Actions of Cyclic AMP and Dibutyryl Cyclic AMP on the Electricl and Mechanical Activities of the Cat Small Intestine

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INTRODUCTION

According to the dynamic receptor theory[1,2], adenyl cyclase is activated by beta-adrenergic stimulants such as isoprenaline, adrenaline and noradrenaline. The activation of adenyl cyclase catalyzes the conversion of adenosine triphosphate to cyclic AMP[3]. On the other hand, it had also been reported that an increase in cyclic AMP correlated positively with relaxation of the intestinal smooth muscle of the guinea-pig, after adrenaline treatment[4]. From those findings, it is suggested tentatively that a content of cyclic AMP relates with a motility of smooth muscle. However, the relaxing action relates to the spike activity of smooth muscle. It is considered that exogenously applied cyclic AMP influences the electrical activity of smooth muscle. In this study, the effect of cyclic AMP on the mechanical activity of the cat small intestine was investigated and also on the electrical activity. The hypothesis that cyclic AMP is an intracellular messenger was discussed on the basis of the results.

METHODS

Small intestine was removed from adult cats which had been anesthetized with isozole. For recording purpose of the electrical and mechanical activities, segment, about 3 cm in length, were used. The arrangements for recordings of both activities and the composition of a modified Krebs solution were the same as those described in the previous paper[5]. Electrical activity of the longitudinal muscle layer was recorded simultaneously in many experiments. The following drugs were used: cyclic 3′,5′-adenosine monophosphate (cyclic AMP), dibutyryl cyclic 3′,5′-adenosine monophosphate (dibutyryl cyclic AMP), adenosine 5′-monophosphate

(AMP), adrenaline hydrochloride, noradrenaline hydrochloride, isoprenaline hydrochloride, salbutamol, propranolol, dichloroisoproterenol (DCI), papaverine hydrochloride, caffeine sodium benzoate, diprophylline and tetracosactide. Abbreviations in the parentheses are used in this paper.

RESULTS

1) Effects of cyclic AMP, dibutyryl cyclic AMP, AMP and beta-adrenoceptive stimulants.

All preparations exhibited spontaneous activity in normal solution. The characteristics of electrical and mechanical activities of the segment were similar to that described in the previous paper[5]. Cyclic AMP produced the inhibition of spike discharge and contraction. At a concentration of 10^{-5}g/ml, spike activity and phasic contraction were slightly decreased. In higher concentration (10^{-4}g/ml), further inhibition of spike discharge and mechanical activity was obtained but slow waves were generated continuously. Slight decrease in tone level was also observed at 10^{-4}g/ml.

Dibutyryl cyclic AMP was also examined. In the low concentrations (10^{-7}–10^{-6}g/ml), considerable inhibitory action on the electrical and mechanical activities were not detected. However, spike discharges were inhibited with increasing the concentrations. In higher concentrations (10^{-5} and 10^{-4}g/ml), sporadic spike generation was still observed but slow waves were not affected. Phasic contractions were reduced gradually and the relaxation was recorded at a concentration of 10^{-4}g/ml. The inhibitory actions of cyclic AMP and dibutyryl cyclic AMP on the spike and mechanical activities were shown in Fig. 1 and 2 respectively.

Addition of AMP caused the inhibition of mechanical activity. Phasic contraction was suppressed at a concentration of 10^{-5}g/ml. In many preparations, complete inhibition of phasic contraction and relaxation were observed in 5×10^{-5}g/ml (Fig. 7A).

Isoprenaline and salbutamol have been shown to have a relatively specific action to beta-adrenoceptors. These agents suppressed the spontaneous spike activity and phasic contractions. Those effects were clearly observed at concentrations between 10^{-6} and 10^{-5}g/ml. Noradrenaline (10^{-6}–10^{-5} g/ml) and adrenaline (10^{-6}–10^{-5} g/ml) also exhibited a strong inhibition on the spike and mechanical activities. The inhibitory actions caused by isoprenaline, adrenaline and noradrenaline produced more rapidly than that by cyclic AMP and dibutyryl cyclic AMP.

