Mechanism of Relaxant Action of Papaverine
VI. Sodium Ion Dependence of Its Effect on $^{45}$Ca-Efflux in Guinea-Pig Taenia Coli

Nobuyoshi SUNAGANE, Toshiaki OGAWA*, Tsutomu URUNO and Kazuhiko KUBOTA
Department of Pharmacology, Faculty of Pharmaceutical Sciences, Science University of Tokyo, Shinjuku-ku, Tokyo 162, Japan
Accepted February 22, 1985

Abstract—The present study was undertaken to investigate the roles of sodium ion and the cyclic AMP system in the relaxant effect of papaverine. The effects of papaverine on the $^{45}$Ca-efflux and the mechanical activity of guinea-pig taenia coli were tested in solutions in which the concentration of sodium ion was varied and compared with those of dibutyryl cyclic AMP. Papaverine dose-dependently caused an acceleration of $^{45}$Ca-efflux and the synchronous relaxation of a depolarized preparation in normal bathing solution. Dibutyryl cyclic AMP mimicked these effects of papaverine. In Na-free solution, papaverine lost its ability to accelerate the $^{45}$Ca-efflux, and its relaxant activity was markedly reduced, while dibutyryl cyclic AMP had neither an effect on the $^{45}$Ca-efflux nor an effect on the muscle relaxation in Na-free solution. Reintroduction of a small amount of sodium ion to the solution, however, recovered these effects of papaverine and dibutyryl cyclic AMP on the $^{45}$Ca-efflux and the muscle relaxation. These findings indicate that the relaxant effect of papaverine may be in part due to an increase in cyclic AMP-mediated Ca-efflux which requires the presence of external sodium ion. The sodium ion dependence of this Ca-efflux process was also discussed.

It has been reported by several authors that papaverine accelerates the Ca-efflux from smooth muscle cells (1-5). The acceleration of Ca-efflux may be one of the possible mechanisms for the papaverine-induced smooth muscle relaxation because this must impair the calcium supply to the contractile element. Meanwhile, there is evidence that papaverine inhibits cyclic nucleotide phosphodiesterase in smooth muscle cells (6-11), implicating that an increase in cellular cyclic AMP level may mediate some actions of papaverine on the cellular processes. Tomyama et al. (1) and Shiba et al. (2) observed that a dibutyryl derivative of cyclic AMP also increased the Ca-efflux. From these findings, it is indicated that papaverine may cause the acceleration of Ca-efflux through the elevation of cellular cyclic AMP level.

Recent studies have shown that the sodium ion gradient across the cell membrane participates in the extrusion of calcium ion from the smooth muscle cells (12-17). In the previous studies, we suggested that the relaxant effect of papaverine is partially due to a mechanism which is only activated in the presence of external sodium ion and that this mechanism is related to the cyclic AMP system (18, 19). It is, therefore, of special interest to know whether the sodium ion gradient is involved in the action of papaverine on the Ca-efflux.

In the present study, effects of papaverine and dibutyryl cyclic AMP on the $^{45}$Ca-efflux from the guinea-pig taenia coli were investigated by using bathing solutions in...
which the concentration of sodium ion was varied by replacing NaCl in their composition with sucrose or choline chloride.

Materials and Methods

Strips of taenia coli, approximately 1.5 cm long, were dissected from the caecum of male guinea pigs weighing 350 to 500 g. The muscle strip was preloaded with 1 g of isotonic tension and suspended in a 3 ml organ bath filled with Locke-Ringer solution by mounting it to a stainless steel hook with nylon thread. The Locke-Ringer solution was kept at 32°C and continuously bubbled with air (pH 7.8). The composition of normal Locke-Ringer solution (normal solution) was as follows (in mM): NaCl, 154; KCl, 5.6; CaCl₂, 2.2; MgCl₂, 2.1; NaHCO₃, 5.9 and glucose, 2.8. Na-free or Na-deficient Locke-Ringer solution (Na-free or Na-deficient solution) was prepared by replacing NaCl and NaHCO₃ in the normal solution by isosmolar amounts of sucrose or choline chloride and KHCO₃, respectively. The potassium ion concentration was adjusted to approximately the level of normal solution by omitting KCl from the solution. Measurement of alteration of ⁴⁵Ca-efflux was carried out by using the apparatus shown in Fig. 1, which was designed so that recording of the mechanical response and quick collection of the washout solution could be made simultaneously.

