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Changes in Cyclic AMP and Cyclic GMP Levels in the Contraction-Relaxation Cycle of Isolated Smooth Muscle Cells from Guinea-Pig Taenia Caeci

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Abstract

Yamada, T. and Obara, K. *Changes in cyclic AMP and cyclic GMP levels in the contraction-relaxation cycle of isolated smooth muscle cells from guinea-pig taenia caeci.* Japanese Journal of Smooth Muscle Research, 1985, **21** (1), 25-37. —— The present study was undertaken to investigate the role of cyclic AMP and cyclic GMP in the regulation of the contraction-relaxation cycle in isolated smooth muscle cells from guinea pig taenia caeci. The contraction of isolated smooth muscle cells induced by both caffeine (4×10^{-3} M) and carbachol (10^{-4} M) consisted of an initial activation phase and a following spontaneous relaxation phase. Isoproterenol (10^{-4} M) inhibited the carbachol-induced contraction and raised intracellular cyclic AMP level of isolated smooth muscle cells. Both the inhibition of the carbachol-induced contraction and the rise in cyclic AMP level elicited by isoproterenol were blocked by propranolol (10^{-4} M). Caffeine increased intracellular cyclic AMP level significantly and contracted isolated smooth muscle cells. Caffeine and carbachol contracted isolated smooth muscle cells and increased intracellular cyclic GMP level. Cyclic GMP was significantly increased in spontaneous relaxation phase. The data suggest that β-adrenergic inhibitory effect on cholinergic excitation in the mammalian isolated smooth muscle cells is mediated by the change in intracellular cyclic AMP level and that cyclic GMP related to the relaxation in these single cells. Thus, it appears that caffeine-induced contraction might be due to the stimulation of Ca^{2+}-induced Ca^{2+} release by cyclic AMP.

Introduction

It is now accepted that myoplasmic free Ca^{2+} ion plays a crucial role in regulating the

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contraction-relaxation cycle in various kinds of smooth muscles. It has been recognized that β-adrenergic agent causes the relaxation of the smooth muscle by activation of receptors located on sarcolemma which leads to a decrease in myoplasmic Ca^{2+} ion concentration. Since the discovery of cyclic AMP-dependent protein kinase (Walsh *et al.*, 1968), there have been many reports that cyclic AMP is closely associated with β-adrenergic-induced relaxation of the smooth muscle (Andersson *et al.*, 1972; Bowman *et al.*, 1970).

On the other hand, since Lee *et al.* (1972) suggested that cholinergic effect was associated with an increased concentration of cyclic GMP in intestinal smooth muscle, the role of cyclic GMP in the regulation of the contraction-relaxation cycle in the smooth muscle has also been investigated.

Despite the intensive study, the role of cyclic AMP in the smooth muscle relaxation is unclear as yet. Furthermore, there is much confusion about the role of cyclic GMP on the contraction-relaxation cycle in the smooth muscle. It is difficult to clarify the relationship between contraction and the change of each nucleotide in multicellular strips of smooth muscle. This is because mechanical and pharmacological properties of multicellular strips, which are used in ordinary studies, do not always give an accurate reflexion of the properties of individual smooth muscle cells. The availability of isolated smooth muscle cells devoid of extracellular space and neural elements permits a more direct examination of the relationship between the contraction and the change of each nucleotide.

Recently, Honeyman *et al.* (1978) and Scheid *et al.* (1979) reported the role of cyclic AMP in smooth muscle relaxation making use of isolated smooth muscle cells from the stomach of an amphibian. However, the change in intracellular cyclic AMP and cyclic GMP in relation to mammalian smooth muscle contraction has not yet been investigated with the use of isolated smooth muscle cells.

In this report, we investigated the role of cyclic AMP and cyclic GMP in the regulation of contraction-relaxation cycle by using isolated smooth muscle cells from guinea pig taenia caeci. To achieve this aim, experiments were performed to observe the effect of caffeine, carbachol, isoproterenol, and propranolol on the free contraction and changes in intracellular cyclic AMP and cyclic GMP levels in isolated smooth muscle cells.

Materials and Methods

Cell preparation

A suspension of isolated smooth muscle cells from guinea pig taenia caeci was prepared by the method of Obara (1984). Male guinea pigs weighing 300-600 g were used as tissue donors. Strips of taenia caeci, 4-5 cm in length, were carefully isolated from freshly killed guinea pigs. They were at 35℃ for 30 min in a modified Tyrode solution bubbled with air and then for 15 min in Ca^{2+}-free modified Tyrode solution. Each strip was minced and then suspended with gentle stirring 2-3 times/sec for 30 min in 2 ml of Ca^{2+}-free modified Tyrode solution containing 0.3% collagenase, 0.6% trypsin inhibitor and 1.0% bovine serum albumin. The suspension was diluted with 8 ml of Ca^{2+}-free modified Tyrode solution containing 1.0% albumin and was centrifuged at 1,000 rpm for 3 min. The precipitate was suspended with gentle stirring for 15 min in 2-4 ml of Ca^{2+}-free modified Tyrode solution and then dispersed by pipetting with a

wide-bored pipette (diameter : 2 mm). The suspension was allowed to stand for 2-3 min and thereafter the dispersed cells in supernatant were subjected to the experiments.

