FURTHER STUDIES OF THE ACTION OF CYCLIC AMP ON THE ELECTRICAL AND MECHANICAL ACTIVITIES OF INTESTINAL SMOOTH MUSCLE

Hiromichi OHKAWA

Department of Physiology, Yamaguchi University School of Medicine, Ube 755, Japan

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Abstract—Effects of externally applied cyclic AMP and other adrenergic stimulants on the electrical and mechanical activities of the cat small intestine were observed by using pressure electrodes. The electrical and mechanical activities were suppressed by cyclic AMP and beta-stimulants. Those inhibitory actions of cyclic AMP and beta-stimulants were potentiated under the treatment with caffeine, theophylline and papaverine which inhibits the phosphodiesterase activity. On the other hand, the inhibitory action of cyclic AMP and beta-stimulants was decreased in imidazole, an agent that increases phosphodiesterase activity. Exogenous applied concanavalin A, an agent that inhibits the adenyl cyclase activity, showed no observable changes in both activities but the effects of beta-stimulants were decreased after treatment with concanavalin A. No obvious changes on both activities were obtained in cyclic GMP and dibutyryl cyclic GMP. These findings tentatively support the hypothesis that cyclic AMP is a second messenger in the inhibitory responses to beta-stimulants on the intestinal smooth muscle. However, it is also concluded that the inhibition of mechanical activity caused by cyclic AMP is partially due to suppression of the membrane activity.

In relation to the relaxation mechanism of smooth muscles, several authors (1, 2, 3) have emphasized two subjects, that is, the activation of adenyl cyclase in cell membrane by beta-adrenergic stimulants and the increase of intracellular level of cyclic 3',5'-adenosine monophosphate (cyclic AMP). In previous papers (4, 5), it has been reported that cyclic AMP and dibutyril cyclic AMP inhibited the spontaneous electrical and mechanical activities of the intestinal smooth muscle. These actions of cyclic AMP and related compounds may thus possibly relate with adenyl cyclase and phosphodiesterase activity in the smooth muscle. On the other hand, it has been acknowledged that the phosphodiesterase activity is affected by externally applied caffeine (3), theophylline (3), papaverine (6, 7) and imidazole (3) and that adenyl cyclase activity is affected by concanavalin A (8).

This report is concerned with the action of cyclic AMP on both activities of intestinal smooth muscle under affecting factors on the enzyme activity.

MATERIALS AND METHODS

The small intestine was removed from adult cats which had been anesthetized with isozole. For the purpose of recording electrical and mechanical activities, a segment, 3 cm to 3.5 cm long, was slipped onto a rectangular Lucite holder and the holder was mounted in the normal solution at 36-37°C in a 200 ml Lucite chamber. One end of the segment was
fixed on the holder and the other which was made by cuttings along the longitudinal direction of segment, 2 cm in length and 3 mm in width, was connected to the force-transducer. The tension development was measured longitudinally. Electrical recordings were made by using pressure electrodes (3M KCl-agar, 0.5-1 mm capillaries) which were handled with a micromanipulator. Time constant of the electrical recording system was 2.0 sec. In many experiments, both activities were recorded simultaneously. The solution was gassed with 95% O2 and 5% CO2. The composition of modified Krebs solution was the same as described in the previous paper (5). The following drugs were used: cyclic 3',5'-adenosine monophosphate (cyclic AMP), adrenaline hydrochloride, noradrenaline hydrochloride, isoprenaline hydrochloride, caffeine sodium benzoate, theophylline, papaverine hydrochloride, imidazole hydrochloride, cyclic 3',5'-guanosine monophosphate (cyclic GMP), dibutyryl cyclic 3',5'-guanosine monophosphate (dibutyryl cyclic GMP), insulin and concanavalin A (Boehringer Mannheim GmbH).

RESULTS

Effects of catecholamines and cyclic AMP

When a pressure electrode was attached to the serosal surface of the segment, slow waves and spikes were recorded. Usually, the spontaneous discharges appeared as repetitive spike discharges superimposed on a slow wave depolarization. General pattern of the slow waves and spikes was similar to that described in previous papers (5, 9).