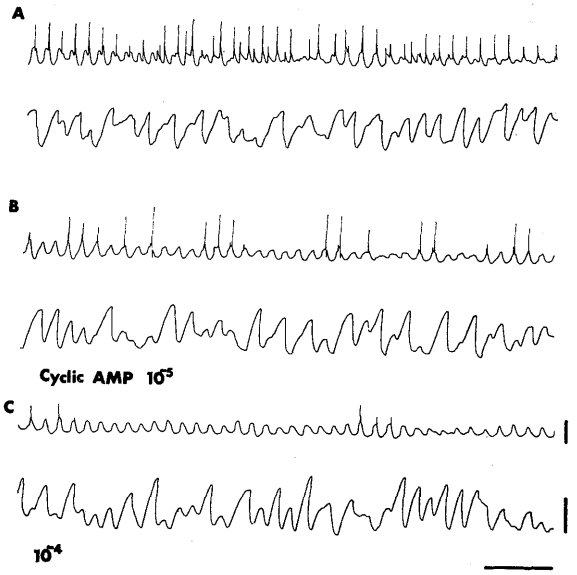

Fig. 1. Effect of cyclic AMP on the electrical (upper) and mechanical (lower) activities of the cat small intestine.
A; control, B; cyclic AMP 10^{-5}g/ml and C; 10^{-4}g/ml. Calibration; 30 sec, 1 mV and 1 g.

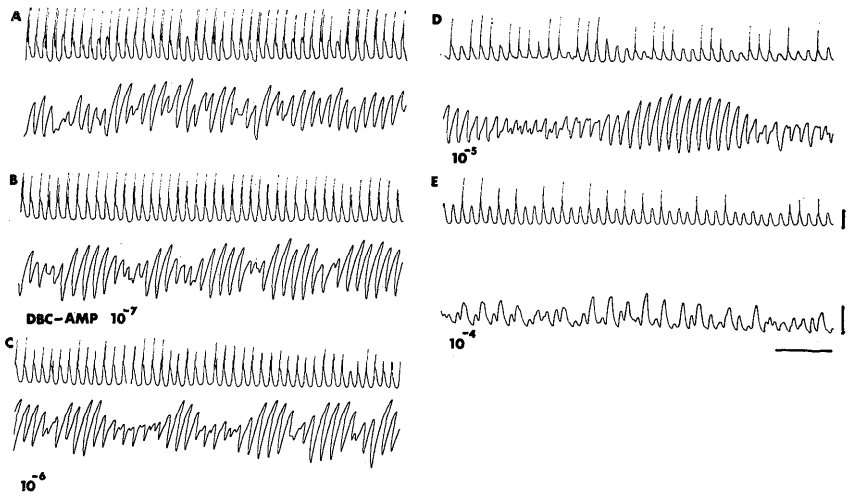

Fig. 2. Effect of dibutyryl cyclic AMP on the electrical (upper) and mechanical (lower) activities of the cat small intestine.
A; control, B; dibutyryl cyclic AMP 10^{-7}g/ml, C; 10^{-6}g/ml, D; 10^{-5}g/ml and E; 10^{-4}g/ml. Calibration; 30 sec, 1 mV and 1 g.

2) Effect of caffeine, diprophylline, papaverine and DCI.

Caffeine caused relaxation and reduced the magnitude of phasic contraction. These effects were depended on the concentrations of caffeine. Spike discharges were reduced but not abolished at a concentration of 2×10^{-4}g/ml. Slow waves were observed continuously in caffeine 2×10^{-4}g/ml. Weaker inhibition on the spike generation was observed at a concentration of 3×10^{-5}g/ml of diprophylline but the contractile activity was not altered. Further inhibition of spike generation produced and contractions were transfered into regular pattern of rhythmic contractions at 3×10^{-4}g/ml. When a concentration of diprophylline was increased to 6×10^{-4}g/ml, spikes were abolished while regular phasic contraction and slow waves were still continued. The tone level was slightly decreased. The decreases in the magnitude of phasic contraction by caffeine and diprophylline were gradually.

