Effects of Nifedipine and Nickel on Plateau Potentials Generated in Submucosal Interstitial Cells Distributed in the Mouse Proximal Colon

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Abstract

The effects of nifedipine and nickel ions (Ni²⁺), known inhibitors of L- and T-type voltage-gated Ca-channels respectively, were investigated on plateau potentials recorded from submucosal interstitial cells distributed in the mouse proximal colon. Plateau potentials were generated at a frequency of about 15 times min⁻¹ and were formed of two components. The primary component had an initial fast rate of rise with a transient potential (rate of rise, 130 mV/s; peak amplitude, 35 mV) and was followed by a secondary plateau component with a sustained potential (amplitude, 25 mV; duration, 2.6 s). Each cell from which recordings were made was injected with neurobiotin. Subsequent morphological examination with a confocal microscope indicated successful visualization of injected cells only in the presence of 18β-glycyrrhetinic acid (an inhibitor of gap junctional connections), suggesting that these cells were dye-coupled with surrounding cells. The cells injected with neurobiotin exhibited an oval-shaped cell body with bipolar processes and were distributed in the submucosal layer, suggesting that they were submucosal interstitial cells of Cajal (ICC-SM). The plateau potentials were not altered by 0.01 µM nifedipine, but were reduced in duration by 0.1 µM nifedipine, and abolished by 1 µM nifedipine. The rate of rise of plateau potentials, but not their amplitude, was reduced by Ni²⁺ (> 10 µM), with no significant alteration of the membrane potential. In the presence of 100 µM Ni²⁺, the plateau potentials were changed to a triangular form. Thus, the plateau potentials were formed by two types of voltage-gated channel current: the initial component was produced by a Ni²⁺-sensitive channel current and the plateau component by a nifedipine-sensitive current. The possible involvement of two different types of voltage-gated Ca²⁺-channels in the generation of submucosal pacemaker potentials was discussed.

Keywords: proximal colon, submucosal cells, plateau potential, nickel ions, nifedipine
Introduction

Gastrointestinal smooth muscle generates spontaneous electrical activity in the form of slow waves or spike potentials (Tomita, 1981). Impairment of the development of interstitial cells of Cajal distributed in the myenteric region (ICC-MY) by inhibiting the expression of c-Kit receptor proteins, either using immunological methods or c-kit gene mutation, induces gastrointestinal disorders. These include disappearance of the rhythmic electrical activity of the smooth muscle (Ward et al., 1994; Torihashi et al., 1995; Huizinga et al., 1995) and are reviewed by a number of authors (Sanders, 1996; Huizinga et al., 1997; Sanders et al., 1999; Suzuki, 2000). The spontaneous electrical activities are therefore considered to be paced by ICC-MY. In smooth muscle of the guinea-pig stomach, excitation of ICC-MY precedes that of smooth muscle cells, which would suggest that ICC-MY are indeed driving smooth muscle activity (Dickens et al., 1999). In the canine colon, however, slow waves recorded in the circular smooth muscle originate from ICC distributed in the submucosal surface of circular muscle layer (submucosal interstitial cells of Cajal, ICC-SM; Smith et al., 1987a). The canine colon also possesses a well-developed network of ICC-MY between the circular and longitudinal muscle layers, and the activities generated in these ICC-MY are propagated to both layers of smooth muscle cells to elicit spike potentials through activation of voltage-gated Ca^{2+}-channels (Smith et al., 1987b). These may reflect the generation of complicated electrical patterns in the circular smooth muscle of the canine colon (Barajas-Lopez and Huizinga, 1989). In the mouse proximal colon, on the other hand, ICC-SM produce electrical activities with rhythms completely different from those of the circular and longitudinal smooth muscle layers, although the latter two are well synchronized (Yoneda et al., 2002). Thus, distribution of pacemaker cells and the mechanisms of signal conduction system are not homogeneous in regions of the gastrointestinal tract (see Sanders, 1996).

In laboratory animals, antiperistaltic activity is initiated in a special region of the proximal colon, termed the “pacemaker area” (Hukuhara and Neya, 1968). In the guinea-pig colon, the pacemaker nodule, similar to the pacemaker area, produces rhythmic contractions with 10–12 cycles min⁻¹, the frequency being different from other regions of the colon (Kobayashi et al., 1996). This area contains bipolar or multipolar cells which express c-Kit proteins in the submucosal layer, in addition to the ICC-MY (Nahar et al., 1998), suggesting the possibility that the antiperistaltic activities appearing in the proximal colon are produced by these submucosal c-Kit protein positive cells. Plateau potentials with rhythms different from those appearing in smooth muscle cells are also generated in the proximal colon of mice (Yoneda et al., 2002).