Adrenaline (10^-9-10^-6 g/ml) inhibited the spontaneous activities of the segment (Fig. 1). At a concentration of 10^-9 g/ml, spike generation was partially suppressed. Higher concentrations of adrenaline (10^-7-10^-7 g/ml) strongly blocked the spike generation but the slow waves were continued. Spike activity was completely abolished at a concentration of 10^-6 g/ml. Mechanical activity was also inhibited by raising the concentrations of adrenaline. At a concentration of 10^-6 g/ml, phasic contraction could not be recorded. Isoprenaline (10^-6 g/ml) and noradrenaline (10^-6 g/ml) also exhibited a strong inhibition on the spike and

![Fig. 1. Effect of adrenaline on the electrical (upper) and mechanical (lower) activities of the cat small intestine. A: Control. B: Adrenaline 10^-9 g/ml. C: Adrenaline 10^-8 g/ml. D: Adrenaline 10^-7 g/ml and E: Adrenaline 10^-6 g/ml. Lines in A-E in the mechanical activity indicate a standard level in tension. The meaning of lines in the following figures is the same. Calibration: 1 mV, 1 g and 30 sec.](image-url)
Cyclic AMP and Intestinal Smooth Muscle

After treatment with cyclic AMP (10^{-5} g/ml), the spike generation was reduced but the frequency of slow wave was not altered. The magnitude of phasic contraction was decreased and the tone level was also decreased slightly as shown in Fig. 2B. These changes in both activities of the intestinal smooth muscle were similar to those in a previous paper (5).

Effects of cyclic AMP and adrenaline under treatment with phosphodiesterase inhibitors and activator

Caffeine, theophylline and papaverine were used as phosphodiesterase inhibitors. Cyclic AMP (10^{-5} g/ml) was added to the organ bath after 15 min during the treatment with various phosphodiesterase inhibitors. Caffeine (10^{-4} g/ml) caused weak inhibition of spike generation, however, slow waves were observed continuously. Stronger inhibition of spike activity by cyclic AMP which was added during the treatment with caffeine was observed. Namely the frequency of spike generation was decreased more than that by cyclic AMP without the treatment with caffeine (Fig. 2, C-E). During treatment with theophylline (10^{-4} g/ml), spike activity was decreased slightly. Cyclic AMP (10^{-2} g/ml) during the treatment with theophylline caused stronger inhibition on slow wave and spike activities. Most of spikes were abolished and mechanical activity was also decreased. This inhibition in theophylline treatment was more considerable than that in normal solution (Fig. 3, A-C).

Papaverine (10^{-5} g/ml) caused the inhibition of phasic contraction. The magnitude of phasic contraction was decreased and the tone level was also reduced after treatment with papaverine. However, the spike activity continued and was similar to that seen when normal solution was used. Spike and mechanical activities in the papaverine treatment were suppressed strongly with an additional application of cyclic AMP (10^{-5} g/ml). As shown in Fig. 3F, spikes were abolished completely and small phasic contractions were observed to be sporadic. The inhibitory action of cyclic AMP in those phosphodiesterase inhibitors...
The effect of adrenaline was examined after treatment with various phosphodiesterase inhibitors. After treatment with theophylline ($10^{-4}$ g/ml), adrenaline exhibited a stronger inhibitory action than that seen in normal solution. As shown in Fig. 4C, the inhibitory action of adrenaline ($10^{-7}$ g/ml) on both activities was considerable, in particular the mechanical activity was completely blocked. However, the inhibitory effect of adrenaline ($10^{-8}$--$10^{-7}$ g/ml) in caffeine ($10^{-4}$ g/ml) was much the same as that seen in normal solution (Fig. 4, D–E).

The activatory action of imidazole on the phosphodiesterase activity is well known (3). The action of cyclic AMP and adrenaline after treatment with imidazole was also examined. When imidazole ($10^{-5}$ g/ml) was added to the organ bath, electrical and mechanical
activities were not altered as shown in Fig. 5B and E. After 15 min of imidazole treatment, cyclic AMP (10^{-5} g/ml) or adrenaline (10^{-7} g/ml) was added. Spike and mechanical activities were not inhibited by cyclic AMP (Fig. 5C) and inhibitory action of adrenaline on spike generation and mechanical activity was not observed (Fig. 5F).

**Effects of catecholamines under treatment with adenyl cyclase inhibitor**

In the dynamic receptor theory, the activation of adenyl cyclase in cell membrane by beta-adrenoceptive stimulants was emphasized (1, 2, 3). It has also been reported that concanavalin A inhibited the adenyl cyclase activity in the various tissues (8). In the present experiment, the effect of externally applied concanavalin A on the electrical and mechanical activities was examined. The preparations were exposed to various concentrations of concanavalin A (10^{-7}-10^{-4} g/ml) for 15 min or more. The spike and mechanical activities were, however, not changed. Neither of the activities was
altered by the externally applied insulin \( (4 \times 10^{-3} - 4 \times 10^{-8} \text{ U/ml}) \). When noradrenaline \( (10^{-7} - 10^{-8} \text{ g/ml}) \) was added to the organ bath after treatment with concanavalin A \( (2.5 \times 10^{-6} \text{ g/ml}) \), the action of noradrenaline on both activities was decreased (Fig. 6). That is, the generation of the spikes was still observed with noradrenaline \( 10^{-6} \text{ g/ml} \) while the spikes disappeared at the same concentration of noradrenaline in normal solution.