Addition of papaverine (10^{-6}–10^{-4}g/ml) caused relaxation and inhibition of spike activity. The amplitude of phasic contraction was reduced and contractions ceased finally at a concentration of 10^{-4}g/ml. The relaxant effect by papaverine was rapidly. Fig. 3 shows the inhibitory action of

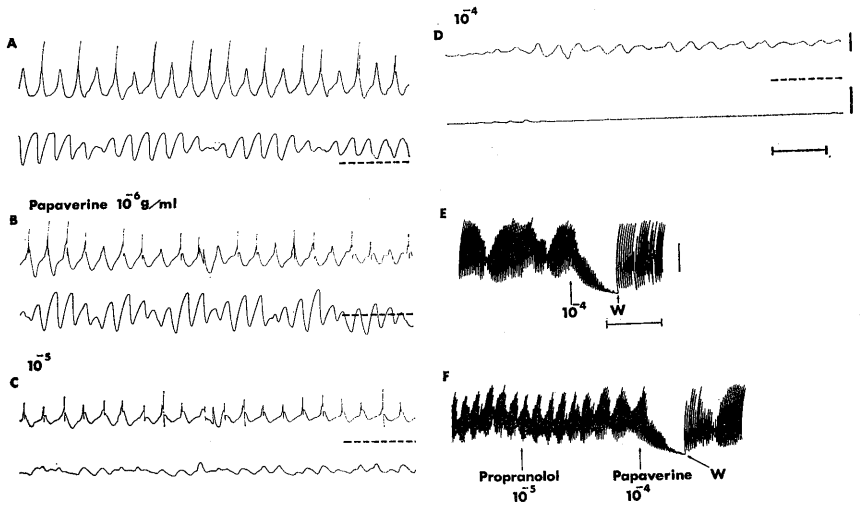

Fig. 3. Effect of papaverine on the electrical and mechanical activities of the cat small intestine.

Electrical activity; upper recordings in A–D, Mechanical activity; lower recordings in A–D. A; control, B; papaverine 10^{-6} g/ml, C; 10^{-5}g/ml and D; 10^{-4} g/ml. E shows the inhibitory action of papaverine 10^{-4} g/ml on the mechanical activity. F shows the effect of propranolol 10^{-5} g/ml on the inhibitory response to papaverine 10^{-4} g/ml. Calibration; 30sec for A–D and 5 min for E and F, 1 mV and 1 g for A–D and 1 g for E and F. Broken lines in A–D indicate a standard level in tension.

papaverine. At a concentration of 10^{-5}g/ml of DCI, spikes and contractions were continued as similar as the control. However, addition of DCI 10^{-4}g/ml caused the abolishment of spike generation and relaxation. This inhibition on both activities was not disappeared during 15min in DCI 10^{-4}g/ml.

3) Effects of various inhibitors on the inhibitory responses to cyblic AMP and adrenergic stimulants.

Propranolol (10^{-5}g/ml) could not block the inhibitory actions of cyclic AMP (10^{-4}g/ml) and dibutyryl cyclic AMP (10^{-4}g/ml). The effect of propranolol on the inhibitory response to dibutyryl cyclic AMP was shown in Fig. 4. On the other hand, after treatment with 10^{-5}g/ml propranolol, the inhibitory actions of isoprenaline (10^{-5}g/ml), salbutamol (10^{-6} and 10^{-5}g/ml) and noradrenaline (10^{-6}g/ml) were blocked. The inhibitory action

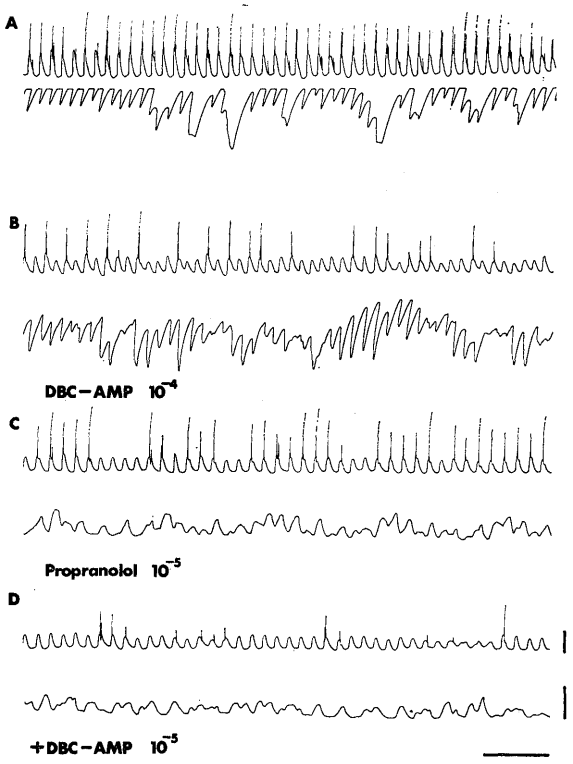

Fig. 4. Effect of propranolol on the inhibitory response to dibutyryl cyclic AMP on the electrical (upper) and mechanical (lower) activities of the cat small intestine.