Before beginning of the Ca-efflux experiments, the taenia coli was equilibrated in the normal solution for 30 min and then repeatedly applied with hypertonic 20 mM KCl at intervals of 15 min until the contractile response of the muscle attained constant level. Thereafter, the muscle strip was immersed in the normal, Na-free or Na-deficient solution for 20 min and then allowed to load with ⁴⁵Ca in the respective solutions containing 10 μCi/ml ⁴⁵CaCl₂ for 20 min. During the latter 10 min period of ⁴⁵Ca-loading, the taenia coli was depolarized with hypertonic 20 mM KCl. Then the muscle was washed out with non-radioactive solution containing 20 mM KCl, according to the time schedule described in the previous study (3). After the final collection of the washout solution, the taenia coli was dissolved by 1 ml of Protosol (New England Nuclear), a tissue solubilizer, with heating at 55°C. The dissolved tissue and a 1 ml portion of the washout solution were poured into vials which contained 10 ml of Bray's
cocktail. The radioactivity of the washout solution and the residual radioactivity of the muscle were counted with an Aloka liquid scintillation counter (Model 670). The $^{45}$Ca-eflux was expressed as the rate constant of $^{45}$Ca-loss from the smooth muscle cells.

Results

The following figures show the effects of papaverine or dibutyryl cyclic AMP on the $^{45}$Ca-eflux from the depolarized taenia coli into the normal, Na-free or Na-deficient solution and typical mechanical responses of the muscle to drugs, which were simultaneously recorded during the $^{46}$Ca-eflux measurement.

When $3 \times 10^{-5}$ M of papaverine was added to the normal solution during an efflux period of 30-50 min, a marked acceleration of $^{45}$Ca-eflux and a synchronous muscle relaxation took place (Fig. 2A). Under this condition, papaverine completely relaxed the taenia coli contracted by 20 mM KCl (Table 1). These effects of papaverine were dose-dependent at concentrations ranging from $10^{-5}$ to $10^{-4}$ M (data not shown). Dibutyryl cyclic AMP ($5 \times 10^{-3}$ M) also produced a significant acceleration of $^{45}$Ca-eflux and a

![Fig. 2. Effects of papaverine (A) and dibutyryl cyclic AMP (B) on $^{41}$Ca-eflux and mechanical activity of taenia coli in normal solution. Upper panel: Typical response of the muscle to the drugs. Lower panel: Effect of the drugs on $^{45}$Ca-eflux. Horizontal bars indicate the duration of drug application. Data were obtained from 6 experiments.](image)

Table 1. Relaxant activities of papaverine and dibutyryl cyclic AMP in normal, Na-free or Na-deficient (10 mM sodium ion-containing) solution

|                    | Normal soln. | Na-free soln. | 10 mM Na soln. |
|--------------------|--------------|---------------|----------------|
| Papaverine         |              |               |                |
| $3 \times 10^{-5}$ M | 123.1±5.0    | 18.3±9.4      | 76.6±3.2       |
| $3 \times 10^{-4}$ M | 82.1±7.1     |               | 105.8±1.1      |
| Dibutyryl cyclic AMP | 117.2±9.1   | 0             | 71.4±8.9       |

Data were obtained from 6 experiments. The extent of relaxation was estimated as the percentage of the complete relaxation of the 20 mM KCl-induced contraction.
synchronous full relaxation of the muscle (Fig. 3B and Table 1).

In the Na-free sucrose solution, the increase in 45Ca-efflux by papaverine was lost even if a higher dose was applied to the muscle (Fig. 3A). Similar results were obtained in the Na-free choline chloride solution (Fig. 4). With regard to the mechanical response, papaverine could still relax the taenia in the Na-free solution (Table 1). On the other hand, dibutyryl cyclic AMP (5×10⁻³ M) caused neither the acceleration of 45Ca-efflux nor the muscle relaxation in the Na-free sucrose solution (Fig. 3B and Table 1). The actions of dibutyryl cyclic AMP on the 45Ca-efflux and the mechanical activity were not restored even when its dose was increased to 10⁻² M.