The composition of the modified Tyrode solution was 137 mM NaCl, 2.7 mM KCl, 0.18 mM $CaCl_2$, 1.0 mM $MgCl_2$, 5.6 mM glucose, and 4.2 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), pH 7.4.

Measurement of the contractile response of the isolated smooth muscle cells.

The measurement of the contractile response of isolated smooth muscle cells were carried out by the method of Momose *et al.* (1981). Single smooth muscle cells were perfused continuously with 1.8 mM Ca^{2+}-modified Tyrode solution ar room temperature on a dichlorodimethylsilane-coated glass slide and some agents were added with the perfusate. The process of free contraction of single smooth muscle cells was observed continuously under direct vision by a 16 mm movie camera (Bolex, H-16 RX-5) provided with an inverted phase contrast microscope (Olympus, IMT). The degree and velocity of the contractile response were determined by this method.

The contractile response of isolated smooth muscle cells was also measured by a modification of the method of Bitar & Makhlouf (1982), i.e. micrometric technique. Aliquots consisting of 5×10^3 cells in 0.5 ml of Ca^{2+}-free modified Tyrode solution were added to 0.1 ml of solution containing the agent or $CaCl_2$ to be tested at room temperature, thereby ensuring rapid mixture. At the end of the reaction time, 0.1 ml of acrolein was added to make a final concentration of 1%. An aliquot of cell suspension, fixed with acrolein, was placed on a glass slide under a coverslip and was randomly scanned on a screen by using a microprojector (Tiyoda, XM 500-II). The length of the first 200-300 cells encountered randomly in successive fields were measured along the curved long axes of the elongated cells. The micrometric technique was validate by measurement in 3-5 experiments.

The contraction was defined as the decrease in the average length of a population of isolated smooth muscle cells exposed to agent.

Determination of cyclic AMP and cyclic GMP levels.

The cyclic AMP and cyclic GMP were determined by a modification of the method of Hazeki & Ui (1980). Before the measurement of these cyclic nucleotides, the cells in suspension were tested for contraction by test agents at room temperature under a phase contrast microscope (Olympus, FHA). Each incubation tube containing 150 μl of cell suspension (4×10^4 cells of taenia caeci) and 50 μl of modified Tyrode solution supplemented with 7.2 mM $CaCl_2$ and test agent (final concentration of 1.8 mM $CaCl_2$). Incubation was carried out at room temperature. After completion of incubation, 50 μl of 0.6 N HCl and 50 μl of 30 mM EDTA (Ethylenediaminetetraacetic acid) were quickly added to make final concentrations of 0.1 N HCl and 5 mM EDTA, respectively and the tube was immersed in boiling water for 5 min to extract cyclic AMP and cyclic GMP. After centrifugation at 3,000 rpm for 20 min, cyclic AMP and cyclic GMP in the supernatant were succinated and then measured by sensitive radioimmunoassay procedure. The nucleotide values were expressed as a percentage of control value or as a function of the protein concentration in the precipitates determined by the method of Lowry *et al.* (1951).

Chemicals

Sources of reagents are as follows: collagenase (Type I), bovine serum albumin (Fraction V), isoproterenol, and propranolol were obtained from Sigma Chemical Co. Soybean trypsin inhibitor came from Miles Laboratories. Carbachol was obtained from Aldrich Chemical Co. Caffeine was purchased from Wako Pure Chemical Industries, Ltd. For measurement of cyclic AMP and cyclic GMP level by the radioimmuoassay procedure, we employed a YAMASA cyclic AMP and cyclic GMP assay kit.

Results

Evaluation of the contractile response of isolated smooth muscle cells under direct vision.

As observed by Obara (1984), freshly dispersed cells of taenia caeci varied widely in length. Some were fully relaxed and others were in various states of contraction. We selected one cell which seemed to be fully relaxed and thus could be easily contracted by test agents. Figure 1 shows the process of carbachol- and caffeine-induced contraction recorded on 16 mm movie film. The time course of these contraction were showed graphically in Fig. 2. As shown in Fig. 2, the contractile responses to caffeine (4×10^{-3} M) and carbachol (10^{-4} M) were prompt, both of them reaching their peaks within 12 sec. The maximal responses to caffeine (4×10^{-3} M) and carbachol (10^{-4} M) expressed as the percent decrease in mean cell length from control were 48% (mean, n=7) and 56% (mean, n=10), respectively. The optimal values for the concentrations of caffeine and carbachol had been determined by preliminary experiments (data not shown).

