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Regional Differences in Calcium Sensitivity in the Guinea-pig Intestine

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Abstract

The effect of the Ca^{2+}-channel antagonist nicardipine on the basal tone of six segments (duodenum, jejunum, ileum, proximal colon, distal colon and rectum) of the guinea-pig intestine has been investigated in muscle preparations.

Nicardipine reduced the basal tone of the proximal and distal colon but not of the duodenum, jejunum, ileum and rectum. Similarly, when each segment was incubated in Ca^{2+}-free medium, the basal tone of the proximal and distal colon, but not that of the other four segments, was reduced. The reduced basal tone recovered after cumulative addition of Ca^{2+} in both colon preparations. The basal tone of the distal colon was partly reduced by tetrodotoxin, atropine and clonidine. Conversely, L-type Ca^{2+}-channel antagonists (Cd^{2+}, verapamil and nicardipine), but not the T-type Ca^{2+}-channel antagonist Ni^{2+}, completely reduced the basal tone of the distal colon.

These results indicate that in the regulation of basal tone there are additional regional differences in the effect of Ca^{2+} influx into the cells from the extracellular fluid which might involve L-type-like Ca^{2+} channels and might partly be because of neuronal factors.

The role of Ca^{2+} as a regulator of smooth muscle tension has already been established. Smooth muscle contraction occurs as a result of an increase in intracellular Ca^{2+}, formation of Ca^{2+}–calmodulin complex and activation of myosin light-chain kinase (Johns et al 1987). The increase in intracellular Ca^{2+} is regulated by the influx of Ca^{2+} into the cells from the extracellular fluid, mainly through Ca^{2+} channels, or the release of Ca^{2+} from intracellular Ca^{2+} stores, or both. The relative importance of the two pathways is dependent on the species or the source of the smooth muscle (Snape 1982; Maggi et al 1985; Barone et al 1986; Arruebo et al 1987; Murillo et al 1994).

There are several reports of regional differences within the intestinal smooth muscle in the blocking action of drugs which interfere with Ca^{2+} mobilization from either the intra- or extracellular Ca^{2+} pool against spontaneous activity or contraction induced by several stimuli (Manzini et al 1984; Maggi et al 1985). Ca^{2+}-channel antagonists are reported to reduce the basal tone of some intestinal smooth muscles, but the regional difference has not been discussed.

Ca^{2+}-channel antagonists are widely used in clinical practice in the management of cardiovascular disease but can cause constipation as a side-effect (Krevsky et al 1991). It is also reported that they can cause constipation in rodents (Nyska et al 1994).

The physiological role of basal tone is not clear, but it is possible that the Ca^{2+} sensitivity of basal tone is reflected in the effects of Ca^{2+}-channel antagonists on intestinal motility. Therefore, we are interested in the effect of Ca^{2+}-channel antagonists on the basal tone of the intestine. The main objective of this study was to investigate whether there are regional differences in the effects of Ca^{2+}-channel antagonists on basal tone. We also examined the properties of Ca^{2+} influx into the cells involved in the regulation of the basal tone.

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Materials and Methods

Materials

The main chemicals used in the experiments were: nicardipine and verapamil (Sigma), atropine, naloxone, clonidine (Nakarai) and tetrodotoxin (Calbiochem). Nicardipine was dissolved in pure dimethylsulphoxide (DMSO) and diluted with sterile distilled water so that the final solution contained less than 1% DMSO. Other compounds were dissolved in sterile distilled water.

Muscle preparation

Male Hartley guinea-pigs (5–10 weeks old) were lightly anaesthetized with ether and then killed by cutting the neck blood vessels. Segments of the duodenum (1–4 cm distal to the pylorus), jejunum (5–10 cm distal to the ligament of Treitz), ileum (5–10 cm proximal to the ileocaecal junction), proximal colon (5–10 cm distal to the caecum), distal colon (15–20 cm distal to the caecum) and rectum (1–5 cm proximal to the anus) were removed. The strips were mounted in a thermostatically controlled 5-mL organ bath containing normal Krebs-Henseleit solution (composition: NaCl 120 mM, KCl 4·7 mM, $CaCl_2$ 2·4 mM, $MgSO_4$ 1·2 mM, $NaHCO_3$ 24·5 mM, KH_2PO_4 1·0 mM and glucose 5·6 mM), at pH 7·4 and 37°C, continuously gassed with 95% O_2–5% CO_2. Mechanical activity, mainly produced from the longitudinal muscle, was isotonically recorded by a strain-gauge transducer connected to a pen recorder, the initial load being 1 g.