In many types of gastrointestinal smooth muscle, the ionic mechanism of the generation of spontaneous activity is equivocal. Slow waves generated in gastric smooth muscle is not inhibited by several types of organic Ca^{2+}-antagonists, such as verapamil (Golenhofen and Lammel, 1978), diltiazem (Ishikawa et al., 1985), D600 (Huizinga et al., 1995) and nifedipine (Liu et al., 1995; Dickens et al., 1999). These observations indicate that the influx of Ca^{2+} through voltage-gated Ca^{2+}-channels may not be the main factor involved in the initiation of the activity. This is directly indicated by recording pacemaker potentials from ICC-MY in the guinea-pig stomach; the pacemaker potentials have two components, an initial fast rising phase which is...
then followed by a plateau component (Dickens et al., 1999). These components may be generated by voltage-sensitive Ca²⁺-permeable channel currents and Ca²⁺-sensitive Cl⁻-channel currents, respectively (Kito et al., 2002). In ICC-MY isolated from the canine colon, there are two types of voltage-gated Ca²⁺-channels with low and high thresholds, and their properties are similar to those of T-type and L-type Ca²⁺-channels, respectively (Lee and Sanders, 1993). However, in ICC isolated from the mouse intestine, the spontaneous depolarization is produced by Ca²⁺-activated Cl⁻-channels (Tokutomi et al., 1995) or by non-selective cation-channels (Thomsen et al., 1998). The possible contribution of a novel type of Ca²⁺ permeable channel is also suggested in cultured ICC of the mouse intestine (Koh et al., 2002). Furthermore, in ICC isolated from the dog colon, the pacemaker potentials are inhibited by Ni²⁺ (Huizinga et al., 1991; Ward and Sanders, 1992), a known inhibitor of T-type Ca²⁺-channels (Tsien and Tsien, 1990).

The present study was aimed to investigate the effects of nifedipine and Ni²⁺, known antagonists of L-type and T-type Ca²⁺-channels respectively, on plateau potentials recorded from the submucosal interstitial cells distributed in the mouse proximal colon. The results have indicated that Ni²⁺ reduces the rate of rise of the initial component and that nifedipine reduces the duration of the plateau component of the plateau potentials. However, increasing concentrations of nifedipine abolished all plateau potentials. The properties of the ion channels contributing to these potentials are discussed. A part of these experimental data was reported briefly to the 43rd Annual Meeting of The Japanese Smooth Muscle Society (Yoneda et al., 2001).

**Materials and Methods**

Male mice (BALB/C strain), weighing 20–25 g, were anesthetized with fluoromethyl 2,2,2-trifluoro-1-(trifluoromethyl) ethyl ether (sevoflurane; Maruishi Pharm., Osaka, Japan) and then decapitated. The animals were treated ethically according to the guidelines for the Care and Use of Animals approved by the Physiological Society of Japan. The proximal colon was isolated, opened along the mesenteric border and the mucosa removed. The preparations were kept in modified Krebs solution with the following ionic composition (in mM): Na⁺ 137.4, K⁺ 5.9, Mg²⁺ 1.2, Ca²⁺ 2.5, HCO₃⁻ 15.5, H₂PO₄⁻ 1.2, Cl⁻ 134, glucose 11.5. The Krebs solution was aerated with O₂ containing 5% CO₂. The pH of the solution was 7.1–7.2.

A small piece of rectangular tissues (2 mm × 3 mm) was isolated and mounted on a silicone rubber plate fixed at the bottom of the recording chamber, with the submucosal layer side uppermost, and immobilized using tiny pins (diameter, 0.1 mm). The recording chamber (8.0 mm wide × 20.0 mm long × 5.0 mm depth, with a capacity of about 1.0 ml) was made from Lucite plate, and the tissue segment was superfused with oxygenated warmed (35°C) Krebs solution at a constant flow rate of about 3 ml min⁻¹. Conventional microelectrode techniques were used to record electrical responses of single cells from each tissue. Glass capillary microelectrodes (borosilicate glass tube with 1.2 mm OD) filled with 3 M KCl had a tip resistance which ranged between 50 and 80 MΩ. The intracellular potentials thus recorded were displayed on a cathode ray oscilloscope (SS-7602, Iwatsu, Tokyo, Japan). The data were also fed into a personal
computer (Dell Computer Inc., Kawasaki, Japan) through an A/D converter (Axon Instruments Inc., CA, USA) at 500 Hz, filtered at 100 Hz, and analyzed with Axoscope 7 (Axon Instruments Inc., CA, USA).

