EFFECTS OF DILTIAZEM ON ELECTRICAL AND MECHANICAL ACTIVITIES OF ISOLATED GUINEA PIG TAENIA COLI

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Abstract—Effects of diltiazem on electrical and mechanical activities of isolated guinea pig taenia coli were studied by means of the double sucrose-gap method. In the spontaneously active preparations, diltiazem (2.2 × 10⁻⁶ M) suppressed both electrical activity and isometric contraction, while electrical and mechanical activities evoked by the depolarizing current pulse were not affected at the concentration of 2.2 × 10⁻⁴ M. In the presence of 2.2 × 10⁻⁵ M diltiazem, the evoked contractile force and the number of repetitive firings during depolarization were reduced, whereas the single spike was almost unchanged or somewhat inhibited. At 2.2 × 10⁻⁴ M diltiazem, both electrical and mechanical activities were almost abolished. The contractile force and single spike suppressed by diltiazem were partly reversed by the addition of 5 mM CaCl₂. There was little significant change in membrane potential and membrane resistance. Similar but somewhat weaker effects were observed when NaCl was replaced with sucrose. In some preparations, 2.2 × 10⁻⁴ M diltiazem reduced the contractile force without significant influence on the electrical activity in Na⁺-free Locke solution. CoCl₂ (3 mM) inhibited the evoked activities in both normal and Na⁺-free solutions. Possible mechanisms for the relaxing effects of diltiazem on isolated guinea pig taenia coli were discussed.

Diltiazem (d-3-acetoxy-cis-2,3-dihydro-5-[2-(dimethylamino) ethyl]-2-(p-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one hydrochloride (1)), a new coronary vasodilator (2), has been shown to have a calcium-antagonistic property in the cardiac ventricular (3, 4) and vascular smooth muscles (5, 6). Results of simultaneous measurements of the transmembrane action potential and contractile force of isolated guinea pig papillary muscle suggested that diltiazem may cause a decrease in the contractile force either by interfering with the transmembrane influx of calcium ion or by intracellularly reducing the free calcium ion concentration available for the contractile system (3, 4). On the other hand, in isolated vascular smooth muscle of dog coronary artery or rabbit aorta, diltiazem antagonizes calcium ion and thus causes a relaxing effect (5). It was also demonstrated that the compound suppresses the contraction of isolated guinea pig taenia coli in both normal and K⁺-rich solutions (7, 8). The membrane effect of diltiazem on the smooth muscle, however, remained unexamined.

In the present experiments, the effects of diltiazem on electrical and mechanical activities

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were studied by means of the double sucrose-gap method in isolated guinea pig taenia coli whose spike activity is due to calcium entry (9-13). Effects of CoCl₂, which is known to inhibit the generation of calcium spike (14, 15), were also examined.

MATERIALS AND METHODS

Male guinea pigs weighing about 300 g were stunned and bled. A length of taenia coli (approx. 20 × 0.5 mm) was dissected from the caecum and mounted in the sucrose-gap apparatus. The double sucrose-gap method for recording the electrical activity and isometric contraction was the same as that described by Kuriyama and Tomita (11) and Magaribuchi et al. (16): a small portion (less than 1 mm) in the central part of the tissue was exposed to the test solution and the remaining parts on both sides were perfused with isotonic sucrose solution. A modified Locke solution with the following composition was used (mM): NaCl 141.9, KCl 5.6, CaCl₂ 2.2, glucose 5.5, Tris-HCl buffer 10 (pH 7.4). The solution was continuously aerated with oxygen. When NaCl in Locke solution was eliminated, such was substituted by an osmotically equivalent sucrose solution. The test compound dissolved in normal or Na⁺-free Locke solution was prepared in a separate reservoir and was applied to the preparation through a small vinyl tubule connected to the apparatus. The final concentration of the test compound is described in 'results'. In experiments with evoked activity, depolarizing current pulse of the order of 10⁻² A (duration 300 msec, 3 sec) was applied to the preparation. Each record shown in this paper was selected out of 4 to 5 experiments. Experiments were carried out at a room temperature of 25 ± 1°C.

