Mechanism of Relaxant Action of Papaverine IV. Roles of Sodium Ion and Cyclic AMP

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It has been suggested that papaverine relaxes the smooth muscle through inhibition of cyclic AMP phosphodiesterase (PDE) and subsequent accumulation of cellular cyclic AMP (1–4). On the other hand, our previous study indicated that the relaxant effect of papaverine on the smooth muscle is partially due to a mechanism which is only activated in the presence of external sodium ion (5). Therefore, it is important to investigate possible interactions between external sodium ion and some smooth muscle relaxants whose relaxant activities are related to the cyclic AMP system. In the present study, the influence of manipulating external sodium ion concentration on the relaxant effects of papaverine, dibutyryl cyclic AMP and deoxycholate, which exhibits its action through partial involvement of the cyclic AMP system (6), were compared in guinea-pig taenia coli.

Strips of taenia coli were dissected from male guinea pigs weighing 300 to 450 g. The strip was mounted in a 10 ml organ bath filled with Locke-Ringer solution (normal solution) which had the following composition (in mM): NaCl, 154; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 2.1; $NaHCO_3$, 5.9 and glucose, 2.8 (pH, 7.8). The bathing solution was kept at 32°C and continuously bubbled with air. The mechanical response of the muscle was isotonically recorded by means of a lever loaded with 1 g. After 30 min equilibration in the normal solution, the taenia was repeatedly applied with hypertonic 20 mM KCl at intervals of 15 min until the contractile response became constant. Thereafter, the muscle preparation was immersed in either the normal solution or Na-free or Na-deficient (10 mM sodium ion containing) Locke-Ringer solution for 20 min. The Na-free or Na-deficient solution was prepared by replacing NaCl and $NaHCO_3$ in the normal solution by isosmolar amounts of sucrose and $KHCO_3$, respectively. The potassium ion concentration was adjusted to the normal level by omitting KCl from the solution. Following the immersion of the taenia, dose-relaxation curves of papaverine (Sigma), dibutyryl cyclic AMP (Sigma) and deoxycholate (Tokyo Tanabe) were obtained on the muscle depolarized by 20 mM KCl in each solution.

When papaverine was cummulatively applied to the taenia, a dose-dependent relaxation took place in either normal, Na-free or 10 mM sodium ion containing solution (Fig. 1A). In the Na-free solution, the dose-relaxation curve was significantly shifted to the right as compared with that in the normal solution. This indicates that the relaxant activity of papaverine is greatly reduced in the absence of external sodium ion. The reduced relaxant activity was partially restored in the presence of a small amount of sodium ion since the dose-relaxation curve was partially shifted back in the solution containing 10 mM sodium ion. Almost complete restoration was achieved in the solution containing 20 mM sodium ion (5).

Dibutyryl cyclic AMP and deoxycholate also elicited dose-dependent relaxations in the normal solution. The dose-relaxation curves for both drugs are illustrated in Figs. 1B and 1C. In contrast with papaverine, the activity of each drug was completely abolished in the Na-free solution. In the solution containing 10 mM sodium ion,

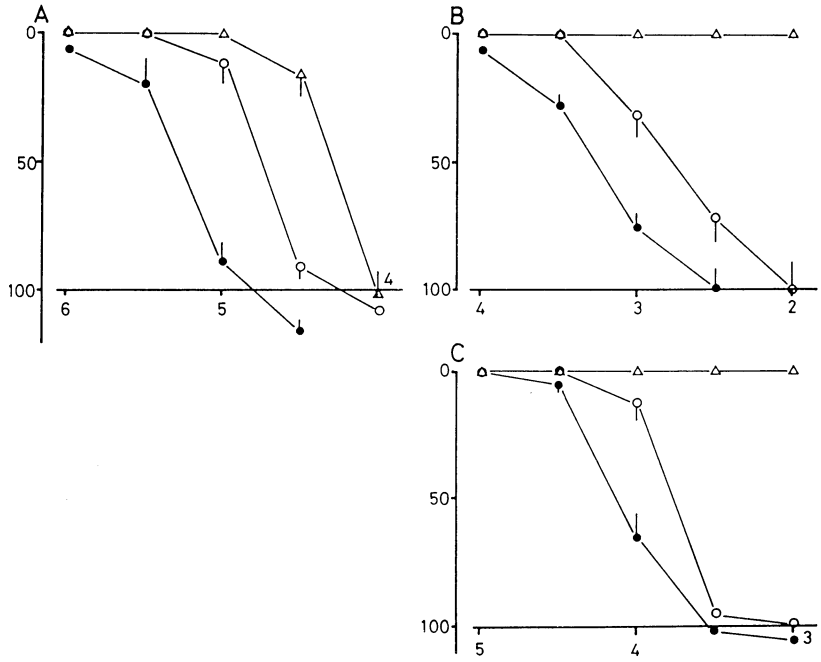

Fig. 1. Influence of manipulating external sodium ion concentration on the relaxant activities of papaverine (A), dibutyryl cyclic AMP (B) and deoxycholate (C). The dose-relaxation curves for each drug on the KCl-depolarized taenia coli were obtained in the normal (●), 10 mM sodium ion containing (○) and Na-free (△) solutions. Percent response for each drug was estimated as the percentage of the complete relaxation of KCl-induced contracture. Each point represents mean±S.E. of 6 experiments. Ordinate: percent relaxation for each drug, Abscissa: dose of each drug (−log M).