Evaluation of the contractile response of isolated smooth muscle cells by micrometric technique.

As shown in Fig. 2, the spontaneous relaxation phase of carbachol- and caffeine-induced contraction could not with certainty be detected by 16 mm movie film recording in all of the experiments as a result of technical difficulty. Thus, we employed the similar method described by Bitar & Makhlouf (1982) in order to evaluate the process of these contractions exactly. Time dependency of the contractile responses to caffeine and carbachol is shown in Fig. 3. The contractile responses to both caffeine (4×10^{-3} M) and carbachol (10^{-4} M) reached their maximum within 15 sec. The slope of the rise was steep and the maximal contractile response to both caffeine and carbachol was a 27% decrease of resting length (mean, n=3). After reaching maximal level, isolated smooth muscle cells relaxed spontaneously to a plateau and this plateau continued to 15 min. The dgree of contraction induced by caffeine and carbachol returned to 84% and 80% of resting length respectively at spontaneous relaxation phase.

Intracellular cyclic AMP level and contraction of isolated smooth muscle cells.

Effect of isoproterenol.

Isolated smooth muscle cells prepared from the same strip of taenia caeci were divided into the following four groups:

a) control.

b) exposure to carbachol (10^{-4} M) alone for 15 sec.

c) exposure to isoproterenol (10^{-4} M) for 3 min prior to challenging with carbachol (10^{-4} M) for 15 sec.