A; control, B; dibutyryl cyclic AMP 10^{-4} g/ml, C; propranolol 10^{-5} g/ml and D; dibutyryl cyclic AMP 10^{-5}g/ml after treatment with propranolol 10^{-5}g/ml. Calibration; 30 sec, 1 mV and 1 g.

of cyclic AMP (10^{-4}g/ml) was not influenced by DCI (10^{-5}g/ml). The effect of dibutyryl cyclic AMP (10^{-4}g/ml) was also not blocked by DCI (10^{-5}g/ml). However, the relaxing action of isoprenaline (10^{-6}g/ml) was completely abolished by addition of DCI (10^{-5}g/ml). Fig. 5 shows the effects of DCI on the inhibitory responses to isoprenaline and to cyclic AMP.

After treatment with diprophylline 6×10^{4}g/ml, the magnitude of slow waves was decreased, smaller phasic contraction and decrease in the tone level were produced by dibutyryl cyclic AMP (10^{-4}g/ml), as shown in Fig. 6. This inhibitory action seems to be more remarkable than that in normal solution. The relaxant effect of cyclic AMP(10^{-4}g/ml) was potentiated by

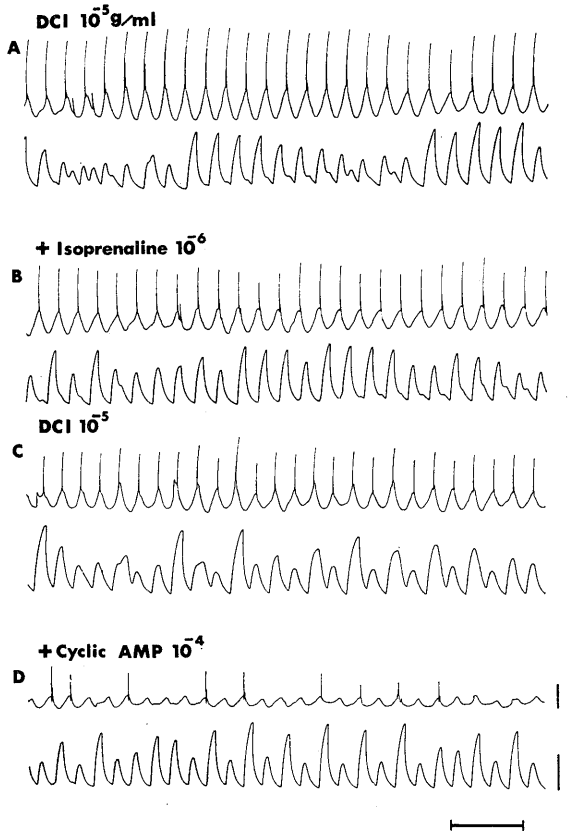

Fig. 5. Effects of DCI on the inhibitory responses to isoprenaline and cyclic AMP on the electrical (upper) and mechanical (lower) activities of the cat small intestine.

A; DCI 10^{-5} g/ml, control for B, B; isoprenaline 10^{-6} g/ml after treatment with DCI 10^{-5} g/ml, C; DCI 10^{-5} g/ml, control for D and D; cyclic AMP 10^{-4}g/ml after treatment with DCI 10^{-4} g/ml. Calibration; 30sec, 1mV and 1g.

diprophylline (10^{-4}g/ml). Caffeine (10^{-4}g/ml) also potentiated the relaxing action of dibutyryl cyclic AMP (10^{-4}g/ml) and cyclic AMP (10^{-4}g/ml).

4) Effect of quinidine on the inhibitory response to cyclic AMP.

Addition of quinidine (10^{-4}g/ml) caused sligh decrease on the phasic contraction, as reported previously[6]. Quinidine (10^{-4}g/ml) reduced the effect of AMP (5×10^{-5}g/ml) and noradrenaline (10^{-6}g/ml) and abolished the effect of cyclic AMP (10^{-4}g/ml). When the concentration of quinidine was rised to 2×10^{-4}g/ml, the effect of noradrenaline was consistently reduced. Fig. 7 shows the effect of quinidine on the inhibitory responses to cyclic AMP, AMP and noradrenaline.

