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Short communication

Ba^{2+} selectively inhibits receptor-mediated contraction of the esophageal muscularis mucosae

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Abstract

The aim of the present study was to examine the effect of Ba^{2+} on acetylcholine- and KCl-induced contractions of the guinea-pig esophageal muscularis mucosae. When the muscularis mucosae was pretreated with nicardipine (1 μM), Ba^{2+} (0.1–30 mM) markedly inhibited the acetylcholine (3 μM)-induced tone, and at 10–30 mM the tone returned to its basal level. In contrast, Ba^{2+} (0.1–30 mM) slightly increased the KCl (60 mM)-induced tone. Moreover, the Ba^{2+} (30 mM)-increased KCl tone was completely inhibited by treatment with nicardipine (0.3–1 μM). In conclusion, Ba^{2+} both selectively inhibits receptor-mediated contraction of the muscularis mucosae and itself permeates through voltage-dependent Ca^{2+} channels.

Keywords: Ba^{2+}; Receptor-activated channel; Voltage-dependent channel; Muscularis mucosae; Acetylcholine; KCl

1. Introduction

The muscularis mucosae, a thin band of smooth muscle located at the base of the gastrointestinal mucosa, has received little attention as compared with the external muscle layers of the gut wall (Kamikawa and Shimo, 1979). We have reported that there are two different mechanisms leading to contraction of the isolated guinea-pig esophageal muscularis mucosae, depolarization-dependent and receptor-mediated contraction mechanisms (Kamikawa et al., 1985; Kamikawa and Shimo, 1987; Uchida et al., 1998). Ca^{2+} channel antagonists can selectively inhibit the depolarization-dependent contraction, but not the receptor-mediated contraction in this tissue (Kamikawa et al., 1985; Kamikawa and Shimo, 1987; Uchida et al., 1997; Uchida et al., 1998). In smooth muscles of the mammalian gastrointestinal tract, extracellular Ca^{2+} largely permeates into smooth muscle cells through voltage-dependent Ca^{2+} channels (VDCs) (Yu and Bose, 1991).

Previous studies have indicated that exogenously applied Ba^{2+} can permeate into the cell through VDCs and behave as a substitute for Ca^{2+}, eliciting contraction of gastrointestinal smooth muscles (Hotta and Tsuzuki, 1968; Yu and Bose, 1991; Murillo et al., 1997). In the esophageal muscularis mucosae, however, the role of Ba^{2+} in depolarization-dependent (KCl-induced) and receptor-mediated (acetylcholine-induced) contractions has not yet been studied. Here we report new evidence showing that Ba^{2+} selectively inhibits the receptor-mediated tonic contraction of the muscularis mucosae isolated from the guinea-pig esophagus.

2. Materials and methods

2.1. Preparations

Adult male guinea-pigs (Hartley strain, 250–400 g) were anesthetized with sevoflurane and were bled from the cervical artery. Then, the esophageal body was excised. The excised esophagus was pinned on a cork mat immersed in a Tyrode solution. The outer striated muscle coat was cut longitudinally and gently peeled away leaving an inner tube (Uchida, 1983). This tube, including the longitudinal muscularis mucosae, was about 10–15 mm long without a load and was immersed in a 10-ml organ bath filled with a Tyrode solution of the following composition (mM): NaCl 136.8, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$

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1.05, $NaHCO_3$ 11.9, NaH_2PO_4 0.42, glucose 5.56 at pH 7.4. The Tyrode solution contained physostigmine (20 nM) to inhibit the cholinesterase activity in this tissue, and the solution was always bubbled with 5% CO_2 and 95% O_2 at 37°C.

2.2. Measurement of response

The esophageal muscularis mucosae thus obtained was suspended under a 0.5-g load, and the experiments were started 60 min later. During this equilibration period, the preparation was washed with fresh Tyrode solution every 20 min. Responses of the longitudinal muscularis mucosae were recorded on a polygraph (RJG-4124, Nihon Kohden, Tokyo, Japan) by an isotonic transducer (TD-112S, Nihon Kohden, Tokyo, Japan). After the 60-min equilibration period, the preparation was maximally contracted with a single concentration of carbachol (10 μM) and was allowed to equilibrate for 30 min after washout. This was repeated until two successive contractions of approximately equal size had been obtained. In experiments on the responsiveness to $BaCl_2$ or $MgCl_2$, the muscularis mucosae was precontracted with either acetylcholine (3 μM) or KCl (60 mM). Both spasmogens caused a tonic contraction which was comparable to the maximum contraction induced by carbachol (10 μM). After the tonic contraction induced by acetylcholine or KCl had reached its plateau, $BaCl_2$ or $MgCl_2$ was added to the tissue as cumulatively increased doses. Statistical analysis of the data was performed with Student's *t*-test. Values of $P < 0.05$ were considered to be significant.