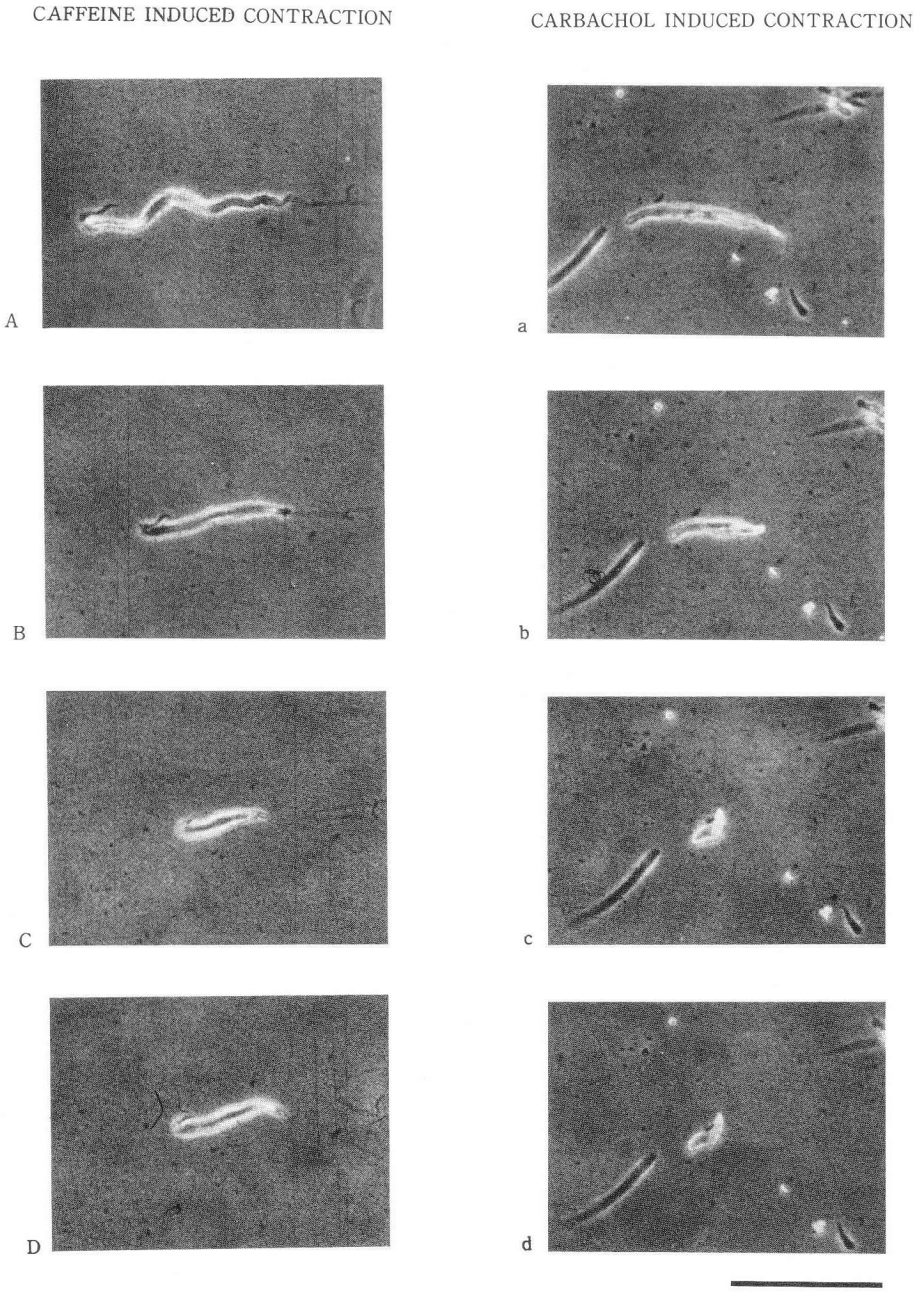

Fig. 1 Caffeine- and carbachol-induced contraction of single smooth muscle cells recorded on 16 mm movie film. A ; before addition of caffeine (4×10^{-3} M). B ; 4 sec after addition of caffeine. C ; 12 sec after addition of caffeine. D ; 28 sec after addition of caffeine. a ; before addition of carbachol (10^{-4} M). b ; 4 sec after addition of carbachol. c ; 16 sec after addition of carbachol. d ; 22 sec after addition of carbachol. In the caffeine-induced contraction, the cell contracted finally to 39% of its resting length (C) and thereafter relaxed spontaneously to 54% of its resting length (D). Carbachol-induced contraction of two single cells was shown in a-d. The cells contracted to 30% of their resting length (c) but the spontaneous relaxation phase was not clear (d). Bar indicates 100 μm.

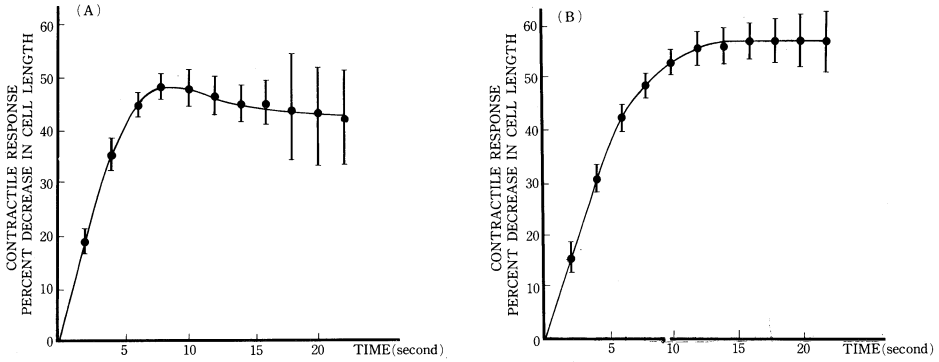

Fig. 2 The time course of caffeine (A)- and carbachol (B)- induced contraction of individual single cell obtained from 16 mm movie film. The contractile response was prompt, rising to their peaks in 10 sec and 12 sec, respectively. Concentrations of caffeine and carbachol are 4×10^{-3} M and 10^{-4} M, respectively. Values are presented as mean with S.E. of 7—10 experiments.

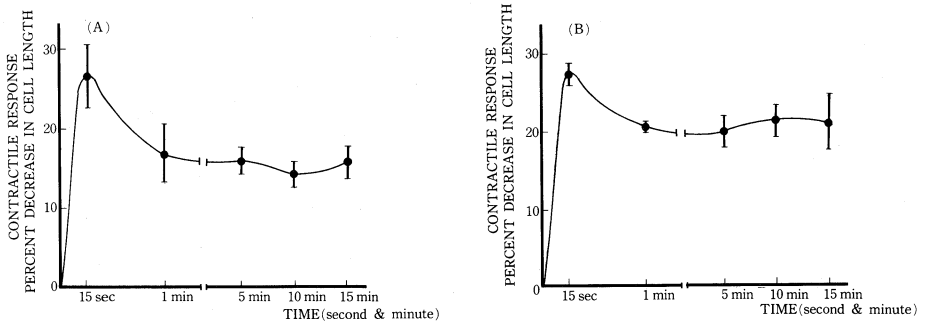

Fig. 3 The time course of caffeine (A)- and carbachol (B)- induced contraction measured by the micrometric method. The contractile response attained it maximum at 15 sec and thereafter it relaxed to a plateaul Concentrations of caffeine and carbachol are 4×10^{-3} M and 10^{-4} M, respectively. Values are presented as mean with S.E. of 3 experiments.

d) exposure to isoproterenol (10^{-4} M) alone for 3 min.

