Mechanisms of Inhibitory Action of Phenylephrine in Guinea-Pig Taenia Coli

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Abstract—The effect of phenylephrine, an α -agonist, on the Ca movements and the influence of removal of external Na^+ on the relaxant activity of phenylephrine were examined in the taenia coli of guinea pigs. Phenylephrine (10^{-7} – 10^{-5} M) caused dose-dependent relaxation of the taenia coli contracted by 20 mM KCl in Locke-Ringer solution. Phenylephrine (10^{-5} M) suppressed the spike discharges of the taenia coli evoked by 20 mM KCl without affecting the membrane potential, and this was accompanied by the muscle relaxation. Phenylephrine also inhibited the cellular ^{45}Ca -uptake in the taenia coli, but had no discernible effect on the ^{45}Ca -efflux from the smooth muscle. These effects of phenylephrine were not observed in a Na-free solution or in the highly depolarized smooth muscle. These findings suggest that the inhibition of Ca-influx in the taenia coli may be involved in the phenylephrine-induced relaxation in the partly depolarized tissue. Reasons for reduction of phenylephrine action encountered under the Na-free condition were also discussed.

It is well established that the contraction-relaxation cycle of the smooth muscle is controlled by cytoplasmic Ca^{2+} (1), and smooth muscle relaxants produce their relaxant action through affecting the Ca movements of the muscle cells (2–5). It is also well-known that the activation of α -adrenoceptor in the intestinal smooth muscle leads to the relaxation of the smooth muscle. However, the relationship between the relaxation and the Ca movements induced by α -adrenoceptor activation remains unclear. Some authors (6–8) suggested that the relaxation of the taenia coli smooth muscle induced by the α -adrenergic action of adrenaline is related to the mobilization of intracellularly bound Ca. In contrast, Iwayama et al. (9) observed that phenylephrine augmented K efflux, but little affected Ca fluxes and Ca uptake in the taenia coli.

There are several reports suggesting that Na^+ plays an important role in regulating the

cytoplasmic Ca^{2+} concentration (10, 11) and modifies the activity of smooth muscle relaxants (4, 5, 12, 13). Bülbring and Tomita (14) found that the α -adrenoceptor-mediated relaxing action of adrenaline was reduced when Na^+ was removed from the bathing fluid, leading them to postulate that the reduction in the adrenaline effect was related to the changes in the Ca distribution within the smooth muscle cells, which had been caused by the removal of external Na^+ . However, den Hertog (8) and Watanabe (15) claimed that external Na^+ is unnecessary for the α -adrenaline action. Thus, it is of interest to examine how the relaxant action of α -agonists will be influenced when the Na^+ gradient across the cell membrane is changed.

In the present study, the authors aimed to explore the mechanisms of the relaxation induced by the activation of α -adrenoceptor through examining the effect of phenylephrine on the Ca movements and to elucidate the role of external Na^+ in the phenylephrine-induced relaxation in the taenia coli.

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Materials and Methods

Strips of taenia coli were dissected from male guinea pigs weighing 300 to 500 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 strip was recorded by an isotonic transducer (ME Commercial Co., Ltd., Tokyo). The smooth muscle preparation was equilibrated for 30 min under loading with 1 g and then repeatedly treated with hypertonic 20 mM KCl at intervals of 15 min until the contractile response of the muscle became constant. In preparing the Na-free or Na-deficient solution, NaCl in the normal solution was replaced isosmotically by sucrose or equimolarly by Tris-Cl or choline-Cl, and NaHCO_3 was also replaced by KHCO_3 . In these solutions, the potassium ion concentration was adjusted to approximately the normal level by omitting KCl from their composition. The pH of each solution was adjusted to 7.8 by adding HCl. On occasion of the use of choline-Cl as a Na substitute, 2×10^{-6} M atropine was added to the solution in order to prevent its cholinergic action.

In order to determine the relaxant activity of phenylephrine in normal, Na-deficient or Na-free solution, phenylephrine was applied cumulatively or in a single dose to the taenia coli precontracted by 20 mM KCl in each solution. The relaxation induced by phenylephrine was calculated as the percentage decrease of the KCl contracture.