The recorded cells were injected with neurobiotin, using the methods reported by Klemm et al. (1999). Briefly, neurobiotin (Vector Laboratories, Burlingame, CA, USA) was dissolved in 3 M KCl solution at a concentration of 4%, and filled into the tip of the recording electrode. After electrical responses had been recorded, a train of depolarizing current pulses (0.1 nA, 1 s duration, 0.2 Hz frequency for 3 min) was applied to the electrode to inject neurobiotin into the cell. Since neurobiotin diffuses to surrounding cells through gap junctions, injection into the recorded cells was performed in the presence of 30 µM 18β-glycyrrhetic acid (Yoneda et al., 2002). Tissues containing neurobiotin-injected cells were pinned on a waxed plate and fixed overnight at 4°C with fresh paraformaldehyde (4% w/v) dissolved with 0.1% phosphate buffer, pH 7.2. The tissues were washed 3 times each for 10 min with 0.1% phosphate buffered saline (PBS) containing 0.3% Triton X-100 (PBS/Triton) and then incubated for 24 h at 4°C with PBS/Triton containing streptavidin-CY3 (Jackson Immunoresearch, PA, USA; concentration, 1: 500). The preparations were washed 3 times with PBS, mounted on glass slides with Dako mounting medium (Dako Corporation, Carpinteria, CA, USA), cover slipped and examined using a confocal microscope (Biorad MRC-1000; BioRad, CA, USA). The confocal microscope, using a krypton/argon laser, allowed the visualization of CY3 (568 nm excitation filter and 605–632 nm emission filter).

Drugs used were as follows: atropine sulphate, 18β-glycyrrhetic acid (18β-GA), nickel ions, nifedipine and tetrodotoxin were purchased from Sigma Chem. (St. Louis, MO, USA). The stock solutions of 18β-GA and nifedipine were dissolved in dimethyl sulfoxide (DMSO), while the others were dissolved at concentrations of 10 mM in distilled water. The stock solutions were kept in a refrigerator and dissolved further with Krebs solution just before use to obtain the desired concentration (the ratios of the dilution were over 1: 1000). The dilution procedures did not alter the pH of the Krebs solution.

Values measured were expressed as the mean ± standard deviation (S.D.), with the value n representing the number of preparations taken from different animals. Differences between values were tested using paired Student t-tests, and probabilities of less than 5% (P<0.05) were considered to be significant.

Results

Properties of plateau potentials recorded from proximal colon

Intracellular recordings made from submucosal layer uppermost preparations of the mouse proximal colon revealed square-shaped potential changes (plateau potentials) with two components; an initial fast transient potential (primary component) and a following plateau potential (plateau component) (Fig. 1A). The plateau potentials were generated at frequencies ranging between 10 and 22 min⁻¹, with a mean frequency of about 15 min⁻¹. The primary component had a peak amplitude ranging between 10 and 40 mV (mean, 20 ± 8 mV, n=20) and the rate of rise ranging between 70 and 330 mV/s (mean, 150 ± 60 mV/s, n=16). The duration
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of plateau components measured at the foot ranged between 2.2 and 3.1 s (mean, 2.6 ± 0.3 s, n=12). The resting membrane potential of cells generating plateau potentials ranged between –42 and –58 mV (mean, –48 ± 6 mV, n=30). These properties of the plateau potentials were similar to those reported previously (Yoneda et al., 2002). Plateau potentials were not altered by either 1 µM tetrodotoxin or 1 µM atropine (data not shown, n=3 for each), suggesting that their generation was not causally related to periodic excitation of cholinergic enteric nerves.