RESULTS

Effects of diltiazem on the spontaneous activity in normal Locke solution

Fig. 1 shows the effects of diltiazem on spontaneous electrical and mechanical activities of isolated taenia coli in normal Locke solution. Diltiazem at the concentration of 2.2 × 10⁻⁷ M decreased the frequency of spontaneous electrical discharge, the slope of slow potential

FIG. 1. Effects of diltiazem on the spontaneous electrical and mechanical activities of isolated taenia coli in normal Locke solution. Upper trace, intensity of applied current pulse; middle trace, potential change; lower trace, isometric contraction. In A, a, control; b and c, 10 min after addition of 2.2 × 10⁻⁷ and 2.2 × 10⁻⁶ M diltiazem, respectively. In B, hyperpolarizing and depolarizing current pulses were applied. a, 2.2 × 10⁻⁶ M diltiazem was added; b, 7 min after addition of diltiazem; c, 20 min after washing out the compound.
The amplitudes of the spike and isometric contraction were slightly reduced. The membrane potential did not change significantly. When the concentration of diltiazem was increased to $2.2 \times 10^{-5}$ M (Fig. 1A c), the spontaneous spike discharge and isometric contraction were abolished and the muscle tone was further decreased. Changes in membrane potential were hardly discernible. The effects of diltiazem were reversed after washing out the compound (Fig. 1B c).

To elucidate whether the suppression of spontaneous spike discharge caused by diltiazem is due to inhibition of the pacemaker potential or to interference with the spike component, the effect of diltiazem was investigated on the spike evoked by electrical stimulation. A depolarizing current pulse (duration 3 sec) was applied to the preparations which exhibited spontaneous activity. The contractile force in response to the evoked spike was also recorded. As shown in Fig. 1B, the evoked spike and the corresponding contractile force were not affected significantly 7 min after the addition of $2.2 \times 10^{-5}$ M diltiazem (Fig. 1B b), while the spontaneous electrical and mechanical activities were abolished about 2 min after addition of the compound. Fig. 1B also shows that the electrotonic potential, which indicates the membrane resistance, induced by applying the hyperpolarizing current pulse (duration 3 sec, approx. $3 \times 10^{-7}$ A) remained practically unchanged in the presence of $2.2 \times 10^{-5}$ M diltiazem.

Effects of diltiazem on evoked electrical and mechanical activities in quiescent preparations

Experiments in normal Locke solution: Diltiazem at a lower concentration ($2.2 \times 10^{-6}$ M) produced no significant influence on spike activity and the contractile force evoked by depolarizing current pulse. On the other hand, $2.2 \times 10^{-5}$ M diltiazem gradually reduced the number of repetitive firings during depolarization of a long pulse (duration 3 sec), whereas

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**Fig. 2.** Effects of $2.2 \times 10^{-3}$ M diltiazem on the evoked activities in normal Locke solution. A: An example of the experimental record. a, control; b, 10 min after addition of diltiazem; c, 5 min after addition of CaCl$_2$ (5 mM) in the presence of $2.2 \times 10^{-3}$ M diltiazem. Hyperpolarizing current pulse (duration 3 sec, approx. $2 \times 10^{-7}$ A) was also applied. B: Time courses for the effect of diltiazem on the electrical and mechanical activities evoked by depolarizing current pulse (duration 3 sec). Data were derived from the experiment shown in Fig. 2A. The amplitude of the initial single spike was estimated from the resting potential level. Results were expressed as $\%$ of control. ——, amplitude of initial single spike; •••, contractile force (C.F.). Stars in the figure (★) indicate the inhibition of repetitive firing.
the initial single spike remained unchanged or was reduced to a certain extent (Fig. 2). The contractile force decreased with time. When a short depolarizing current pulse (duration 300 msec) was applied, a single spike without repetitive firing was evoked, the generation of which was somewhat inhibited by $2.2 \times 10^{-5} \text{M}$ diltiazem (Fig. 2A). The contractile force was also reduced. The membrane resistance and membrane potential were not affected significantly. When $\text{CaCl}_2$ (5 mM) was added to the preparation exposed to diltiazem, the contractile force was partly recovered (Fig. 2A c).

A higher concentration of diltiazem ($2.2 \times 10^{-4} \text{M}$) caused a more rapid and pronounced

![Graph](image)

**Fig. 3.** Effects of $2.2 \times 10^{-4} \text{M}$ diltiazem on the evoked activities in normal Locke solution. a, $2.2 \times 10^{-4} \text{M}$ diltiazem was added; a, b, continuous recordings; c, 10 min after addition of diltiazem; d, 12 min after addition of diltiazem. A conditioning depolarization was applied. e and f, 6 min and 7 min after addition of $\text{CaCl}_2$ (5 mM) in the presence of diltiazem, respectively.