In order to examine the effects of phenylephrine on the electrical activity of the taenia coli, the membrane potential change and developed tension were measured with the single sucrose-gap method. The organ chamber of the apparatus was continuously circulated with the solution which was kept at 32°C and bubbled with 100% O_2 . Phenylephrine was infused to the circulating solution. The exchange of the circulating solution in the chamber was carried out so that it was completed within 1 min.

For determining the cellular ^{45}Ca -uptake, the taenia coli equilibrated in the normal solution for 30 min was rinsed for 10 min in the Ca-free depolarizing solution, which was prepared by adding hypertonic 20 mM or isotonic 50 mM KCl and by removing CaCl_2 to and from the normal or Na-free solution, and then it was immersed for 10 min in the depolarizing solution containing 1 mM of CaCl_2 and ^{45}Ca ($1 \mu\text{Ci/ml}$) in the presence or absence of phenylephrine. After the tissue was washed with the ice-cold Ca-free solution containing 10 mM LaCl_3 for 10 min, it was blotted with ashless filter paper (Whatman No. 42) and quickly weighed.

^{45}Ca -efflux was measured according to the method described previously (5). In this experiment, a specially designed apparatus was set up and the recording of the mechanical response and quick collection of the washout solution were simultaneously made. The ^{45}Ca -efflux was expressed as the rate constant of ^{45}Ca -loss from the muscle.

The radioactivity of the washout solution or in the taenia coli which was digested with a tissue solubilizer, Soluene-350 (Packard Instrument Co.), was counted in a liquid scintillator, Univergel II (Nakarai Chemicals), with a liquid scintillation counter (Aloka, model 651). Drugs and chemicals used were as follows: L-phenylephrine hydrochloride (Sigma), atropine sulfate (Merck), and $^{45}\text{CaCl}_2$ (New England Nuclear). The other reagents used herein were of the highest purity available.

Results

Dose-relaxation curves for phenylephrine in the normal or Na-deficient or Na-free sucrose solution in the smooth muscle precontracted by 20 mM KCl are shown in Fig. 1. When the muscle bathed in the normal solution was immersed in Na-deficient or Na-free sucrose solution, the muscle tone rose transiently and returned to the initial level during 10–20 min washing with each solution. Thus the muscle was preincubated in the Na-deficient or Na-free sucrose solution for 20 min or more prior to the application of KCl. Phenylephrine was applied cumulatively to the muscle when 20 mM KCl-induced contracture reached a

plateau level approximately 10 min after the application of KCl. Phenylephrine (10^{-7} – 10^{-5} M) caused dose-dependent relaxation of the taenia coli depolarized by 20 mM KCl in the normal solution. The dose-relaxation curve was significantly shifted to the right

Fig. 1. Influence of changing external Na^+ concentrations on the relaxing activity of phenylephrine in guinea-pig taenia coli. The dose-relaxation curves for phenylephrine in the 20 mM KCl-depolarized taenia coli were obtained in the normal (●, number of experiments=24), Na-free (○, $n=6$), 10 mM Na^+ (△, $n=6$), 50 mM Na^+ (▲, $n=6$), or 100 mM Na^+ (□, $n=6$) solution, in which NaCl was replaced by sucrose. The relaxation by phenylephrine was calculated as the percentage decrease of the KCl-induced contracture.

by an α -blocker, phentolamine (3×10^{-6} M), but unaffected by a β -blocker, propranolol (10^{-6} M) (data not shown). In the Na-free sucrose solution, phenylephrine failed to cause the relaxation of the taenia coli, while the relaxant activity of phenylephrine was recovered by about 20% in the presence of 10 mM Na^+ . Although the extent of recovery of the relaxation was dependent upon the amount of Na present in the solution, the complete recovery was not obtained even with 100 mM Na^+ (Fig. 1).

The time courses of the changes in the relaxant activity of phenylephrine in the three different solutions, sucrose-, choline- and Tris-substituted Na-free solutions, were compared since the effects of Na-free solutions on the responsiveness of the taenia coli are variable with the substitutes used for replacing Na^+ and the length of the periods during which the taenia was exposed to the Na-free solution (16). The changes in the muscle tone after the exposure of the taenia to each Na-free solution were uneven. When the taenia was exposed to each Na-free solution, the muscle tone was initially increased and the increased tone in both the Na-free Tris and choline solution, unlike in the Na-free sucrose solution, did not return to the initial level and persisted at a considerably high level even after washing with them for over 20 min. In order to examine the