Experimental procedure

In the investigation of the effects of various antagonists on basal tone, each strip was left to equilibrate in normal Krebs-Henseleit solution for 30 min before use, and the reactivity of

each segment to acetylcholine (10^{-5} M) was then confirmed. Cumulative concentration–response curves to the various antagonists were constructed. The antagonists were added directly to the bath and the concentration was increased as soon as there was a stable response.

In the examination of the effects on high K^+-induced contraction, the strips were exposed to Ca^{2+}-free Krebs-Henseleit solution containing a high concentration of K^+ for 30 min and contraction was induced by addition of 2 mM $CaCl_2$. The effects of compounds were investigated by cumulative addition to the bath after stable response to high K^+ concentration was observed. Each strip was used for only one concentration–response curve.

Data analysis

The concentration of the drug required to reduce the basal tone or high K^+ concentration-induced contraction by 50% (IC50) was calculated using Litchfield-Wilcoxon's method (Litchfield & Wilcoxon 1949).

Results

Effect of nicardipine on the basal tone of guinea-pig intestine segments

After the 30-min incubation period in normal Krebs-Henseleit solution, each segment of guinea-pig intestine was equilibrated. Fig. 1 shows a typical set of data obtained after addition of nicardipine to the guinea-pig intestine. Nicardipine ($10^{-8} - 3 \times 10^{-5}$ M) resulted in a concentration-dependent reduction of the basal tone of the proximal and distal colon, and its effect reached plateau at 3×10^{-5} M. Even at 3×10^{-5} M, however, nicardipine had no effect on the basal tone of the duodenum, jejunum, ileum and rectum.

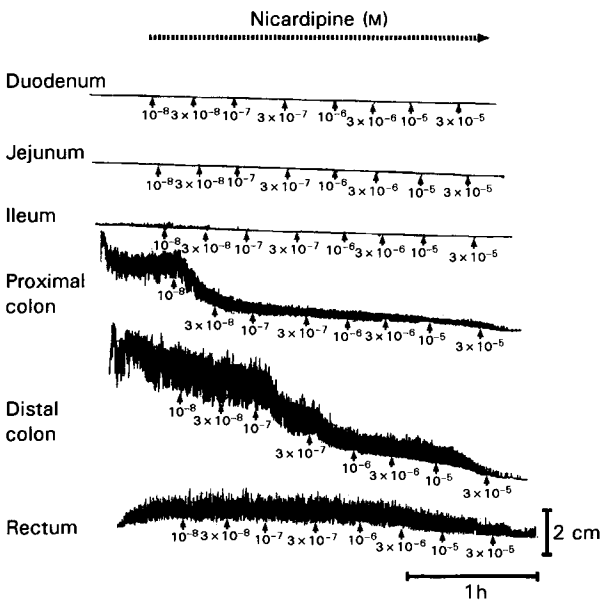

FIG. 1. Effect of nicardipine on the basal tone of the guinea-pig duodenum, jejunum, ileum, proximal colon, distal colon and rectum. Arrow heads denote additions of nicardipine at the concentrations indicated.