![Graph](image)

**Fig. 4.** Time courses for the effect of $2.2 \times 10^{-4} \text{M}$ diltiazem in normal Locke solution. Explanations are the same as in Fig. 2B. Data were derived from the experiment shown in Fig. 3.
inhibitory effect on the evoked activities as compared to the action of $2.2 \times 10^{-5}$ M. As represented in Figs. 3 and 4, the initial single spike and repetitive firing were suppressed. In parallel to these electrical changes, the contractile force decreased and finally disappeared. The membrane potential and membrane resistance remained practically unchanged. Application of conditioning depolarization (Fig. 3d) produced a partial recovery of the single spike, whereas repetitive firing and isometric contraction were not reversed. On the other hand, the contractile force as well as the single spike were partly recovered by the addition of CaCl$_2$ (5 mM) (Figs. 3 e, f).

Experiments in Na$^+$-free Locke solution: To verify that sodium ion is not involved in the membrane activity, experiments were carried out in Na$^+$-free Locke solution. When NaCl in Locke solution was substituted by osmotically equivalent sucrose solution, the muscle tone increased transiently and then returned to the initial resting level. Simultaneously with the increase in muscle tone, spontaneous burst discharge appeared. It was also evident that in Na$^+$-free Locke solution, duration of the isometric contraction increased and the rate of relaxation decreased.

In the present study, experiments were started 30 min or more after replacing NaCl, i.e., after the evoked electrical and mechanical activities attained an equilibration in Na$^+$-free Locke solution.

The effects of diltiazem in Na$^+$-free Locke solution were similar to, but somewhat weaker than those in normal Locke solution. A lower concentration of diltiazem ($2.2 \times 10^{-6}$ M) had no influence on the evoked spike and isometric contraction in Na$^+$-free solution. At $2.2 \times 10^{-5}$ M diltiazem, the repetitive firing during depolarization was reduced but the generation of initial single spike was not greatly affected. The contractile force was gradually decreased with time. In the presence of $2.2 \times 10^{-4}$ M diltiazem (Fig. 5), the number of repetitive firings and contractile force were reduced. The amplitude of initial single spike was slightly suppressed. There was little significant change in the membrane potential. It was also observed that addition of CaCl$_2$ (5 mM) partly reversed the contractile force and single spike reduced by diltiazem (not shown in the figure).

In some preparations, diltiazem decreased the contractile force without producing any significant influence on the evoked electrical activity in Na$^+$-free solution. A typical example of the results is shown in Fig. 6. The contractile force was decreased by approx. 50% of the control about 5 min after the addition of $2.2 \times 10^{-4}$ M diltiazem (Fig. 6A c) while

![Fig. 5. Effects of 2.2 $\times 10^{-4}$ M diltiazem on the evoked activities in Na$^+$-free Locke solution. a, control; b, c and d, 2 min, 5 min and 8 min after addition of diltiazem, respectively.](image-url)
no significant change was observed on the initial single spike and on the repetitive firing. Thereafter, the repetitive firing was inhibited gradually, whereas the single spike still retained a normal magnitude.

Effects of CoCl₂ on evoked electrical and mechanical activities

As shown in Fig. 7, 3 mM CoCl₂ abolished not only the repetitive firing but also the initial single spike in both normal and Na⁺-free Locke solutions. The contractile force was also inhibited by CoCl₂. Application of the conditioning depolarization (Fig. 7a) did not restore the original amplitude of the action potential, suggesting that the inhibitory action of CoCl₂ may not be due to the increase in electrical threshold. In the presence of CoCl₂, the membrane potential was not affected, or, the membrane was slightly hyperpolarized in

Fig. 6. Effects of 2.2 × 10⁻⁴ M diltiazem on the electrical and mechanical activities evoked by depolarizing current pulse in Na⁺-free Locke solution. A: Experimental record. a, control; b, c, d and e, 2.6 min, 5.2 min, 7.8 min and 11.7 min after addition of diltiazem, respectively. B: Time courses for the effect of 2.2 × 10⁻⁴ M diltiazem. Explanations are the same as in Fig. 2B. Data were derived from the experiment shown in Fig. 6A.