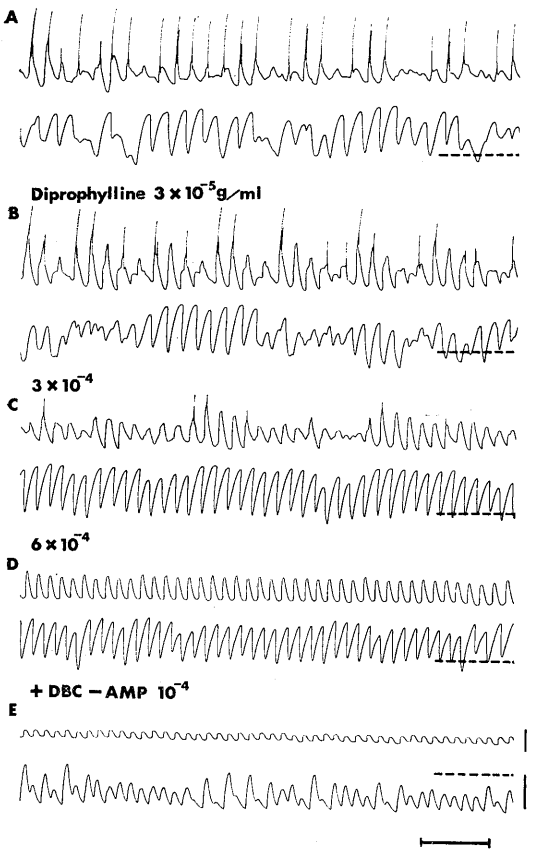

Fig. 6. Effect of diprophylline on the inhibitory response to dibutyryl cyclic AMP on the electrical (upper) and mechanical (lower) activities of the cat small intestine.

A; control, B; diprophylline 3×10^{-5} g/ml, C; 3×10^{-4} g/ml, D; 6×10^{-4} g/ml and E; dibutyryl cyclic AMP 10^{-4} g/ml after treatment with diprophylline 6×10^{-4} g/ml. Calibration; 30sec, 1 mV and 1 g. Broken line indicates a standard level in tension.

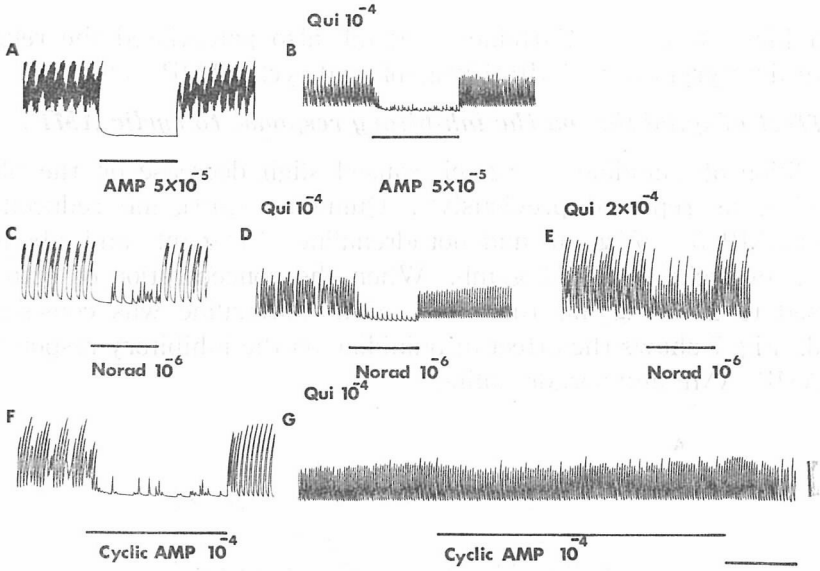

Fig. 7. Effect of quinidine on the inhibitory responses to AMP, noradrenaline and cyclic AMP on the mechanical activity of the cat small intestine.

A; AMP 5×10^{-5} g/ml, B; AMP 5×10^{-5} g/ml after treatment with quinidine 10^{-4} g/ml during 20 min. C; noradrenaline 10^{-6} g/ml, D; noradrenaline 10^{-6} g/ml after treatment with quinidine 10^{-4} g/ml during 70min, E; noradrenaline 10^{-6} g/ml after treatment with quinidine 2×10^{-4} g/ml during 20min, F; cyclic AMP 10^{-4} g/ml and G; cyclic AMP 10^{-4} g/ml after treatment with quinidine 10^{-4} g/ml during 45 min. Agents were applied as indicated by horizontal bars. Calibration 5 min and 1 g.