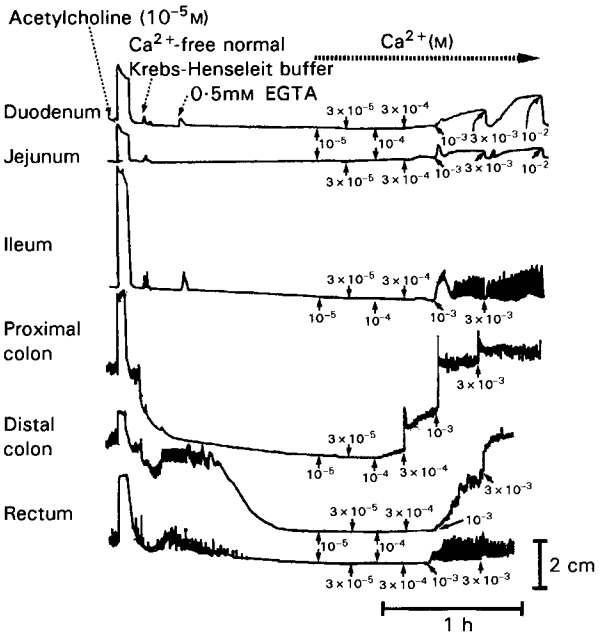

FIG. 2. Effect of extracellular Ca^{2+} on the basal tone of the guinea-pig duodenum, jejunum, ileum, proximal colon, distal colon and rectum. Arrow heads denote additions of Ca^{2+} at the concentrations indicated.

Effect of extracellular Ca^{2+}

The reduced basal tone of the proximal and distal colon was restored to the normal level by addition of Ca^{2+} at 10^{-3} M for the proximal colon and at 3×10^{-3} M for the distal colon (Fig. 2). Addition of Ca^{2+} to the duodenum, jejunum, ileum and rectum preparations resulted in a transitory increase of basal tone only. The relative potency of the decrease of basal tone induced by removal of extracellular Ca^{2+} compared with the acetylcholine-induced contraction was 0·1, 0·1, 0·1, 0·83, 2·07 and 0·43, respectively, for the guinea-pig duodenum, jejunum, ileum, proximal colon, distal colon and rectum. Therefore, the order of potency in each segment was distal colon > proximal colon > rectum > duodenum = jejunum = ileum.

Effects of tetrodotoxin, atropine, clonidine and naloxone on the basal tone of guinea-pig distal colon

Tetrodotoxin, atropine and clonidine inhibited the basal tone of guinea-pig distal colon in a concentration-dependent manner, but the maximum reduction was only 29·8 ± 3·1, 44·6 ± 4·9 and 30·2 ± 14·9%, respectively (Fig. 3). Even at a concentration of 30 μM naloxone had no effect on basal tone.

Effects of Ca^{2+}-channel antagonists on the basal tone or high-K^+-concentration-induced contraction of guinea-pig distal colon

Cd^{2+}, nicardipine and verapamil reduced the basal tone of guinea-pig distal colon in a concentration-dependent manner with IC50 values of 0·42 mM, 0.155 μM and 1·71 μM, respectively, but Ni^{2+}, even at 3 mM, had no effect (Fig. 4).

Nicardipine inhibited high K^+-concentration (40, 60, 80 and 100 mM)-induced contraction, in a concentration-dependent manner, with IC50 values of 108, 1·74. 0·99 and 1·88 nM, respectively. Verapamil inhibited high-K^+-concentration-

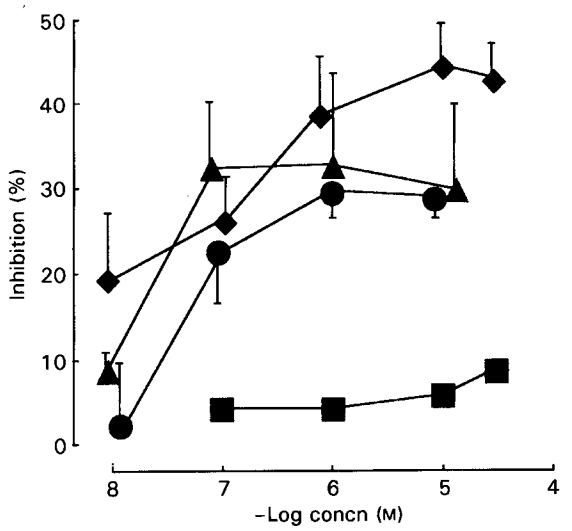

FIG. 3. Inhibitory effects of tetrodotoxin (●), atropine (◆), clonidine (▲) and naloxone (■) on the basal tone of guinea-pig distal colon. Each point represents the mean ± s.e. of results from five experiments.