The contraction of isolated smooth muscle cells in each group were measured by micrometric technique. Then, under the conditions shown in a)-d), we also measured the cyclic AMP levels of the isolated smooth muscle cells by radioimmunoassay procedure. Figure 4 shows the effect of isoproterenol on cyclic AMP level and contractile response of isolated smooth muscle cells. The control value of cyclic AMP was measured to be 5.13 ± 0.53 pmol per mg protein (mean$\pm$ S.E., n=8). Both contractile response and cyclic AMP level of the cells are expressed as a percent of control value. As shown in Fig. 3, isolated smooth muscle cells contracted maximally in 15 sec when exposed to carbachol (10^{-4} M). So we judged 15 sec to be the optimal reaction period for carbachol-induced contraction.

In response to carbachol (10^{-4} M) alone, isolated smooth muscle cells contracted significantly (71% of resting length, mean, n=5), but failed to produce any significant increase in cyclic AMP level compared to the control value. When isolated smooth muscle cells were exposed to isoproterenol (10^{-4} M) for 3 min prior to challenging with carbachol (10^{-4} M), they

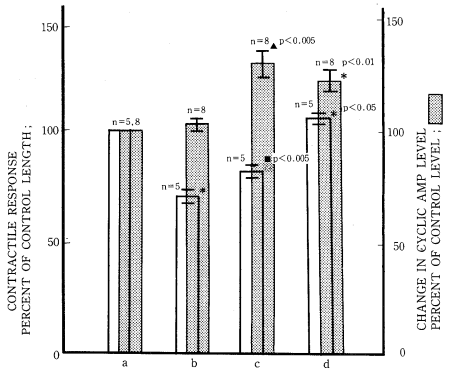

Fig. 4 The effect of isoproterenol on the cyclic AMP level and contractile response of isolated single smooth muscle cells in the presence of carbachol. a; control. b; exposure to carbachol (10^{-4} M) alone for 15 sec. c; exposure to isoproterenol (10^{-4} M) for 3 min prior to challenging with carbachol for 15 sec. d; exposure to isoproterenol alone for 3 min. Values are presented as mean with S. E. of 5—8 experiments.
* significantly different from the control value (100%).
▲, ■: significantly different from the level obtained in response to carbachol alone (▲: $p<0.005$, ■: $p<0.005$).

contracted to 82% of resting length (mean, n=5). Therefore, isoproterenol decreased the potential for carbachol-induced contraction significantly ($p<0.005$). Under the same conditions as those in which the inhibitory effect of isoproterenol on carbachol-induced contraction was observed, we determined the cyclic AMP levels of isolated smooth muscle cells. Exposure to isoproterenol (10^{-4} M) for 3 min prior to challenging with carbachol (10^{-4} M) gave rise to a significant increase in the cyclic AMP level (130.0±5.7% of control level, mean±S.E., n=8, $p<0.005$) and this increase level of cyclic AMP was significantly greater than that elicited by carbachol alone ($p<0.005$). On the other hand, exposure to isoproterenol (10^{-4} M) alone for 3 min produced a small but significant relaxation (107% of resting length, mean, n=5, $p<0.05$), and the cyclic AMP level of the cells was also significantly increased (121.0±5.3% of control level, mean±S.E., n=8, $p<0.01$). The increase in cyclic AMP seemed to cause the inhibition of carbachol-induced contraction, as can be seen in Fig. 4.

Effect of propranolol.

We used propranolol, a β-adrenergic blocker, in order to ascertain the relation between cyclic AMP and β-adrenergic-induced relaxation. Just as was the case in the experiment using isoproterenol, the single cells in suspension prepared from the same strip of taenia caeci were divided into four groups:

a) control.
b) exposure to carbachol (10^{-4} M) alone for 15 sec.
c) exposure to isoproterenol (10^{-4} M) for 3 min prior to challenging with the carbachol (10^{-4} M) for 15 sec.
d) exposure to propranolol (10^{-4} M) for 3 min before incubation with isoproterenol (10^{-4} M) for 3 min, and then successively challenged with carbachol (10^{-4} M) for 15 sec.