2.3. Drugs

The following drugs were used: acetylcholine chloride (Dai-ichi Seiyaku, Tokyo, Japan), carbamylcholine chloride (carbachol) (Sigma, St. Louis, USA), $BaCl_2 \cdot 2H_2O$, $MgCl_2 \cdot 6H_2O$, nicardipine hydrochloride, physostigmine sulphate (Wako, Osaka, Japan), KCl (Kanto Chemical, Tokyo, Japan), sevoflurane (Maruishi Pharmaceutical,

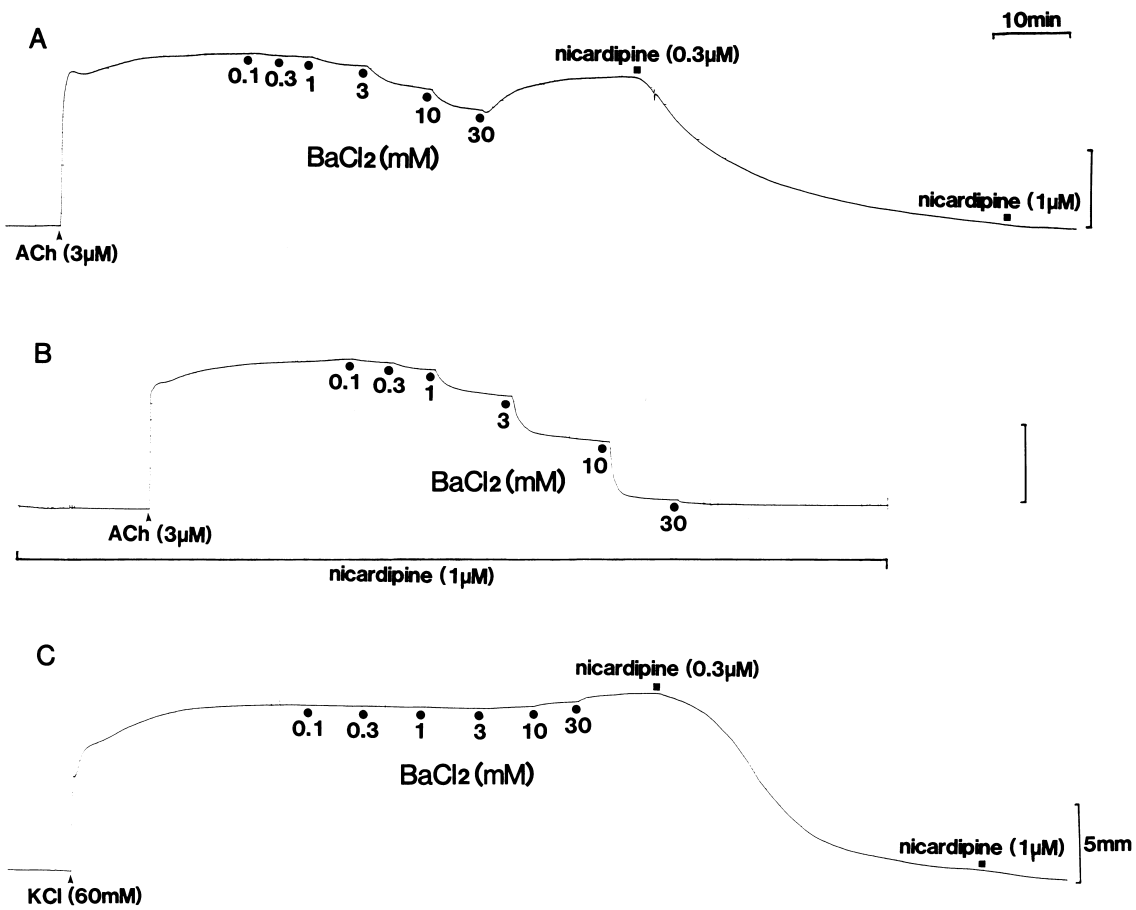

Fig. 1. Typical traces showing the influence of cumulatively increased concentrations of $BaCl_2$ on the acetylcholine (ACh 3 μM, A and B)- or KCl (60 mM, C)-precontracted muscularis mucosae isolated from the guinea-pig esophagus. A: $BaCl_2$ slightly relaxed the muscularis mucosae precontracted with ACh (3 μM), but at its higher concentration $BaCl_2$-induced relaxation rather reversed to a contraction. B: After pretreatment with nicardipine (1 μM), $BaCl_2$ produced a concentration-dependent relaxation of the muscularis mucosae precontracted with ACh (3 μM) and at 10–30 mM abolished the ACh-induced tone. C: $BaCl_2$ slightly elevated the tone of the KCl (60 mM)-precontracted muscularis mucosae. Vertical calibrations show 5 mm shortening of the tissue. Horizontal calibration shows 10 min.