Fig. 2. Effect of phenylephrine (10^{-5} M) on the 20 mM KCl depolarized taenia coli exposed to normal (○, $n=12$) and Na-free solutions in which NaCl was replaced by sucrose (●, $n=4$), Tris-Cl (△, $n=4$) or choline-Cl (▲, $n=4$). The relaxation by phenylephrine was calculated as the percentage decrease of the KCl-induced contracture.

differences in the phenylephrine action with three Na substitutes, the taenia was depolarized by 20 mM KCl at the beginning of the exposure of the muscle to each Na-free solution, the muscle tone was kept in an increased state, and then phenylephrine was applied to the taenia. Figure 2 shows the time course of changes in the relaxant activity of phenylephrine (10^{-5} M) 1, 5, 10, 20 or 40 min after exposure of the taenia to each Na-free solution. Although 10^{-5} M phenylephrine caused a full relaxation of the depolarized taenia coli within 1 min in the normal solution, its relaxant activity was reduced with the time exposed to each Na-free solution and finally abolished. The reduction in the relaxant activity of phenylephrine was slower in the Tris-substituted solution than in the sucrose- or choline-substituted solution.

Figure 3 represents the time-relaxation curves for 10^{-5} M phenylephrine in the solutions containing different concentrations of Na^+ in which NaCl was replaced with sucrose, choline or Tris. Phenylephrine was applied to the depolarized taenia 10 min after the exposure to the sucrose- or choline-substituted solution and 20 min after to the Tris-substituted solution. The relaxant action of phenylephrine was abolished in each Na-free solution. The relaxant effect of phenylephrine was dose-dependently restored by the readmission of Na^+ into the respective Na-free solution (Fig. 3a, b and c). In contrast, the effect of Tris on the phenylephrine action was different from those of sucrose or choline. The relaxant activity of phenylephrine was more sensitively restored in the presence of Na^+ in the Tris-substituted solution (Fig. 3c), as compared with the sucrose- and choline-substituted solutions (Fig. 3a and b).

The effects of phenylephrine on the electrical and mechanical activities in the taenia coli are shown in Fig. 4. In the normal solution, an application of 20 mM KCl to the taenia caused a depolarization of the membrane with spike discharges and a sustained contraction. When phenylephrine (10^{-5} M) was applied to the muscle during the depolarization, the membrane potential was little affected, while the spike discharges were completely suppressed and a full relaxation

was observed (Fig. 4a). On the other hand, 20 mM KCl evoked the contraction and the

Fig. 3. Effects of sodium ion on the relaxing activity of phenylephrine. Time-relaxation curves for phenylephrine (10^{-5} M) in the taenia coli depolarized by 20 mM KCl were obtained in the normal (●, $n=16$), Na-free (○, $n=4$), 10 mM Na^+ (△, $n=4$), 50 mM Na^+ (▲, $n=4$), or 100 mM Na^+ (□, $n=4$) solution, in which NaCl was replaced by sucrose (a), choline-Cl (b) or Tris-Cl (c). Phenylephrine was applied at 10 min (a, b) and 20 min (c) after exchanging the normal solution to the respective Na-deficient one noted above. The relaxation by phenylephrine was calculated as the percentage decrease of the KCl-induced contracture.

a) Normal b) Na-free

Fig. 4. Effect of phenylephrine (10^{-5} M) on the mechanical (lower trace) and electrical (upper trace) activities of the taenia coli depolarized by 20 mM KCl in normal (a) and Na-free (choline substitution) (b) solutions. Horizontal bars show the duration of phenylephrine application. At W, the drug was washed out with the normal solution.

Table 1. Effect of phenylephrine on the cellular ^{45}Ca -uptake of depolarized taenia coli in normal or Na-free solution

		Inhibition % (mean $\pm$ S.E.) ^a in		
		Normal soln. + 20 mM KCl	Na-free soln. + 20 mM KCl	Normal soln. + 50 mM KCl
Phenylephrine	10^{-6} M	15.1 $\pm$ 5.2 ^b (6)	—	—
	10^{-5} M	16.8 $\pm$ 4.9 ^b (4)	6.4 $\pm$ 8.9 (4)	-0.5 $\pm$ 7.4 (4)
	10^{-4} M	—	-9.2 $\pm$ 3.1 (4)	—