In these conditions both contractile response and cyclic AMP level were determined by micrometry and radioimmunoassay procedure. Figure 5 shows the effect of isoproterenol and its antagonist, propranolol, on cyclic AMP level and contractile response of isolated smooth muscle cells. The control value of cyclic AMP was measured to be 4.22±0.20 pmol/mg protein (mean±S.E., n=8). When the cells in suspension were challenged with carbachol (10^{-4} M)

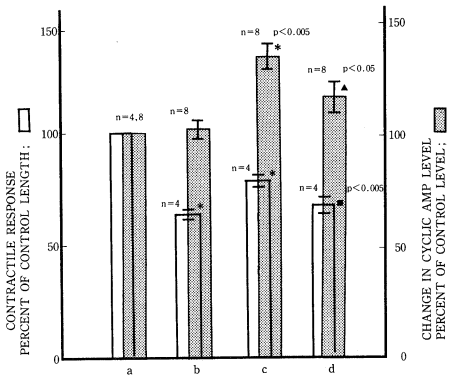

Fig. 5 The effect of isoproterenol and its antagonist, propranolol on the cyclic AMP level and contractile response of isolated smooth muscle cells in the presence of carbachol. a; control. b; exposure to carbachol (10^{-4} M) alone for 15 sec. c; exposure to isoproterenol (10^{-4} M) for 3 min prior to challenging with the carbachol for 15 sec. d; exposure to propranolol (10^{-4} M) for 3 min before incubation with isoproterenol for 3 min, and then successively challenged with carbachol for 15 sec. Values are presented as mean with S.E. of 4—8 experiments.
* significantly different from the control level (100%).
▲, ■: Propranolol significantly inhibited the effect of isoproterenol on both contractile respnse and cyclic AMP generation (▲: $p<0.005$, ■: $p<0.05$).

alone, they contracted significantly, to 65% of resting length (mean, n=4), and there was no significant change in cyclic AMP level compared to the control level. Exposure to isoproterenol (10^{-4} M) prior to challenging with carbachol (10^{-4} M) produced significant inhibition of carbachol-induced contraction (80% of resting length, mean, n=4) and induced a significant increase in cyclic AMP level (135.0±6.5% of control level, mean±S.E., n=8, $p<0.005$). However, when the cells in suspension were preincubated with propranolol (10^{-4} M) before exposure to isoproterenol (10^{-4} M) and the successive addition of carbachol, they contracted to 69% of resting length (mean, n=4) and the cyclic AMP level was 117.0±7.3% of the control level (mean±S.E., n=8).

Thus, both the increase in cyclic AMP and the decrease in the carbachol-induced contraction, which were elicited by isoproterenol, were inhibited significantly by propranolol ($p<0.005$ and $p<0.05$, respectively).

Effect of caffeine.

Caffeine was employed as a smooth muscle stimulant of isolated smooth muscle cells (Figs. 1-3). However, Butcher & Sutherland (1962) reported that caffeine inhibited the activity of phosphodiesterase, so we investigated the caffeine-induced cyclic AMP generation of isolated smooth muscle cells during caffeine-induced contraction. The time course for the effect of the caffeine (4×10^{-3} M) on cyclic AMP generation is shown in Fig. 6. At 15 sec after the addition of caffeine (4×10^{-3} M), the cyclic AMP level was found to increase remarkably (187.0±9.4% of control level, mean±S.E., n=4), and at 10 min the cyclic AMP level was still significantly increased (180.0±12.8% of control level, mean±S.E., n=4).

Relation between cyclic GMP level and contractile response of isolated smooth muscle cells.

We investigated the time course of the caffeine (4×10^{-3} M)-and carbachol (10^{-4} M)-induced cyclic GMP generation under the same conditions in which the time courses of contractions

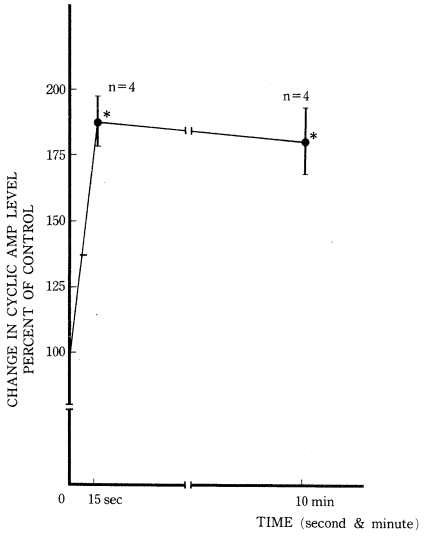

Fig. 6 The time course for the effects of caffeine on cyclic AMP levels in isolated smooth muscle cells. At 15 sec and 10 min, caffeine (4×10^{-3} M) gave rise to a marked increase in cyclic AMP level. Values are presented as mean with S.E. of 4 experiments.
* significantly different from the control level (100%)