In each of the submucosal layer uppermost preparations, neurobiotin was injected into 3 cells, after the generation of plateau potentials had been confirmed. Experiments were carried out in the presence of 20 µM 18β-glycyrrhetinic acid, a known inhibitor of gap junctions (Yamamoto et al., 1998). This prevented diffusion of the neurobiotin into surrounding cells through gap junctional connections (Yoneda et al., 2002). In the 5 preparations examined, the application of 18β-glycyrrhetinic acid depolarized the membrane by 7 ± 2 mV thus reducing the amplitude of the plateau potentials (mean, 15 ± 3 mV). It also increased the frequency of plateau potentials (control, 15 ± 3 times min\(^{-1}\); in 18β-glycyrrhethinic acid, 18 ± 2 times min\(^{-1}\); P<0.05). Confocal microscopic examination revealed that the injected cells were distributed in the submucosal layer (data not shown) and had morphological properties similar to those reported
by Yoneda et al. (2002), i.e., they had oval shaped cell bodies with the longer diameter ranging between 20 and 30 µm, with a thickness ranging between 5 and 8 µm, and with bipolar processes of between 50 to 150 µm in length. Thus, the results confirmed that the plateau potentials were recorded from submucosal interstitial cells.

**Effects of nifedipine and Ni²⁺ on plateau potentials**

Experiments were carried out to test the effects of nifedipine and Ni²⁺ on plateau potentials recorded from ICC-SM. Application of 0.01 µM nifedipine did not produce any alteration to these electrical activities (n=3, data not shown). The plateau potentials were generated in the presence of 0.1 µM nifedipine with frequencies similar to those in the absence of nifedipine (control, 16 ± 5 times/min; in nifedipine, 15 ± 4 times/min; n=10; P>0.05), but with a significant reduction in the duration of the plateau component (control, 3 ± 0.3 s; in nifedipine, 2 ± 0.3 s; n=10; P<0.05; Fig. 1, B and C). The amplitude (control, 20 ± 7 mV; in nifedipine, 20 ± 6 mV; n=10; P>0.05) and the rate of rise of the initial component of plateau potentials (control, 160 ± 70 mV/s; in nifedipine, 150 ± 70 mV, n=10; P>0.05) were not altered by 0.1 µM nifedipine. All plateau potentials were abolished in the presence of 1 µM nifedipine (Fig. 1, D). The resting membrane potential was reduced slightly in the presence of 1 µM nifedipine (control, −49 ± 3 mV; in nifedipine, −44 ± 2 mV; n=5; P<0.05). These data confirmed the previous observations (Yoneda et al., 2002), and it was considered that the plateau potentials were all produced by activation of voltage-gated L-type Ca²⁺-channels.

The effects of Ni²⁺ on plateau potentials were also observed to investigate a possible participation of voltage-gated T-type Ca²⁺-channels, and the quantified results are summarized in Table 1. In the presence of 1 µM Ni²⁺, the amplitude and frequency of plateau potentials and the rate of rise of the initial component of plateau potentials were not altered. Increasing the concentration of Ni²⁺ to 10 µM caused reduction of the rate of rise of the initial component, with no alteration to the amplitude and frequency of plateau potentials (Fig. 1, E and F). In the presence of 100 µM Ni²⁺, the frequency and rate of rise of plateau potentials were reduced, with associated alteration of the plateau potential to a triangular form (Fig. 1, G). The peak amplitude of the plateau potentials was not altered by 100 µM Ni²⁺. The resting membrane

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**Table 1  Effects of Ni²⁺ on plateau potentials**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Ni²⁺</th>
<th>n</th>
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<tbody>
<tr>
<td>a. 1 µM Ni²⁺</td>
<td>Rate of rise</td>
<td>149 ± 59 mV/s</td>
<td>6</td>
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<tr>
<td></td>
<td>Amplitude</td>
<td>16.5 ± 4.2 mV</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>13.3 ± 1.0 min⁻¹</td>
<td>6</td>
</tr>
<tr>
<td>b. 10 µM Ni²⁺</td>
<td>Rate of rise</td>
<td>159 ± 35 mV/s</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Amplitude</td>
<td>24.3 ± 3.8 mV</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>13.2 ± 1.0 min⁻¹</td>
<td>5</td>
</tr>
<tr>
<td>c. 100 µM Ni²⁺</td>
<td>Rate of rise</td>
<td>133 ± 27 mV/s</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Amplitude</td>
<td>25.6 ± 2.5 mV</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Frequency</td>
<td>13.2 ± 1.3 min⁻¹</td>
<td>5</td>
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Plateau potentials were recorded from submucosal layer uppermost preparations, in the absence (Control) and presence of Ni²⁺ (a, 1 µM; b, 10 µM; c, 100 µM). Mean value ± S.D. is shown. *, significantly different to control (P<0.05). n = number of observed tissues.
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potential was also not altered by 100 µM Ni\textsuperscript{2+} (control, −50 ± 4 mV, in Ni\textsuperscript{2+}, −50 ± 5 mV; n=6; P>0.05). These results are consistent with the idea that the initial component of plateau potentials is produced by an activation of voltage-gated T-type Ca\textsuperscript{2+}-channels.