Osaka, Japan). Acetylcholine, carbachol and physostigmine were dissolved in and diluted with 0.9% NaCl solution. Nicardipine, $BaCl_2$, $MgCl_2$ and KCl were dissolved in and diluted with distilled water. Concentrations of drugs described in this paper refer to the final bath concentrations.

3. Results

The esophageal muscularis mucosae responded to an application of acetylcholine (3 μM) or KCl (60 mM) with a tonic contraction (Fig. 1). The amplitude of the tonic contraction induced by acetylcholine (3 μM) was almost the same as that of the contraction induced by KCl (60 mM). When the tonic contraction induced by acetylcholine or KCl reached its plateau after 20–30 min, $BaCl_2$ was added cumulatively to the organ bath. $BaCl_2$ (0.1–10 mM) slightly concentration dependently inhibited the acetylcholine-induced tone (Fig. 1A, Fig. 2A). At the highest concentration (30 mM), however, the $BaCl_2$-induced relaxation was reversed to a contraction. This $BaCl_2$ (30 mM)-reversed contraction was completely inhibited by the L-type VDC blocker, nicardipine (0.3–1 μM) (Fig. 1A). Although pretreatment of the esophageal muscularis mucosae with nicardipine (1 μM) did not modify the acetylcholine (3 μM)-induced tonic contraction, subsequently applied $BaCl_2$ (0.1–30 mM) produced only a concentration-dependent relaxation and at 10–30 mM abolished the acetylcholine-induced tone (Fig. 1B, Fig. 2A). In contrast, $BaCl_2$ (0.1–30 mM) did not produce any relaxation of the esophageal muscularis mucosae precontracted with KCl (60 mM), but very slightly elevated the KCl-induced tone (Fig. 1C, Fig. 2A). The $BaCl_2$ (30 mM)-elevated KCl tone was completely inhibited by treatment with nicardipine (0.3–1 μM) (Fig. 1C). Exogenously applied $MgCl_2$ (3–30 mM) produced a concentration-dependent relaxation of the muscularis mucosae precontracted with acetylcholine (3 μM) or KCl (60 mM), and at 30 mM $MgCl_2$ abolished the acetylcholine- and KCl-induced tone (Fig. 2B). Relaxing responses induced by $MgCl_2$ were not affected by nicardipine (1 μM).

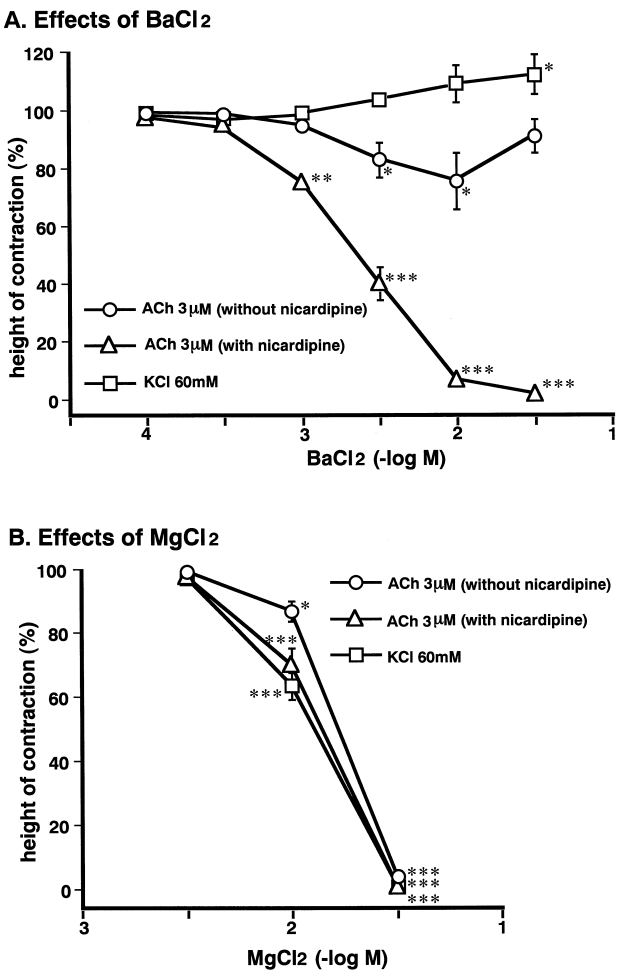

Fig. 2. Cumulative concentration–response curves to $BaCl_2$ (A) and $MgCl_2$ (B) of the esophageal muscularis mucosae. ○, Effect on acetylcholine (ACh, 3 μM)-precontracted preparations without nicardipine pretreatment. △, Effect on ACh (3 μM)-precontracted preparations with nicardipine (1 μM) pretreatment. □, Effect on KCl (60 mM)-precontracted preparations. Ordinate scales show the amplitude of contraction as a percentage of the plateau level of precontraction. Each point represents the mean ± S.E.M. of 4–7 observations. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; significantly different from the plateau level of ACh- or KCl-induced tone.