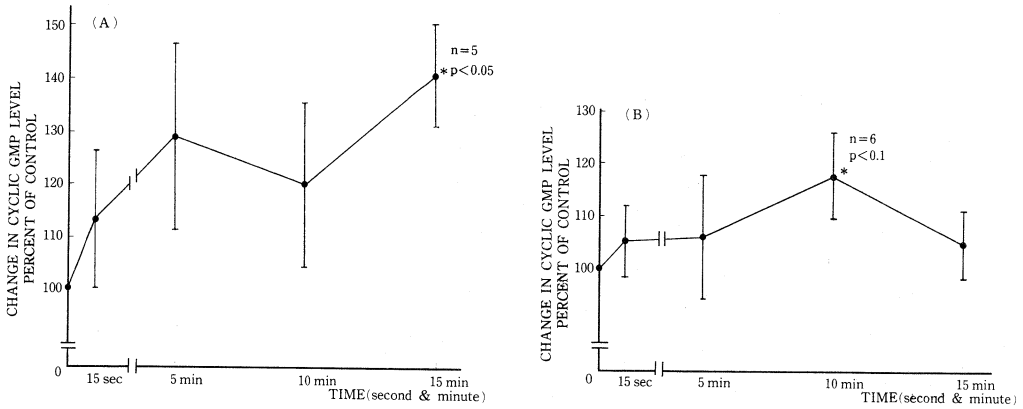

Fig. 7 The time course of caffeine (A)- and carbachol (B)- induced cyclic GMP generation in isolated smooth muscle cells. Aliquots of cells were exposed for period ranging from 15 sec to 15 min to caffeine (4×10^{-3} M) and carbachol (10^{-4} M). Values are presented as mean with S.E. of 5—6 experiments.
* significantly different from the control level (100%).

induced by these excitatory stimulants were examined (Fig. 3). The time courses of caffeine (4×10^{-3} M)-and carbachol (10^{-4} M)-induced cyclic GMP generation are shown in Fig. 7. Caffeine induced a gradual increase in cyclic GMP level, as compared to the control value (1.94±0.33 pmol/mg protein, mean±S.E., n=5). At 5 min after the addition of caffeine (4×10^{-3} M), the cyclic GMP level was 129.0±17.7% of the control level (mean±S.E., n=5, $p>0.1$) and at 15 min cyclic GMP level increased to statistically significant level (141.0±10.2% of the control level, mean±S.E., n=5, $p<0.05$). Just as with the caffeine-induced cyclic GMP generation, carbachol increased cyclic GMP level significantly. Within 15 sec after the addition of carbachol (10^{-4} M), cyclic GMP level was found to be slightly higher than the control value (1.65±0.26 pmol/mg protein, mean±S.E., n=6) but was not significant. At 10 min, the cyclic GMP level had just increased to a significant level (118.0±8.2% of the control level,

mean±S.E., n=6, $p<0.1$) and it thereafter declined.

Discussion

Though the regulatory role of cyclic AMP and cyclic GMP in the contraction-relaxation cycle has been proposed in various kinds of smooth muscles, the exact physiological role of them is not yet elucidated and still remaines to be determined. There are many reports suggesting that cyclic AMP is closely associated with the smooth muscle relaxation (Bowman *et al.*, 1970 ; Andersson *et al.*, 1972 ; Honeyman *et al.*, 1978 ; Scheid *et al.*, 1979). On the other hand, acetylcholine and other muscarinic agonist cause the contraction of intestinal smooth muscle and increase the cyclic GMP level (Lee *et al.*, 1972). And yet, some have reported that intracellular cyclic GMP elevation is associated with smooth muscle relaxation (Napori *et al.*, 1980 ; Gruetter *et al.*, 1981). Whereas, Diamond *et al.* (1983) suggested that cyclic GMP had no relation to the relaxation of smooth muscle. Thus there has been some confusion about the role of this cyclic nucleotide.

With the use of isolated smooth muscle cells, it seems possible to make clear the physiological role of these two cyclic nucleotides on the contraction-relaxation cycle in smooth muscle, since they are devoid of extracellular space and nerve elements. For this purpose, we investigated the changes in cyclic AMP and cyclic GMP levels in the contraction-relaxation cycle of isolated smooth muscle cells.

Though Fay *et al.* (1982) were able to measure the isometric tension in single isolated smooth muscle cell of an amphibian stomach, in the present study, we could not measure the isometric tension in the single isolated smooth muscle cell from guinea pig taenia caeci due to technical difficulty. Therefore, the contractile and relaxing effects of some agents were assessed by the measurement of their free contraction or its inhibition.

The contractions of single smooth muscle cells elicited by caffeine (4×10^{-3} M) and carbachol (10^{-4} M) reached their peaks within 15 sec and thereafter declined spontaneously to a plateau (Figs. 1-3). According to Momose *et al* (1981), it took only 2 sec to reach the peak of the free contraction of single smooth muscle cells from guinea pig taenia caeci elicited by acetylcholine (10^{-4} M). As they observed, the time to the peak of the contraction seemed to be very rapid at 35℃. However, in our experiments, we measured the contraction of isolated smooth muscle cells at room temperature (20-25℃). Therefore, the time to the peak of the contraction was longer than that shown by Momose *et al.*

As shown in Fig. 3, maximal responses to both carbachol (10^{-4} M) and caffeine (4×10^{-3} M) measured by micrometric technique were 27% but those detected from movie film observation were 48% and 56% decrease of resting length, respectively. The contractile responses measured by the latter method were greater than those by the former method. This was because, using the micrometric method, we simultaneously measured the length of fully contracted smooth muscle cells and non contractile cells.