Discussion

In smooth muscle preparations of the mouse proximal colon, visualization of the recorded cells using neurobiotin injection indicated that plateau potentials were generated in cells distributed in the submucosal layer. These cells were structurally similar to the pacemaker cells distributed in the submucosal layers of the colon of the guinea-pig (Kobayashi et al., 1996; Nahar et al., 1998) or the dog (Kobayashi et al., 1995). Yoneda et al. (2002) found that in the mouse proximal colon, both the circular and longitudinal smooth muscle produce periodic bursts of spike potentials and oscillatory potentials, respectively. However, the plateau potentials recorded from submucosal cells differed from electrical activities recorded from circular and longitudinal muscles, in both form and frequency. The frequency of plateau potentials was much higher than that of the bursts of excitation generated in circular and longitudinal smooth muscle cells, although the frequency of the latter two was similar. Thus, in the mouse, the plateau potentials generated in the submucosal cells do not couple with the bursts of excitation in the smooth muscle. This is in contrast to the dog proximal colon, where the activity of smooth muscle is produced by the summation of inputs from two discrete populations of pacemakers, the submucosal pacemaker cells and ICC-MY (Smith et al., 1987b; Barajas-Lopez and Huizinga, 1989; Keef et al., 1997).

Isolated smooth muscle of the guinea-pig proximal colon contracts with a frequency of between 10–12 times min\textsuperscript{−1} (Kobayashi et al., 1996), and this is comparable to the frequency of the plateau potentials recorded in the mouse proximal colon (equal to 14.8 times min\textsuperscript{−1}; Yoneda et al., 2002), suggesting the possibility that the submucosal pacemaker cells are driving the spontaneous contraction of the colonic smooth muscle. In circular smooth muscle of the colon of the dog and pig, the spontaneous electrical activity is in the form of slow waves (Smith et al., 1987a; Huizinga et al., 1987; Keef et al., 1997). Structural examination reveals that in the canine colon, gap junctional networks are formed between submucosal pacemaker cells and smooth muscle cells (Smith et al., 1987a; Sanders and Smith, 1989; Kobayashi et al., 1995). This may also be the case in the mouse proximal colon. In the present study, neurobiotin-injected cells are successfully visualized only in the presence of 18β-glycyrrhetinic acid, an inhibitor of gap junctional communications (Yamamoto et al., 1998), indicating that neurobiotin-injected cells may form gap junctional networks with surrounding cells. However, in the mouse proximal colon, rhythmic activities with similar frequencies were observed in circular and longitudinal smooth muscle cells, either in the presence or absence of submucosal layers (Yoneda et al., 2002), indicating that the electrical activity of smooth muscle cells may be derived from other pacemaker cells. Uncoupling of the spontaneous electrical activity between submucosal cells and smooth muscle cells further indicates that the submucosal cells are not functionally connected to smooth muscle cells, and signals arising in the submucosal cells cannot directly drive electrical activity of smooth muscle cells in the mouse proximal colon. The proximal colon
possesses properties specific to this region such as anti-peristaltic movements (Hukuhara and Neya, 1968), and unidentified special roles related to some physiological functions are considered for submucosal pacemaker cells in the mouse.