4. Discussion

The present study provided new evidence that exogenously applied Ba^{2+} has different modulating effects on receptor-mediated and depolarization-dependent contractions of the guinea-pig esophageal muscularis mucosae. Ba^{2+} concentration dependently inhibited acetylcholine-induced tonic contraction of this tissue in the presence of nicardipine, but raised the tone, presumably by permeating into the cell through VDCs which were inhibited by treatment with nicardipine. It is well known that, in most smooth muscle preparations, Ba^{2+} can permeate into the smooth muscle cell through VDCs and produce a contraction as a substitute for Ca^{2+} (Hotta and Tsuzuki, 1968; Baba et al., 1985; Karaki et al., 1986; Murillo et al., 1997). Recently, non-selective cation channels which are activated by receptor stimulation have been found in electrophysiological studies of some smooth muscles (Inoue, 1995; Karaki et al., 1997). Non-selective cation channels are permeable to Ba^{2+} (Inoue, 1995), therefore Ba^{2+} presumably opened VDCs through membrane depolarization and Ba^{2+} itself also permeated into the cell through VDCs, eliciting contractions of smooth muscle. The relaxing action of Ba^{2+} on the acetylcholine-induced tonic contraction was stronger in the presence of nicardipine,

because Ba^{2+} is thought not to permeate into the cell through VDCs and not to elevate the tone of this tissue.

Our previous studies on the esophageal muscularis mucosae of guinea-pig have indicated that Ca^{2+} channel antagonists such as verapamil or nicardipine selectively inhibit the KCl-induced contraction of this tissue, but not the acetylcholine-induced one (Kamikawa et al., 1985; Uchida et al., 1997). In addition, the acetylcholine-induced contraction is fully dependent on the presence of extracellular Ca^{2+} (Kamikawa et al., 1985), and this contraction was strongly inhibited by the general Ca^{2+}-entry blocker, SK & F96365 (1-{β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl}-1*H*-imidazole hydrochloride), and slightly inhibited by the protein kinase C inhibitor, H-7 (1-(5-isoquinolinyl-sulfonyl)-2-methylpiperazine) (Uchida et al., 1997). We had suggested that the acetylcholine-induced contraction is coupled mainly to receptor-activated Ca^{2+} influx and partly to Ca^{2+} sensitization to contractile elements, but not intracellular Ca^{2+} release (Karaki et al., 1997). Therefore, mechanisms of the relaxing action of Ba^{2+} possibly involve inhibition of Ca^{2+} influx and/or a decrease in Ca^{2+} sensitivity of contractile elements. There are some reports of a modulating effect of Ba^{2+} on receptor-activated Ca^{2+} influx. Murray and Kotlikoff (1991) have reported that a novel Ca^{2+} channel antagonist-insensitive receptor-activated Ca^{2+} influx pathway is located on human airway smooth muscle cells and that Ba^{2+} inhibits this novel Ca^{2+} influx pathway. Moreover, capacitative Ca^{2+} entry, which is the transmembrane Ca^{2+} influx through store-operated Ca^{2+} channels in the plasma membrane in response to Ca^{2+} depletion within the endoplasmic reticulum, is known to occur following agonist activation of smooth muscle (Gibson et al., 1998). Recently, Ohta et al. (1995) reported that Ba^{2+} inhibits capacitative Ca^{2+} entry in the rat ileal smooth muscle. Thus, it seems likely that Ba^{2+} inhibits the receptor-activated Ca^{2+} influx in the esophageal muscularis mucosae of guinea-pig. Ba^{2+} is a useful tool for differentiating receptor-mediated and depolarization-dependent contractions in this tissue.

In contrast to Ba^{2+}, Mg^{2+} acts as inhibitor of every Ca^{2+} influx pathway (Karaki, 1989; Wallnofer et al., 1989). Exogenously applied Mg^{2+} non-selectively inhibited both the contraction induced by acetylcholine and that by KCl in the esophageal muscularis mucosae. The relaxing action of Mg^{2+} was not dependent on the presence of nicardipine. These results indicate that Mg^{2+} acts as a non-selective inhibitor of receptor-mediated and depolarization-dependent contractions in the esophageal muscularis mucosae.

In conclusion, exogenously applied Ba^{2+} selectively inhibits receptor-mediated contraction of the guinea-pig esophageal muscularis mucosae. Ba^{2+} may, however, elevate the tone of this tissue by permeating into the smooth muscle cell through VDCs. The esophageal muscularis mucosae is a useful preparation to examine the Ca^{2+} entry mechanism through receptor-activated channels with unique characteristics.

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