When 10 mM of sodium ion was reintroduced to the Na-free sucrose or choline chloride solution, the action of papaverine to accelerate the 45Ca-efflux was revived (Figs. 4 and 5A). The revival of the papaverine action on the 45Ca-efflux was dependent upon the amount of sodium ion reintroduced (data not shown). The reduced relaxant activity of papaverine under the Na-free condition was also partially restored in the presence of 10 mM sodium ion (Table 1). Almost complete restoration of the relaxant activity was achieved in the solution containing 20 mM sodium ion (18). Reintroduction of 10 mM of sodium ion to the solution also recovered the actions of dibutyryl cyclic AMP to accelerate the 45Ca-efflux and to relax the taenia coli (Fig. 5B and Table 1).

Discussion

It has been presumed that the early phase of 45Ca-efflux may be predominantly
Sodium Ion and Papaverine Effect on Ca-Efflux

Fig. 5. Effects of papaverine (A) and dibutyryl cyclic AMP (B) on $^{45}$Ca-efflux and mechanical activity of taenia coli in Na-deficient (10 mM sodium ion-containing) sucrose solution. For legend see Fig. 2.

Previous studies have demonstrated that papaverine accelerated the Ca-efflux from smooth muscle cells (1-5). However, most of these studies have not simultaneously monitored the changes in the mechanical activity of the muscle and changes in the Ca-efflux from the muscle. The present study was designed to permit the simultaneous measurement of changes in the $^{45}$Ca-efflux and the mechanical activity, revealing that papaverine caused the acceleration of $^{45}$Ca-efflux and the synchronous muscle relaxation of guinea-pig taenia coli. This finding well supports the concept that the acceleration of Ca-efflux is responsible for the papaverine-induced smooth muscle relaxation. The present results also showed that dibutyryl cyclic AMP also evoked the acceleration of $^{45}$Ca-efflux and the synchronous muscle relaxation. This result is in good agreement with those of Tomiyama et al. (1) and Shiba et al. (2) who suggested that the action of papaverine on the Ca-efflux might be mediated by the elevation of cellular cyclic AMP level. On the other hand, recent studies of Huddart and his co-workers offered opposing results to us concerning the effects of papaverine (22, 23). They found that papaverine depressed the $^{45}$Ca-efflux from the ileum, vas deferens and urinary bladder of rats and inhibited the high K-stimulated $^{45}$Ca-efflux. Discrepancies between their findings and our present results may be attributed to differences in tissues,
species and experimental conditions.

The present results provided further evidence as to the mechanism by which papaverine induces the acceleration of Ca-efflux. The findings that the action of papaverine to accelerate the $^{45}$Ca-efflux was abolished in the Na-free solution, but easily revived in the solution containing a small amount of sodium ion indicate that the presence of external sodium ion is prerequisite for the production of papaverine action on the Ca-efflux. Moreover, the action of dibutyryl cyclic AMP accelerating the $^{45}$Ca-efflux was found to be affected in the same way as that for papaverine when sodium ion was removed from or reintroduced to the washout solution. These findings suggest that a certain process of the cyclic AMP-mediated Ca-efflux requires the presence of external sodium ion, and this may explain the sodium ion dependence of papaverine action on the $^{45}$Ca-efflux.

Papaverine still could relax the taenia coli in the Na-free solution without causing the acceleration of $^{45}$Ca-efflux, although its relaxant activity was markedly reduced. Therefore, papaverine has an ability to relax the smooth muscle through mechanisms other than the acceleration of Ca-efflux, which are activated even in the absence of external sodium ion. Sunagane et al. (18) have suggested that such mechanisms include the inhibition of Ca-influx into the muscle cells. Meanwhile, the reduced relaxant activity in the Na-free solution was restored in the solution containing a small amount of sodium ion with similar sodium ion dependence to that of the revival of the action on the $^{45}$Ca-efflux. Moreover, it was found that dibutyryl cyclic AMP entirely failed to reveal the relaxant activity in the Na-free solution, but easily recovered from the failure in the solution containing a small amount of sodium ion. Therefore, it is likely that the reduction of the relaxant activity of papaverine in the Na-free condition may mainly be due to the inhibition of cyclic AMP-mediated Ca-efflux induced by the removal of external sodium ion.