The ionic mechanism of the electrical activity generated in gastrointestinal smooth muscle tissues is partly solved. Spike potentials generated in most smooth muscle cells are inhibited by organic Ca²⁺-antagonists, such as verapamil (Golenhofen and Lammel, 1978), diltiazem (Ishikawa et al., 1985), D600 (Huizinga et al., 1995) and nifedipine (Liu et al., 1995; Dickens et al., 1999), suggesting that the potentials are produced by activation of voltage-gated L-type Ca²⁺-channels. In antrum smooth muscle of the dog, the plateau components, but not the initial fast components, of the slow action potentials are inhibited by organic Ca²⁺-antagonists (verapamil, el-Sharkaway et al., 1978; nicardipine, Fujii et al., 1985; Ozaki et al., 1991), suggesting an involvement of voltage-gated L-type Ca²⁺-channels. However, slow waves generated in the guinea-pig stomach are not inhibited by organic Ca²⁺-antagonists (Golenhofen and Lammel, 1972; Ishikawa et al., 1985; Huizinga et al., 1995; Tomita et al., 1998; Dickens et al., 1999; Huang et al., 1999; Suzuki and Hirst, 1999). Slow waves generated in intestinal and colonic smooth muscles are also insensitive to Ca²⁺-antagonists (Smith et al., 1987a; Huizinga et al., 1991; Huizinga et al., 1995; Takano and Suzuki, 1999), suggesting that the potentials are not produced by activation of voltage-gated L-type Ca²⁺-channels. In the present experiments, the plateau potentials recorded from submucosal interstitial cells were abolished by nifedipine in a manner similar to activities recorded from circular and longitudinal smooth muscle of the mouse proximal colon (see Yoneda et al., 2002). These results suggest that the plateau potentials recorded from submucosal cells are generated by activation of voltage-gated L-type Ca²⁺-channels. The threshold concentration of nifedipine to inhibit the plateau potential (1 µM) was about 10 times higher than that required for inhibiting the spontaneous activities generated in smooth muscle cells (equal to 0.1 µM; Yoneda et al., 2002), suggesting that, although both may be categorized as a voltage-gated Ca²⁺-channel, the pharmacological identity of the channels that are involved is different.

In circular smooth muscle of the dog colon, Ni²⁺ reduces the initial fast component of slow waves (Huizinga et al., 1991; Ward and Sanders, 1992). Ni²⁺ is an inhibitor of T-type Ca²⁺-channels (Tsien and Tsien, 1990), and thus it is reasonable to speculate that the initial component of plateau potentials is produced by an activation of voltage-gated T-type Ca²⁺-channels, as in the case of ICC-SM of the dog colon (Lee and Sanders, 1993). In the guinea-pig stomach antrum, Ni²⁺ (100 µM) reduced the duration and the rate of rise of the square-shaped large potentials (possibly pacemaker potentials, see Dickens et al., 1999) and increased the frequency of their occurrence (Tomita et al., 1998), and these results agree with the idea that the initial component of pacemaker potential is formed by an activation of voltage-gated T-type Ca²⁺-channels (Lee and Sanders, 1993). The effects of Ni²⁺ on slow waves generated in the guinea-pig stomach are more complicated, and comparable concentrations (10–100 µM) of Ni²⁺ abolish slow waves generated in smooth muscle cells distributed at the border between the antrum and the pyloric region (Huang et al., 1999) but not in the antrum region (Tomita et al., 1998). Thus, the contribution of T-type Ca-channels in the generation of spontaneous activity cannot be evaluated simply from the actions of Ni²⁺ in the guinea-pig stomach. In submucosal
cells of the mouse proximal colon, nifedipine reduced the duration of plateau component at a concentration of 0.1 µM and abolished the plateau component when the concentration was increased to 1 µM. These data suggest that the voltage-gated L-type Ca\(^{2+}\)-channels are playing the major role in the generation of plateau potentials. Alternatively, nifedipine has an ability to inhibit T-type Ca\(^{2+}\)-channels at 1 µM concentration, although this does not seem to be supported from the molecular pharmacology of Ca\(^{2+}\)-channels (Mori et al., 1996; Heady et al., 2001).

In summary, the effects of nifedipine and Ni\(^{2+}\) on plateau potentials generated in submucosal ICC of the mouse proximal colon indicated that at subthreshold concentrations, Ni\(^{2+}\) reduces the rate of rise of the initial component and nifedipine reduced the duration of the plateau component. These results are consistent with the idea that the initial and plateau components of plateau potentials are produced by activation of T-type and L-type Ca\(^{2+}\)-channels, respectively. However, it is also possible to speculate that plateau potentials are produced by only L-type Ca\(^{2+}\)-channel currents, since higher concentrations of nifedipine abolish plateau potentials completely. The results indicate that subtypes of Ca\(^{2+}\)-channels involved in each component of plateau potentials are not clearly identified by the effects of nifedipine and Ni\(^{2+}\).

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References


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