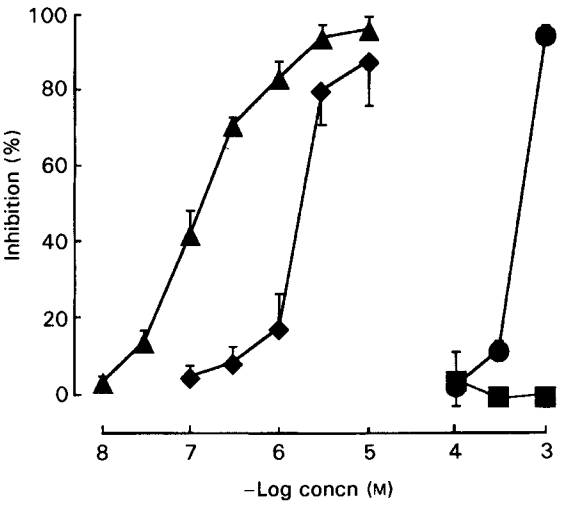

FIG. 4. Inhibitory effects of Ni^{2+}(■), Cd^{2+} (●), nicardipine (▲) and verapamil (◆) on the basal tone of guinea-pig distal colon. Each point represents the mean ± s.e. of results from five experiments.

induced contraction in a concentration-dependent manner with IC50 values of 864, 110, 44·1 and 61·0 nM, respectively (Fig. 5).

Discussion

In this investigation of whether there are regional differences in the regulatory mechanism of basal tone in the guinea-pig intestine, we have demonstrated that nicardipine reduced the basal tone of the proximal and distal colon, but not of the other segments of the intestine. Results for verapamil were similar to those for nicardipine. Our results for the inhibitory effect of Ca^{2+}-channel antagonist on the basal tone of the proximal and distal colon are in good agreement with those found in similar studies using the circular muscle of guinea-pig colon (Marcoli et al 1989; Lecchini et al 1991). However, this is the first report that indicates a regional difference. Furthermore, to determine whether the inhibition of extracelluar Ca^{2+} influx is responsible for these results, we examined the effect of external Ca^{2+}. The removal of Ca^{2+} from the medium reversibly reduced the basal tone of the proximal and distal colon but not that of other regions. Thus, it was clarified that the regional difference is dependent on the Ca^{2+} influx into the cells as the mechanism regulating the basal tone.

In our examination of the cause of the regional difference between the colon and the other intestinal segments, we used the distal colon rather than the proximal colon, because the Ca^{2+} influx into the cells seemed to play a more important role in the basal tone of the distal colon than in that of the proximal colon. The basal tone was partially inhibited by neuronal blockage with tetrodotoxin, muscarinic blockade with atropine and activation of adrenergic alpha-2 receptor with clonidine. Opiate antagonism with naloxone did not affect it. These results suggested that a neuronal factor involving cholinergic