The actual process leading to the acceleration of Ca-efflux in response to papaverine, however, remains to be elucidated. Recent studies have shown that the Na-Ca exchange mechanism in which the downhill movement of sodium ion along its electrochemical gradient across the cell membrane drives the outward movement of calcium ion is involved in the extrusion of calcium ion from smooth muscle cells (12–17). Moreover, it was found that dibutyryl cyclic AMP entirely failed to show relaxant activity in the Na-free solution, but easily recovered from the failure in the solution containing a small amount of sodium ion. Therefore, it is likely that the reduction of the relaxant activity of papaverine in the Na-free condition may mainly be due to the inhibition of cyclic AMP-mediated Ca-efflux induced by the removal of external sodium ion.

In the present results, the actions of papaverine and dibutyryl cyclic AMP on the $^{45}$Ca-efflux was found to be abolished in the Na-free solution in which the Na-Ca exchange mechanism would be inhibited and found to be revived when sodium ion is present in the amount that has been reported by Katase and Tomita (12) to be sufficient to activate the Na-Ca exchange. Thus, the hypothesis proposed by Sheid et al. (24, 25) may also explain the process of the acceleration of Ca-efflux in response to papaverine. On the contrary, evidence for the existence of a Ca-extrusion pump in smooth muscle cells has also been provided by some authors (26–28). According to Bülbring and den Hertog (28), isoproterenol can stimulate the Ca-extrusion pump of guinea-pig taenia coli. Since the roles of sodium ion and cyclic AMP on such a Ca-extrusion pump are not clear, the involvement of such Ca-extrusion in the action of papaverine on the Ca-efflux may not be ruled out.

References

1 Tomiyama, A., Takayanagi, I. and Takagi, K.: Relaxation of intestinal smooth muscle and calcium movements. J. Pharm. Pharmacol. 25, 65–68 (1973)

2 Shiba, T., Uruno, T., Kubota, K. and Takagi, K.: Effects of papaverine, benactyzine and D600 on Ca$^{2+}$ efflux, determined by means of a Ca$^{2+}$-selective electrode, from guinea pig taenia coli
into a Ca²⁺-free physiological solution, Japan. J. Pharmacol. 31, 553–561 (1981)

3 Sunagane, N., Uruno, T. and Kubota, K.: Mechanism of relaxant action of papaverine. II. Difference between modes of relaxant effects of papaverine and amytal in guinea-pig taenia coli. J. Pharmacobiodyn. 6, 209–215 (1983)

4 Hattingberg, M.V., Kuschinsky, G. and Rahn, K.H.: Der Einfluß von Pharmak auf Calciumgehalt und ⁴⁵Calciumaustausch der glatten Muskulatur der Taenia Coli vom Meerschweinchen. Naunyn Schmiedebergs Arch. Exp. Pathol. Pharmacol. 253, 438–443 (1966)

5 Banerjee, A.K.: Effects of drugs on ⁴⁷Ca²⁺ movements in depolarized longitudinal smooth muscle. Arch Int. Pharmacodyn. Ther. 207, 148–161 (1974)

6 Triner, L., Vulliemoz, Y., Schwartz, I. and Nahans, G.G.: Cyclic phosphodiesterase activity and the action of papaverine. Biochem. Biophys. Res. Commun. 40, 64–69 (1970)

7 Poch, G. and Kukovetz, W.R.: Papaverine-induced inhibition of phosphodiesterase activity in various mammalian tissues. Life Sci. 10, 133–144 (1971)

8 Uruno, T., Takayanagi, I., Kubota, K. and Takagi, K.: Actions of papaverine, aspaminol, and bile salts and intracellular cyclic AMP level. Japan. J. Pharmacol. 24, 681–689 (1974)

9 Inatomi, N., Takayanagi, I., Uchida, M. and Takagi, K.: Intracellular cyclic AMP level and intestinal smooth muscle relaxation. Eur. J. Pharmacol. 26, 73–76 (1974)