Fig. 7. Effects of CoCl₂ (3 mM) on the evoked activities in either normal or Na⁺-free Locke solution. In a, conditioning depolarization was applied at the end of the experiments. The last panel in record b was taken 5 min after washing out the compound.
DISCUSSION

In the present experiments, it was demonstrated that cobalt ion, which is known to be an inhibitor of calcium spike (14, 15), suppressed evoked electrical and mechanical activities of guinea pig taenia coli in both normal and Na⁺-free Locke solutions. Inhibition of electrical activity could not be ascribed to increase in the electrical threshold for the excitation of cell membrane. Thus, the present data are consistent with the hypothesis that spike activity of taenia coli is due to the transmembrane influx of calcium ion (9-13), although the mechanism underlying the generation of the repetitive firing is still uncertain.

When NaCl was eliminated from normal Locke solution, the duration of isometric contraction was increased and the rate of relaxation was reduced. It was thus assumed that according to the Na-Ca exchange mechanism (17, 18), the efflux of calcium ion from the myoplasm is reduced in Na⁺-free solution, thereby causing an increase in intracellular concentration of free calcium ions.

In the spontaneously active preparations of taenia coli, diltiazem (2.2 × 10⁻⁷ M) reduced the frequency of spikes and the slope of pacemaker potential as well as the muscle tone. The amplitudes of the spike and isometric contraction were slightly decreased. When the concentration of the compound was increased to 2.2 × 10⁻⁶ M, both the spontaneous electrical activity and isometric contraction were abolished. Since diltiazem (2.2 × 10⁻⁶ M) produced no influence on the spikes evoked by electrical stimulation, it is possible that inhibition of the spontaneous spike discharge caused by diltiazem is not due to the suppression of the spike component but rather can be ascribed primarily to the inhibition of the pacemaker potential. As shown in the present experiments, the membrane potential was not significantly affected by diltiazem, suggesting that hyperpolarization of the cell membrane is not responsible for the inhibition of pacemaker potential. It has been reported that under physiological conditions, the mechanical tone of taenia coli is maintained and controlled by the spontaneous spike discharge (19). Therefore, it is quite feasible that diltiazem suppresses the pacemaker potential of spontaneous electrical activity, thereby causing a relaxing effect on the smooth muscle of taenia coli.

Papaverine (20) and calcium-antagonists such as verapamil (21) and D 600 (22) have also been shown to inhibit the pacemaker potential. Tashiro and Tomita (20) suggested that papaverine may bind with a site at the membrane with which calcium normally binds, thus reducing the pacemaker potential and the contraction. Such a mechanism may be involved in the inhibitory action of diltiazem in the spontaneously active preparations.

As shown in the present experiments, diltiazem decreased the contractile force evoked by depolarizing current pulse. This decrease in contractile force was accompanied by inhibition of the single spike and the repetitive firing during depolarization in both normal (Figs. 2, 3, 4) and Na⁺-free (Fig. 5) Locke solutions. When CaCl₂ (5 mM) was added to the preparation in which electrical and mechanical activities had been suppressed by diltiazem,
the contractile force partly regained its original strength concurrently with partial recovery of the single spike (for example, Fig. 3). Since the spike activity of taenia coli is assumed to be due to calcium entry, these results suggest that a reduction of the transmembrane influx of calcium ion, as well as an inhibition of repetitive firing, may be responsible for the decrease in the contractile force caused by diltiazem.

On the other hand, it was found that in some preparations, diltiazem reduced the contractile force without having significant influence on either the single spike or the repetitive firing in Na+-free Locke solution containing calcium ion (Fig. 6). In addition, decrease in the contractile force was observed when an initial single spike of almost normal amplitude could be evoked. Therefore, the following assumption may also be presented: 1. Diltiazem may act as an excitation-contraction uncoupler in the smooth muscle of taenia coli. Inhibition of the excitation-contraction coupling is also suggested by the finding that application of a conditioning depolarization to the preparation exposed to diltiazem partly reversed the single spike, whereas the reduced contractile force did not recover (Fig. 3d). Thus, it is inferred that diltiazem may cause a decrease in the contractile force by intracellularly reducing the concentration of free calcium ion essential for muscle contraction. 2. Diltiazem may inhibit an influx of a small amount of calcium ion which cannot be estimated from the change in spike activity measured in the present experiments. As reported for skeletal (23-25) and cardiac (26-28) muscles, such a small amount of calcium ion may function as a trigger for regenerative release of calcium ion which activates the contractile system.

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