Isoproterenol (10^{-4} M), a β-adrenergic agent, inhibited the carbachol-induced contraction and simultaneously increased the cyclic AMP level significantly (Fig. 4). This result is consistent with the reports of Andersson *et al.*, (1972), Honeyman *et al.* (1978) and Scheid *et al.* (1979). Both the increase in cyclic AMP and the decrease in the contractile response of isolated smooth

muscle cells to carbachol, which were induced by isoproterenol, were blocked by propranolol (10^{-4} M) (Fig. 5). As shown in Fig. 4, exposure to isoproterenol (10^{-4} M) alone produced a small but significant relaxation and the cyclic AMP level increased significantly. These data support the belief that the relaxation of isolated smooth muscle cells which was induced by β-adrenergic agent was mediated by a cyclic AMP-dependent process. However, exact mechanism of cyclic AMP-dependent relaxation is unclear. Scheid *et al.* (1979) suggested that cyclic AMP enhanced Na^+/K^+ transport and that finally, the decrease of intracellular Ca^{2+} through Na^+/Ca^{2+} exchange induced the relaxation of smooth muscle. On the other hand, Conti & Adelstein (1980) suggested that relaxation occurred through cyclic AMP-dependent phosphorylation of smooth muscle myosin light chain kinase.

However, caffeine (4×10^{-3} M) increased the intracellular cyclic AMP level significantly (Fig. 6) and yet it contracted isolated smooth muscle cells (Figs. 1-3). These data were inconsistent with the notion of cyclic AMP mediated relaxation. This discrepancy may be explained as follows. Caffeine releases Ca^{2+} ion from intracellular Ca^{2+} stores (Nasu & Urakawa, 1974) and this release is inhibited by procaine in guinea pig taenia caeci (Obara *et al.*, 1983). Procaine inhibits the Ca^{2+}-induced Ca^{2+} release mechanism in vascular smooth muscle (Saida & Van Breemen, 1984a). These findings suggested that caffeine can release Ca^{2+} from intracellular stores by enhanching the Ca^{2+}-induced Ca^{2+} release mechanism. Moreover, Saida & Van Breemen (1984b) reported that cyclic AMP stimulated the Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum in arterial smooth muscle. Therefore, it is possible that in caffeine-induced contraction, cyclic AMP predominantly stimulates the Ca^{2+}-induced Ca^{2+} release mechanism rather than the extrusion mechanism of Ca^{2+} or having a suppression effect on the myosin light chain kinase.

On the other hand, numerous contractile agents, including cholinergic agents, have been shown to increase cyclic GMP levels in a variety of smooth muscle tissue preparations (Rapoport & Murad, 1983). Actually, as shown by our results, both carbachol and caffeine stimulated cyclic GMP elevation in isolated smooth muscle cells (Fig. 7). Therefore, caffeine-induced contraction is assumed to be mediated by cyclic GMP. However, comparing the time course of carbachol- and caffeine-induced contraction with that of cyclic GMP elevation induced by these two stimulants (Figs. 1 and 7), cyclic GMP increased significantly in the spontaneous relaxation phase. Napori *et al.* (1980) reported that exogeneous application of cyclic GMP to bovine coronary arterial smooth muscle induced relaxation. Gruetter *et al.* (1981) also showed, with use of nitroso compounds, a close relationship between cyclic GMP elevation and relaxation of smooth muscle. From our results and the reports described above, cyclic GMP seemed to be related to smooth muscle relaxation. We were unable to demonstrate a correlation between the initial activation phase of the contraction and significant changes of cyclic GMP levels elicited by caffeine.

There are many reports on the role of intracellular cyclic AMP and cyclic GMP in the contraction-relaxation cycle of smooth mucsle strips. We observed the relaxing effects of both cyclic AMP and cyclic GMP with the use of isolated smooth muscle cells of a mammalian visceral tissue. However, as shown in caffeine-induced contraction, the rise of the cyclic AMP level did not always induce the relaxation of smooth muscle cells. Therefore, insofar as the

contractile response of smooth muscle is investigated, studies comparing intracellular cyclic AMP and cyclic GMP levels with Ca^{2+} movement, the activity of myosin light chain kinase and cyclic nucleotide-depentent protein kinase will be necessary in future.

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