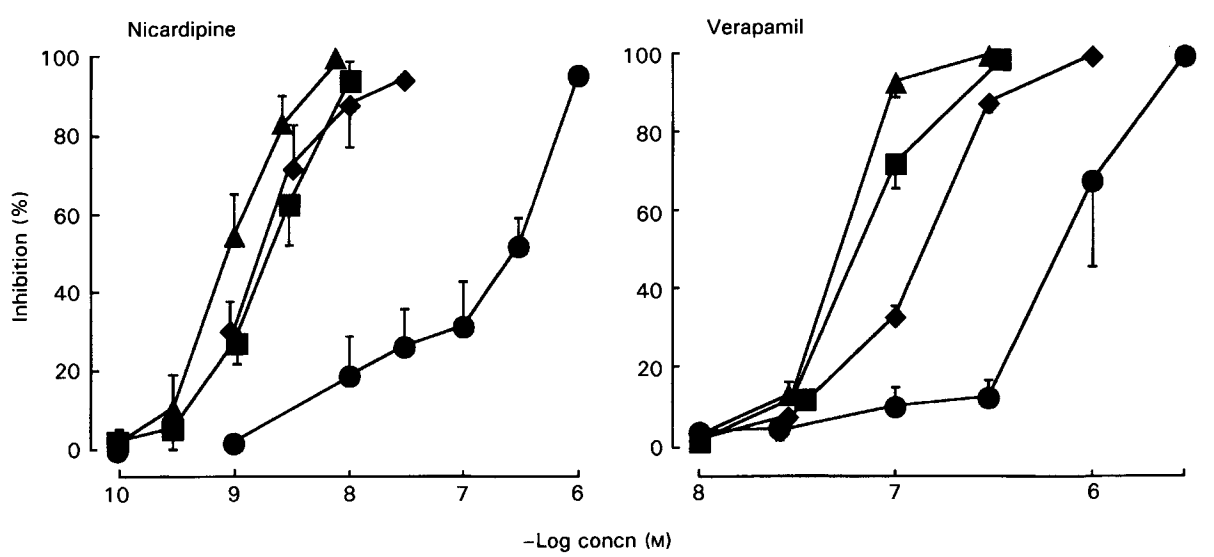

FIG. 5. Effects of nicardipine and verapamil on contraction of guinea-pig distal colon induced by 40 (●), 60 (◆), 80 (▲) and 100 (■) mM K^+. Each point represents the mean ± s.e. of results from 4–6 experiments.

transmission but not the opiate receptor might be partly related to the regulation of basal tone, because both tetrodotoxin and clonidine, but not naloxone, were reported to inhibit resting endogenous acetylcholine overflow in the guinea-pig colon (Marino et al 1994). These antagonists and agonists had no effects on the basal tone of the duodenum, jejunum, ileum and rectum (data not shown).

Inhibition of the L-type, but not T-type, Ca^{2+} channel completely reduced the basal tone of the distal colon. However, this effect was less potent than that on 80 mM K^+-induced contraction of the distal colon.

We speculated that there are species or state differences between Ca^{2+} channels involved in basal tone and those involved in 80 mM K^+-induced contraction, and we accordingly examined the effect of Ca^{2+}-channel antagonists on 40, 60 and 100 mM K^+-induced contraction. We found that the effect of Ca^{2+}-channel antagonists on 40 mM K^+-induced contraction was nearly equal to that on basal tone but less potent than that on 80 mM K^+-induced contraction. Gurney (1994) similarly reported that the inhibitory effect of nifedipine on 20 mM K^+-induced contraction was less potent than that on 50 mM K^+-induced contraction in rabbit main pulmonary artery. He noted that the less potent effect in moderate depolarization (20 mM K^+) than in large depolarization (50 mM K^+) arose because nifedipine binds with higher affinity to inactivated channels than to closed (resting) or open channels. Therefore, it might be assumed that in the guinea-pig distal colon, external 40 mM K^+-induced moderate depolarization and the states of Ca^{2+}channels differed from those involved in 60, 80 or 100 mM K^+-induced contraction. As the effects of Ca^{2+}-channel antagonists on basal tone and 40 mM K^+-induced contraction were nearly equal, we assume that the species of Ca^{2+} channels involved in basal tone are the same as those involved in 80 mM K^+-induced contraction but that the states differ.

In contrast with the distal colon, in the ileum the effect of Ca^{2+}-channel antagonists on 40 mM K^+-induced contraction was nearly equal to that on 80 mM K^+-induced contraction (data not shown). Thus, in the ileum, external 40 mM K^+ seems to induce large depolarization but not moderate depolarization. This finding is another regional difference. We intend to test our results by electrobiological analysis.

These results indicate that in the regulation of basal tone, there are additional regional differences in the effect of Ca^{2+} influx into the cells from the extracellular fluid which might involve L-type-like Ca^{2+} channels and might be partly a result of neuronal factors.

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