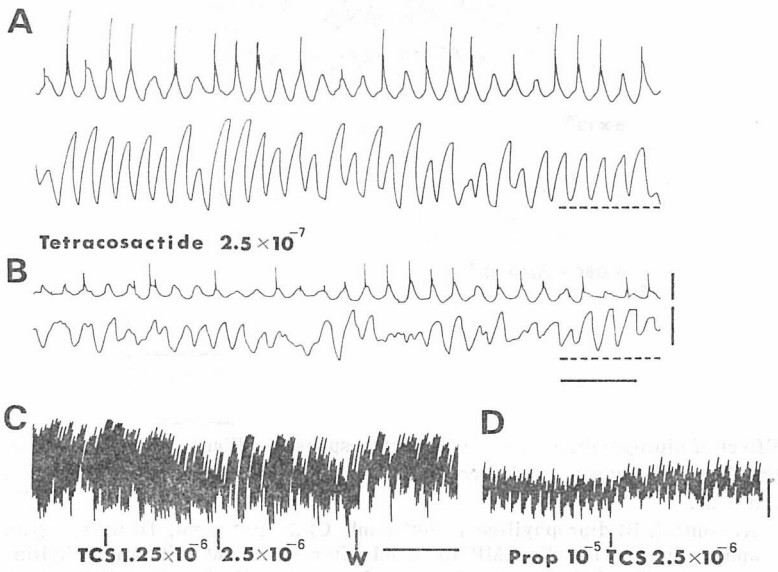

Fig. 8. Effect of tetracosactide on the electrical and mechanical activities of the cat small intestine.

Electrical activity; upper recordings in A and B. Mechanical activity; lower recordings in A and B. A; control and B; effect of tetracosactide (2.5×10^{-7}g/ml). Broken lines indicate a standard level in tension. Calibration; 30 sec, 1 mV and 1g. C; effect of tetracosactide (TCS, 1.25–2.5×10^{-6} g/ml) on the mechanical activity. D; effect of propranolol (Prop., 10^{-5}g/ml) on the inhibitory response to tetracosactide (TCS, 2.5×10^{-6} g/ml). Calibration; 5 min and 1 g.

5) Effect of tetracosactide.

Tetracosactide produced weaker inhibitory effect on the spike and mechanical activities (Fig. 8). At a concentration of 2.5×10^{-7}g/ml which is equivalent to 0.1 U/ml, the spike generation and the phasic contraction were reduced gradually. At higher concentrations of tetracosactide (1.25–2.5×10^{-6}g/ml), the magnitude of phasic contraction was decreased gradually and the tone level was also reduced slightly but not abolished the phasic contraction during 20 min in tetracosactide 2.5×10^{-6}g/ml. After treatment with propranolol 10^{-5}g/ml, the relaxing effect caused by tetracosactide (2.5×10^{-6}g/ml) was blocked.

DISCUSSION

The differences between the inhibitory actions of catecholamines and cyclic AMP (and dibutyryl cyclic AMP) were followings: (1) Effective concentrations of catecholamines were lower. Higher concentrations of cyclic AMP and dibtyryl cyclic AMP were required to produce an inhibitory action. (2) Time courses of inhibitory actions were very different. The inhibitory action of cyclic AMP and dibutyryl cyclic AMP appeared slowly. (3) Catecholamines abolished the spike activity of smooth muscle completely without the inhibition of generation of slow waves while the spike activity was observed in higher concentration of cyclic AMP and dibutyryl cyclic AMP and (4) The inhibitory action of catecholamines was blocked by propranolol and DCI while that of cyclic AMP and dibutyryl cyclic AMP was not abolished by these beta-blocking agents. The final results are in good agreement with those obtained by the guinea-pig taenia coli[7] and the rat ileum[8]. It is clearly from above results that the inhibitory actions of cyclic AMP and dibutyryl cyclic AMP were not mediated by beta-receptors. In many recent studies[9,10,11], it had been reported that the accumulation of cyclic AMP in cells inhibits the motility of intestinal smooth muscles. Results obtained in this study support above hypothesis.