10 Inatomi, N., Takayanagi, I. and Takagi, K.: Antiphosphodiesterase activity and nonspecific smooth muscle relaxation tested on intestinal smooth muscle. Japan. J. Pharmacol. 25, 63–69 (1975)

11 Miyamoto, M., Takayanagi, I., Ohkubo, H. and Takagi, K.: Actions of papaverine on intestinal smooth muscle and its inhibition of cyclic AMP and cyclic GMP phosphodiesterases. Japan. J. Pharmacol. 26, 114–117 (1976)

12 Katase, T. and Tomita, T.: Influences of sodium and calcium on the recovery process from potassium contracture in guinea pig taenia coli. J. Physiol. (Lond.) 224, 489–500 (1972)

13 Reuter, H., Blauenstein, M.P. and Haeusler, G.: Na-Ca exchange and tension development in arterial smooth muscle. Philos. Trans. R. Soc. Lond. [Biol.] 265, 87–94 (1973)

14 Ma, T.S. and Bose, D.: Sodium in smooth muscle relaxation. Am. J. Physiol. 232, C59–C66 (1977)

15 Brading, A.F.: Calcium-induced increase in membrane permeability in the guinea-pig taenia coli. Evidence for involvement of a sodium-calcium exchange mechanism. J. Physiol. (Lond.) 275, 65–84 (1978)

16 Brading, A.F. and Widdicombe, J.H.: Interaction between sodium and calcium movements in smooth muscle. In Smooth Muscle Pharmacology and Physiology. Edited by Worcel, M. and Vassort, G., p. 235–245, Inserm, Paris (1976)

17 Hirata, M., Itoh, T. and Kuriyama, H.: Effects of external cations on calcium efflux from single cells of the guinea-pig taenia coli and porcine coronary artery. J. Physiol. (Lond.) 310, 321–336 (1981)

18 Sunagane, N., Sakata, T., Uruno, T. and Kubota, K.: Mechanism of relaxant action of papaverine, III. Comparison of sodium ion dependencies on the relaxant effects of papaverine, aspaminol and benactyzine in guinea-pig taenia coli. J. Pharmacobiodyn. 6, 466–474 (1983)

19 Sunagane, N., Fujihara, R., Uruno, T. and Kubota, K.: Mechanism of relaxant action of papaverine. IV. Roles of sodium ion and cyclic AMP. Japan. J. Pharmacol. 35, 461–464 (1984)

20 Deth, R.C.: Effect of lanthanum and reduced temperature on ⁴⁵Ca-efflux from rabbit aorta. Am. J. Physiol. 234, C139–C145 (1978)

21 Deth, R.C. and van Breemen, C.: Agonist induced release of intracellular Ca²⁺ in the rabbit aorta. J. Mem. Biol. 30, 363–380 (1977)

22 Huddart, H., Langton, P.D. and Saad, K.H.M.: Inhibition by papaverine of calcium movements and tension in smooth muscles of rat vas deferens and urinary bladder. J. Physiol. (Lond.) 349, 183–194 (1984)

23 Huddart, H. and Saad, K.H.M.: Papaverine-induced inhibition of electrical and mechanical activity and calcium movements of rat ileal smooth muscle. J. Exp. Biol. 86, 99–114 (1980)

24 Scheid, C.R. and Fay, F.S.: ⁵⁻Adrenergic effects of transmembrane ⁴⁵Ca fluxes in isolated smooth muscle cells. Am. J. Physiol. 246 (Cell Physiol. 15), C431–C438 (1984)

25 Scheid, C.R., Honeyman, T.W. and Fay, F.S.: ⁵⁻Adrenergic relaxation of smooth muscle. Nature 277, 32–36 (1979)

26 Casteels, R. and van Breemen, C.: Active and passive Ca²⁺ fluxes across cell membranes of the guinea-pig taenia coli. Pfluegers Arch. 359, 197–207 (1975)

27 Uchida, M.: Active extrusion of calcium ions by smooth muscle mitochondria. Biochem. Biophys. Res. Commun. 73, 127–132 (1976)

28 Bulbring, E. and den Hertog: The action of isoprenaline on the smooth muscle of the guinea-pig taenia coli. J. Physiol. (Lond.) 304, 277–296 (1980)