^a ^{45}Ca -uptake was determined after 10 min incubation in the ^{45}Ca containing solutions. ^{45}Ca -uptake of the control tissues in the normal + 20 mM KCl, Na-free + 20 mM KCl and normal + 50 mM KCl solutions were 0.439 ± 0.024 , 1.191 ± 0.181 and 0.370 ± 0.010 mmol/kg wet wt., respectively. Numbers of experiments are presented in parentheses. ^b Significant difference from the control, $P < 0.05$.

depolarization of the muscle in the Na-free choline solution, but caused no spike discharges. Phenylephrine exerted no appreciable effect on the 20 mM KCl-evoked changes in the membrane potential and the tension of the muscle (Fig. 4b). The similar results were obtained in the sucrose- or Tris-substituted solution (data not shown).

Since it is generally accepted that the spike generation in the smooth muscle is dependent upon the influx of extracellular

Ca^{2+} (17, 18), the effect of phenylephrine on the cellular ^{45}Ca -uptake in the depolarized taenia coli was examined. Phenylephrine (10^{-6} M, 10^{-5} M) significantly inhibited the cellular ^{45}Ca -uptake in the muscle depolarized by 20 mM KCl in the normal solution, but exerted no effect on that in the Na-free sucrose solution (Table 1). Even a higher dose of phenylephrine (10^{-4} M) failed to inhibit the cellular ^{45}Ca -uptake in the Na-free solution.

Fig. 5. Effect of phenylephrine on the depolarized taenia coli by hypertonic 20 mM KCl (●, $n=12$), by hypertonic 40 mM KCl (▲, $n=6$) and by isotonic 50 mM KCl (■, $n=6$). Phenylephrine was applied cumulatively to the muscle 10 min after the application of KCl. The relaxation by phenylephrine was calculated as the percentage decrease of the KCl-induced contracture.

The effects of phenylephrine on the muscle relaxation and the cellular ^{45}Ca -uptake in the highly depolarized taenia were also examined. Phenylephrine (10^{-5} M) showed no relaxant activity (Fig. 5) and little influenced the cellular ^{45}Ca -uptake (Table 1) in the taenia depolarized by isotonic 50 mM KCl in the normal solution. Furthermore, in the taenia coli depolarized by hypertonic 40 mM KCl, phenylephrine also failed to induce the relaxation of the muscle (Fig. 5), indicating that the disappearance of phenylephrine action in the muscle depolarized by isotonic 50 mM KCl is not due to osmotic changes.

Figure 6 shows the effects of phenylephrine on the ^{45}Ca -efflux from the depolarized taenia coli by 20 mM KCl and the mechanical response of the taenia coli which was recorded simultaneously with the ^{45}Ca -efflux measurement. Phenylephrine (10^{-4} M) applied for 20 min which started 30 min after the initiation of the ^{45}Ca -efflux measurement produced muscle relaxation, but had no effect on the ^{45}Ca -efflux.

Discussion

In the present study, phenylephrine suppressed the spike discharge induced by 20

Fig. 6. Effect of phenylephrine on the ^{45}Ca -efflux and mechanical activity of the depolarized (20 mM KCl) taenia coli of guinea pigs in normal solution. The upper panel shows the response of the muscle to phenylephrine (10^{-4} M). The lower panel shows the effect of phenylephrine (10^{-4} M) on the ^{45}Ca -efflux. Horizontal bars indicate the duration of phenylephrine application. Data were obtained from 6 experiments, respectively.

mM KCl accompanied by a synchronous relaxation of the taenia coli (Fig. 4). Magaribuchi and Kuriyama (12) and Weisbrodt et al. (19) also obtained similar results by using α -agonists such as adrenaline and noradrenaline. In addition, we observed that phenylephrine significantly inhibited the cellular ^{45}Ca -uptake in the taenia coli partly depolarized by 20 mM KCl. As described previously (18, 19) in the results, the spike generation in the smooth muscle of the guinea-pig taenia coli is due to the inward movement of Ca^{2+} . Thus, it is likely that the relaxant action of an α -agonist may be primarily due to the inhibition of the inward Ca movement. On the other hand, phenylephrine exerted no effect on the ^{45}Ca -efflux from the partly depolarized taenia coli of guinea pigs, indicating that the α -agonist-

induced relaxation of the smooth muscle is not related to the acceleration of Ca-efflux.