however, both dibutyryl cyclic AMP and deoxycholate induced dose-dependent relaxations, and each dose-relaxation curve was partially shifted back to the left. The restoration of their relaxant activities appears to be dependent on the amount of sodium ion contained in the solution since the activities were more effectively restored in the solution containing 20 mM sodium ion (data not shown). For the purpose of examining further the effect of sodium ion on the relaxation of taenia in response to dibutyryl cyclic AMP or deoxycholate, a small amount of sodium ion, which of itself induced no detectable relaxation, was applied to the tissue after confirmation that no relaxant effect had been elicited by each drug in the Na-free solution. As shown in Fig. 2, the application of 10 mM NaCl in the presence of dibutyryl cyclic AMP or deoxycholate produced a marked relaxation of the taenia. These findings may also support the sodium ion dependence of the relaxant effects of dibutyryl cyclic AMP and deoxycholate.

The present results showed that papaverine evoked the relaxation of taenia coli even in the Na-free solution. This indicates that papaverine has the ability to relax the smooth muscle in the absence of external sodium ion. However, the relaxant effect of papaverine was also dependent on the presence of sodium ion since its relaxant activity was markedly reduced in the Na-free solution, but significantly restored in the solution containing a small amount of sodium ion. Sunagane et al. (5) claimed that papaverine induces the smooth muscle relaxation not only through mechanisms independent of the presence of external sodium ion, but also through other mechanisms dependent on the presence of sodium ion, on the basis of similar observations to those obtained in the present experiments. While the sodium ion-independent mechanisms have been sug-

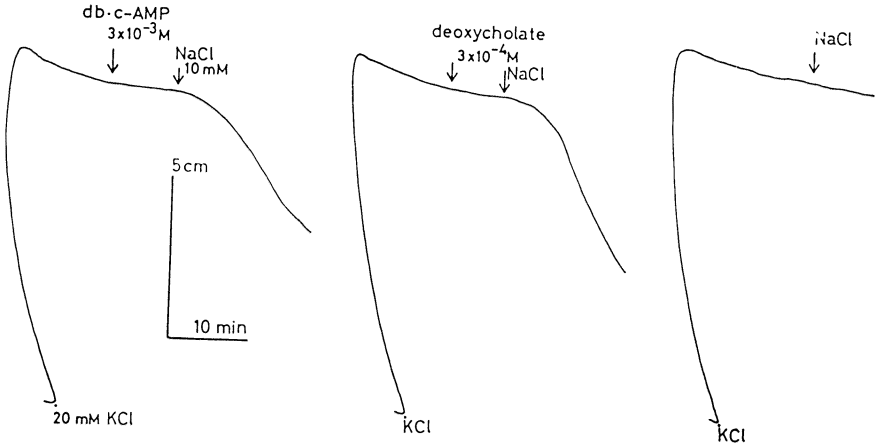

Fig. 2. Effects of addition of sodium ion on the relaxant responses of the taenia coli to dibutyryl cyclic AMP (db·c-AMP) and deoxycholate in the Na-free solution. Drugs were used at doses which induced full relaxation of KCl-induced contracture in the normal solution.

gested to include the inhibition of Ca-influx into the muscle cells (5), the sodium ion-dependent mechanisms have not yet been explained.

In the present results, it was found that the relaxant activity of dibutyryl cyclic AMP was completely lost in the Na-free solution, but revived in the presence of 10 mM sodium ion, suggesting that the presence of sodium ion may be essential for the action of dibutyryl cyclic AMP on the smooth muscle relaxation. Since dibutyryl cyclic AMP is known to qualitatively exert the same effect as cyclic AMP on the cell functions (7), the action of cyclic AMP inducing the smooth muscle relaxation may also be dependent on the presence of sodium ion. This hypothesis is supported by the observations that the relaxations induced by other drugs which increase the cellular cyclic AMP level were affected by external sodium ion. It was observed in the present study that the relaxant effect of deoxycholate, which has been shown to inhibit the PDE (6), was completely abolished in the Na-free solution, but easily revived in the presence of a small amount of sodium ion. Moreover, the relaxation in response to a beta-adrenoceptor stimulant which is known to activate adenylate cyclase has reported to be seriously affected by the removal of external sodium ion (8–10).

It has been well documented that the smooth muscle relaxation induced by papaverine is closely related to the increase of cellular cyclic AMP (1–4). In addition, Sunagane et al. (5) found that the relaxant effects of Aspaminol and benactyzine, synthetic smooth muscle relaxants, which have been demonstrated to inhibit the Ca-influx (11), but not to change the cellular cyclic AMP level (12) were much less dependent on the presence of external sodium ion than that of papaverine. Thus, it is likely that the sodium ion-dependent mechanism of the relaxant action of papaverine involves the increase in cellular cyclic AMP, and the reduction of its relaxant activity in the Na-free solution results from the inactivation of a cyclic AMP-mediated mechanism by the removal of external sodium ion.

However, because our discussion is based on only data obtained from the experiments with replacement of medium sodium ion with sucrose, more experiments in which sodium ion is replaced by other materials such as LiCl and Tris will be necessary in order to define the role of sodium ion in the action of papaverine.

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