Effects of cyclic GMP and dibutyrly cyclic GMP

Cyclic GMP \( (7 \times 10^{-6} - 7 \times 10^{-5} \text{ g/ml}) \) and dibutyril cyclic GMP \( (10^{-5} - 10^{-4} \text{ g/ml}) \) had no striking action on the electrical and mechanical activities. A slight inhibition of the spike activity caused by cyclic GMP at a concentration of \( 7 \times 10^{-5} \text{ g/ml} \) was recorded while there was no considerable change in the mechanical activity. The inhibitory action of noradrenaline \( (10^{-8} - 10^{-7} \text{ g/ml}) \) in dibutyril cyclic GMP \( (10^{-5} \text{ g/ml}) \) was similar to that seen in normal solution.

DISCUSSION

Cyclic AMP and beta-stimulants inhibited the electrical and mechanical activities of the intestinal smooth muscle. According to the dynamic receptor hypothesis, beta-stimulant increases the adenyl cyclase activity in cell membrane and consequently the intracellular level of cyclic AMP increases. This increase of cyclic AMP content relates positively to the relaxation of intestinal smooth muscle. As shown in results, spike activity was suppressed by cyclic AMP which indicates that cyclic AMP affects the membrane activity of intestinal smooth muscle in a manner similar to that of beta-stimulants. Relaxation caused by cyclic AMP may be partially due to such inhibition of the membrane activity. In previous papers \( (4, 5) \), the effect of dibutyrly cyclic AMP on the membrane activity was reported. The effects of cyclic AMP and its dibutyrly derivative on the membrane activity of intestinal smooth muscle were found to be similar.

The phosphodiesterase involved in cyclic AMP breakdown is reportedly inhibited by theophylline \( (3) \), caffeine \( (3) \) and papaverine \( (6, 7) \) and the inhibitory action of papaverine on the phosphodiesterase activity is apparently greater than that of theophylline \( (6) \). These data suggest that the actions of beta-stimulants and cyclic AMP are potentiated under treatment with those inhibitors. In fact, results obtained in the present experiment showed that the inhibitory action of cyclic AMP on the mechanical activity of intestinal smooth muscle was indeed potentiated after treatment with caffeine, theophylline and papaverine. Imidazole is considered to have an activatory action on the phosphodiesterase activity \( (3) \). Under treatment with imidazole, the effect of cyclic AMP and adrenaline was decreased herein. Results as mentioned above appear to support the dynamic receptor hypothesis. However, the interaction between these phosphodiesterase inhibitors, activators and cyclic AMP or beta-stimulants on the membrane activity cannot be excluded because these inhibitors and activators, in themselves, modified the electrical activity.

Adenyl cyclase plays an important role in the relaxation mechanism caused by beta-stimulants \( (1, 2, 3) \). It had been reported that in various tissues this enzyme activity was inhibited by concanavalin A, however, the inhibitory action on adenyl cyclase activity by
this agent was limited to within a certain range (8). In the present work, concentrations of $10^{-7}$-$10^{-4}$ g/ml were used. Electrical and mechanical activities were not affected at various concentrations of concanavalin A. However, after concanavalin A, the inhibitory action of noradrenaline was decreased suggesting that the inhibition of adenyl cyclase activity may be caused by externally applied concanavalin A.

It had been reported that the intracellular level of cyclic AMP is reciprocally related to the intracellular content of cyclic GMP (10). Though the excitatory action of dibutryl cyclic GMP on the smooth muscle strip of guinea-pig ileum and rat stomach has been reported (11, 12), cyclic GMP and dibutryl cyclic GMP showed no obvious effects on either activity in the present experiment. The relationship of cyclic GMP to cholinergic transmission (12) and $Ca^{2+}$ supply to the contractile element during dibutryl cyclic GMP treatment (11) was suggested for the mechanism of action of cyclic GMP. The discrepancy between the obtained results and the former data on the effect of cyclic GMP was not elucidated and further study concerning the action of cyclic GMP on the activity of smooth muscle is now underway in our laboratory.

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