Iwayama et al. (9) have studied the effect of phenylephrine on the cellular calcium movements in the polarized taenia coli, demonstrating that phenylephrine does not inhibit the cellular ^{45}Ca -uptake. In addition, they observed that phenylephrine caused the hyperpolarization in the polarized taenia coli smooth muscle. Other α -agonists such as adrenaline and noradrenaline were also reported to cause the hyperpolarization in the polarized muscle preparations (7, 8, 14, 20, 21). In the present study, however, phenylephrine produced little effect on the membrane potential of the taenia coli partly depolarized by 20 mM KCl, although it suppressed the spike discharges as shown in Fig. 4. The discrepancy between their results and ours may be mainly attributed to the differences in the experimental conditions. In the depolarized muscles, the Ca-influx should increase, and phenylephrine may act predominantly on the Ca-influx.

Den Hertog (8) reported that α -receptor-mediated action of adrenaline little influenced the cellular ^{45}Ca -uptake of the taenia depolarized by isotonic 50 mM KCl. In the present study, phenylephrine also failed to inhibit the cellular ^{45}Ca -uptake in the taenia coli depolarized by isotonic 50 mM KCl (Table 1). Furthermore, the relaxant action of phenylephrine was completely inhibited in the taenia depolarized by isotonic 50 mM or hypertonic 40 mM KCl. These results suggest that in the highly depolarized smooth muscle the inhibitory action of α -agonists on the inward movement of calcium might become ineffective and the relaxant activity of α -agonists eventually disappear. Meanwhile, it is known that the spike generation is ceased in the highly depolarized muscle, probably because of the depolarization block (12, 19). In fact, the spike discharges disappeared in the taenia coli depolarized by isotonic 50 mM or hypertonic 40 mM KCl (data not shown). Thus, the inhibitory action of α -agonists on the inward calcium movement seems to be effective only in such conditions as when the spike discharges are being generated.

It is well documented that the relaxation of the taenia coli of guinea pigs in response to

α -agonists is related to the acceleration of K^+ flux (8, 9, 22), while the role of Na^+ on the α -adrenergic action remains unclear. The present results demonstrated that the relaxant action of phenylephrine was completely lost in the Na-free solution in which Na^+ was replaced by substitutes such as sucrose, choline and Tris, but restored in proportion to the amount of Na^+ readded to the Na-free solution. These findings suggest that the presence of external Na^+ may be essential for the operation of the relaxation mechanisms mediated via α -adrenoceptors. Meanwhile, the fact that the relaxant action of phenylephrine completely disappeared in the Tris- and choline-substituted Na-free solutions containing normal Cl^- concentration may indicate that the role of chloride ion in the relaxing action of phenylephrine is, if any, minimal. This view is in line with that of Jenkinson and Morton (23).

Since the external Na^+ is known to be important for the generation of the spike potential (24), the removal of external Na^+ should influence the KCl-induced spike discharges. In fact, the spike discharges of the taenia coli could not be generated in the Na-free solution even when the muscle was depolarized by 20 mM KCl. This fact indicates that the smooth muscle contraction in the Na-free solution may be evoked via some mechanisms other than those related to the Ca-spikes. The present study further showed that phenylephrine failed to inhibit the cellular ^{45}Ca -uptake in the partly depolarized taenia coli in the Na-free solution. These facts may account for the loss of the relaxant action of phenylephrine in the Na-free solution, that is, the calcium mobilizing pathways associated with muscle contraction may be converted from phenylephrine-sensitive ones to phenylephrine-insensitive ones with the removal of the external Na^+ . Some authors (25, 26) postulated that under the external Na-depleted condition, calcium can enter into the vascular smooth muscle cells through the channel which is usually occupied by sodium. If it is the case in the guinea-pig taenia coli, the channel may function as the calcium mobilizing pathway for the contraction induced by KCl in the Na-free solution. In this context, phenylephrine

is supposed to be insensitive to the channel.

In conclusion, phenylephrine causes the relaxation of the 20 mM KCl-depolarized taenia coli through inhibiting the inward movement of Ca^{2+} in the muscle cells in the medium containing a normal concentration of Na^+ , but loses its relaxant action in the Na -free media probably because it becomes insensitive to the altered Ca -mobilization under Na -free conditions.

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