Spike activity of the intestial smooth muscle was inhibited by treatment with higher concentration of cyclic AMP and dibutyryl cyclic AMP. The inhibition of spike activity also caused the inhibitory effect on mechanical activity. Therefore, it is considered that the inhibitory action of exogenously applied cyclic AMP on the mechanical activity is bring by its metabolic action in the cell and by inhibition of spike activity partially.

Phosphodiesterase catalyzes the conversion of cyclic AMP to 5′-AMP[12,13]. Caffeine, diprophylline and papaverine are known as phospho-

diesterase inhibitors. It had been reported that in the uterine and vascular smooth muscles, papaverine inhibited phosphodiesterase and increased the amount of cyclic AMP in the cells[9,14]. A part of the inhibitory action of papaverine on the mechanical activity of cat small intestine may due to such metabolic action. However, the spike activity was also inhibited by papaverine. The relaxing action of papaverine may due to the inhibition of spike activity partially. The inhibitory action on spike generation by papaverine is not mediate beta-receptors because the effect of papaverine was not antagonized by propranolol.

Caffeine and diprophylline produced also the relaxant action but the time course of inhibitory process was different from that by papaverine. Furthermore, the spike activity was still observed in caffeine and diprophylline. Therefore, the inhibition caused by caffeine and diprophylline seems to be bring by the metabolic action which inhibits phospho diesterase activity[3]. Caffeine potentiated the relaxant action of cyclic AMP in rat ileum[8] and guinea-pig taenia coli[5] and diprophylline potentiated the relaxant action by catecholamines in guinea-pig trachea[16]. Similar results were obtained in the effects of caffeine and diprophylline with cyclic AMP and dibutyryl cyclic AMP. Those results suggest that an accumulation of cyclic AMP in cells is rised with the inhibition of phosphodiesterase activity by caffeine or diprophylline.

Of a series of pyrimidine derivatives tested for inhibitory action on the cat small intestine[6] and the guinea-pig taenia coli[17], AMP produced the strong inhibitory action. Burnstock et al[17] estimated that ATP and ADP are transmitter substances from non-adrenergic inhibitory nerves in gut. However, the inhibition mechanism of AMP is still uncertain. The inhibitory action caused by AMP was more rapidly than that by cyclic AMP and the effective concentration of AMP was lower. These results suggest that AMP acts at the surface of the cell membrane.

Quinidine was reported to antagonize the relaxing effect of ATP on the rabbit ileum[18], the guinea-pig taenia coli[17] and the cat samll intestine[6]. Quinidine reduced or abolished the effect of noradrenaline[17]. In this study, similar results on the effect of quinidine on the inhibitory responses to AMP and noradrenaline were obtained. Moreover, the inhibitory action of cyclic AMP was also antagonized completely by quinidine. The membrane stabilizing action of quinidine seems to be non-specific but its mechanism is not clear.

Haynes et al[19] had been reported that ACTH rised a content of cyclic AMP in adrenal cortex. According to the two-messenger system[20], tropic hormone such as ACTH acts as a first messenger. In this study,

tetracosactide, synthetized ACTH, was used. Exogenously applied tetacosactide exhibited a weaker relaxant action. The time course of inhibition was similar to that of dibutyryl cyclic AMP but the effect was blocked by propranolol. It is not clear whether ACTH increases a content of cyclic AMP intestinal smooth muscle. However, from the results, tetracosactide seems to act a weaker beta-adorenoceptive stimulant.

SUMMARY

Cyclic AMP and dibutyeyl cyclic AMP produced the inhibitory action on the spike activity and contraction of the cat small intestine Beta-stimulants also exhibited the inhibitory action. Caffeine, diprophylline, papaverine and DCI produced the inhibitory effect. The inhibitions caused by caffeine, diprophylline and papaverine were depended on those metabolic action but a part of inhibitory action by papaverine seems to caused by the inhibition of spike activity. The relaxing effects of cyclic AMP and dibutyryl cyclic AMP were not blocked by propranolol and DCI and were potentiated by caffeine and diprophylline. Quiuidine blocked the inhibitory action of noradrenaline, AMP and cyclic AMP. Those findings support the hypothesis that cyclic AMP in cells relates the motility of intestinal smooth muscle. The spike activity was little influenced by exogenously applied cyclic AMP and dibutyryl cyclic AMP. Therefore, the inhibitory action of those agents may due to the inhibition on electrical activity partially.

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