and after the treatment with 1 mM Mg (a), 100 mU relaxin (b), combination of Mg and relaxin (c), and Ca-free solution containing 15 mM Mg and 40 mM K (d). In record d, electrical stimulation was discontinued for the period between the dots. Experiments were made on different muscle strips obtained from different rats.

dibutyryladenosine 3':5'-cyclic monophosphate) was obtained from Sigma, and porcine relaxin (NIH RXN-P1) was given by National Hormone and Pituitary Program, USA.

Spontaneous contractions disappeared after about 20-min incubation with warm Mg-free Krebs solution, and thereafter electric stimulation generated phasic contractions of nearly constant amplitude. The maximum depression was reached by the application of $30\text{ }\mu\text{M}$ db cAMP in 12–15 min. The contractile depression was not altered after the pretreatment with 1 mM Mg for 40 min (Fig. 1a). After the pretreatment with 100 mU relaxin (15 min), the contractile depression caused by db cAMP was enhanced, and the enhancement became attenuated by a further exposure to control solution (Fig. 1b). In Fig. 1c, 100 mU relaxin was applied in the presence of 1 mM Mg. After the pretreatment with relaxin and Mg, db cAMP caused a strong depression of contractions, and enhanced contractile depression was again produced: depression by db cAMP persisted even after further exposure to Mg-free Krebs solution. To compare with Fig. 1c, the effects of db cAMP were examined in Fig. 1d before and after the muscle strip was exposed to the Ca-free solution containing 15 mM Mg and 40 mM KCl, where 62.5 mM NaCl was reduced to maintain osmolarity. Contractile depression by db cAMP was slightly enhanced after combined perfusion of 15 mM Mg and 40 mM K, but became attenuated again by a further exposure to the control solution.

Fig. 2. Effects of the pretreatment with 1 mM Mg (a, arrows), 100 μ U relaxin (b, broken lines), and combination of Mg and relaxin (c) on the phasic contraction during the application of 30 μ M db cAMP (15 min) was expressed as % response of the amplitude of phasic contractions evoked in Mg-free solution (ordinates). Responses obtained from individual muscle strips are indicated by different symbols, and are connected by lines.

The results shown in Fig. 1a, b, and c are graphically summarized in Fig. 2. The degree of contractile depression by db cAMP differed from preparation to preparation even when they were obtained from the same rat, and the response to inhibitory agents (relaxin, forskolin, db cAMP, 8-bromo cGMP) varied with the incubation period [2–4]. Therefore, the results are summarized according to the incubation period (abscissae) for individual muscle strips. In Fig. 2b, contractile depression by db cAMP was enhanced in 4 muscle strips out of 7, and the means ($\pm$ SD) of the % response of contraction in the presence of 30 μ M db cAMP were 72 ± 18 and 64 ± 15 before and after the treatment with relaxin, respectively. In Fig. 2c, contractile depression was enhanced in all 7 muscle strips examined, and the means ($\pm$ SD) of the % response of contraction were 89 ± 6 and 22 ± 30 , respectively, before and after the combined treatment with Mg and relaxin. It is noted that the enhancement of contractile depression in response to db cAMP still remained for about 2 h in the control solution (Mg-free Krebs solution).

It is evident that a coexistence of external Mg and relaxin was needed to generate a long-lasting effect to respond strongly to db cAMP. One role of Mg is to promote the binding of relaxin with receptor [5] to enhance the relaxin action. However, it is to be pointed out that even when contractile depression was strong enough in Mg-free Krebs solution, the long-lasting effect was not markedly developed (Fig. 1b). On the other hand, the intracellular free Mg concentration ($[Mg]_i$) in intact smooth muscle cells is estimated to be around 0.5 mM, and it could be elevated by mild or severe changes in the extracellular environment [6, 7]. An increase in $[Mg]_i$ could be assumed to be responsible for the enhancement of cAMP-dependent relaxation of the myometrium because biochemical studies indi-

cate that protein kinase A is activated by Mg ions ranging from 1 to 10 mM [8]. In fact, an increase in $[Mg]_i$ by $40 \mu M$ has been reported for the myometrium by the ionic maneuver described for Fig. 1d [4]. However, the above concept is not applicable to relaxin because changes in $[Mg]_i$ was not detected by the measurement using mag-fura 2 (data not shown). Therefore, why external Mg was needed to produce the long-lasting effect caused by relaxin remains open to question.

Although activation of protein kinase A is commonly proposed for adrenergic β agonists and relaxin [9], a different physiological manifestation has also been noticed: isoprenaline hyperpolarizes the membrane, whereas relaxin does not [1]. Furthermore, the isoprenaline-induced relaxation was enhanced, whereas relaxin-induced relaxation was attenuated when the tissue was treated with 0.6 mM Mn [2]. The strong and long-lasting enhancement of contractile depression by db cAMP was characteristic for pretreatment with relaxin in the presence of Mg, but that was not achieved for relaxation produced by isoprenaline or forskolin (unpublished observation). For the convenience of following description, the phenomenon is tentatively called a "relaxin-induced memory." It may be presumed that some putative factor, a candidate is a substance which inhibits phosphodiesterase(s) activity, is produced intracellularly, so that the muscle strip is conditioned to respond strongly to db cAMP.

The contractile depression caused by $10 \mu M$ 8-bromo cGMP was also enhanced during the period of relaxin-induced memory (unpublished observation). Even though the above discussion is full of speculations and concerns only a part of relaxin actions, it is possible that relaxin initiates a Mg-dependent positive feedback system in operating myometrial relaxation mediated by a pathway which crosses over both cAMP- and cGMP-dependent processes.

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