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Effects of Noradrenaline on the Spontaneous Contraction and Ionic Current in the Antral Circular Muscle of Guinea-pig Stomach

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= ABSTRACT=

There is evidence that noradrenaline enhances spontaneous contractions dose-dependently in guinea-pig antral circular muscle. To investigate the mechanism of this excitatory action, slow waves and membrane currents were recorded using conventional microelectrode techniques in muscle strips and the whole cell patch clamp technique in isolated gastric myocytes. On recording slow waves, noradrenaline (10^{-6} M) induced the hyperpolarization of the membrane potential, although the shape of the slow waves became tall and steep. Also, spike potentials occurred at the peaks of slow waves. These changes were completely reversed by administration of phentolamine (10^{-5} M), an α-adrenoceptor blocker. Noradrenaline-induced hyperpolarization was blocked by apamin (10^{-7} M), a blocker of a class of Ca^{2+}-dependent K^+ channels. To investigate the mechanisms for these effects, we performed whole cell patch clamp experiments. Noradrenaline increased voltage-dependent Ca^{2+} currents in the whole range of test potentials. Noradrenaline also increased Ca^{2+}-dependent K^+ currents, and this effects was abolished by apamin. These results suggest that the increase in amplitude and the generation of spike potentials on slow waves was caused by the activation of voltage-dependent Ca^{2+} channel via adrenoceptors, and hyperpolarization of the membrane potential was mediated by activation of apamin-sensitive Ca^{2+}-dependent K^+ channels.

Key Words: Noradrenaline, Slow waves, Voltage-dependent Ca^{2+} currents, Ca^{2+}-dependent K^+ currents, Apamin.

INTRODUCTION

Guinea-pig antral circular muscle has spontaneous mechanical activities, which are generated by the regular and slow oscillation of membrane potential (slow waves) (Komori & Suzuki, 1986; Ohba & Sakamoto, 1977). The gastric motility is regulated by these slow waves which are modulated by extrinsic and intrinsic autonomic nervous systems as well as circulating hormones and drugs (Demol et al, 1989).

Endogenous catecholamines as a humoral agent play an important role in regulating smooth muscle contractility. In gastric smooth muscle, noradrenaline produces dual actions: contraction and relaxation depending on the presence of mucosa, regions and species (Bülbring & Tomita, 1987).

We have previously reported about the excitatory effects of noradrenaline on the contractility and membrane potential of guinea-pig gastric

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smooth muscle (Lee, Kim & Kim, 1991). In that report, noradrenaline showed excitatory effects both on the spontaneous contractions and slow waves mediated through α-adrenoceptors not via the enteric nervous system. As isoprenaline can inhibit the spontaneous contractions and slow waves (Lee, Kim & Kim, 1991), there are also inhibitory mechanisms mediated through β-adrenoceptors but exogenously applied noradrenaline usually acts through α-adrenoceptors. Although the effects of catecholamines on the mechanical activity and the membrane potential of guinea-pig gastric smooth muscle have been studied (Yamaguchi & Tomita, 1974), the underlying ionic mechanisms have not been investigated. Therefore, to clarify the underlying mechanisms of excitatory response of noradrenaline, we investigated the electrical activities by use of intracellular recording and the whole cell patch clamp technique.

MATERIALS AND METHODS

Preparation of tissue and intracellular recording

Albino guinea-pigs of either sex, weighing 200~250 g, were stunned and bled. The stomach was isolated and cut in the longitudinal direction along the lesser curvature. The contents of the stomach were removed, and the mucosal layer was separated from the muscle layers in phosphate-buffered Tyrode solution (NaCl 147, KCl 4, $MgCl_2 \cdot 6H_2O$ 1.05, $CaCl_2 \cdot 2H_2O$ 0.42, $NaH_2PO_4 \cdot 2H_2O$ 0.42, $Na_2HPO_4 \cdot 2H_2O$ 1.81, glucose 5.5 mM, pH 7.35) at room temperature. Strips of muscle (2 mm wide, 10 mm long) were mounted in a 2 ml horizontal chamber, The strips were pinned out at one end with tiny pins on a rubber plate, and the other end was connected to a force transducer (Isometric Transducer, Harvard Bioscience, USA). The strip was constantly perfused at a rate of 2-3 ml /min with Tris-buffered normal Tyrode solution (NaCl 147, $CaCl_2 \cdot 6H_2O$ 1.05, tris · HCl 5, glucose 5.5 mM, pH 7.35) bubbled with 100 % O_2 and maintained at 35℃. Electrical responses of smooth muscle cells were recorded by means of glass microelectrodes filled with 3M KCl. Microelectrodes with a tip resistance of 40~80 MΩ were used. Mechanical and electrical responses of smooth muscle cells were simultaneously recorded on a pen recorder (MX-6, Device Ltd, Britain).

Patch clamp experiment

Isolation of cells: The circular muscle was dissected from the longitudinal layer and small segments of the tissues were made. The muscles were incubated in Ca^{2+}-free physiological salt solution (PSS) for 30 min at room temperature. Then, small segments were incubated for 20~30 min in Ca^{2+}-free PSS containing 0.1% collagenase, 0.1% trypsin inhibitor, and 0.2% bovine serum albumin at 35℃. After digestion, single cells were dispersed by gentle agitation with a glass pipette in the Krafts-Brühe (KB) solution. Isolated gastric myocytes were kept in KB medium at 4℃. All experiments were carried out within 12 hours of harvesting cells and performed at room temperature.

Membrane current measurement: Isolated cells were transferred to a small chamber on the stage of an inverted microscope (IMT-2, Olympus, Japan). The chamber was perfused with PSS (2-3 ml/min). Glass pipettes with a resistance of 2-5 MΩ were used to make a giga seal of 5-10 GΩ. Standard patch clamp techniques were used (Hamill et al., 1981). An axopatch-1C patch-clamp amplifier (Axopatch-1C, Axon instrument, USA) was used to record membrane currents. The data were displayed on a digital oscilloscope (PM 3350, Philips, Netherland), a pen recorder (Recorder 220. Gould, USA) and stored on videotape recorder (BR-6400, Victor, USA) with a pulse code modulator (RP-880, NF, USA).

Solutions: Ca^{2+}-free PSS contained (mM) NaCl 134.8, KCl 6.2, $CaCl_2$ 0, glucose 12.2, HEPES 0.4 and pH was adjusted to 7.3 by Tris. PSS contained 2.3 mM $CaCl_2$ in the Ca^{2+}-free PSS, KB solution contained (in mM) L-glu-

tamate 50, KCl 50, taurine 20, KH_2PO_4 20, $MgCl_2$ 3, glucose 10, HEPES 10, EGTA 0.5 and pH was adjusted to 7.3 by KOH. Pipette solution consisted of (in mM) K^+-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 5, KCl 20, $MgCl_2$ 1, ethyleneglycol-bis (β-aminoethyl ether)-N, N, N′,N′-tetraacetic acid (EGTA) 0.1, HEPES 5, pH 7.4. For studies in which K^+ currents were blocked, pipette solution contained (in mM) Cs-aspartate 110, Mg-ATP 5, di-Tris-creatine phosphate 2.5, di-Na-creatine phosphate 2.5, $MgCl_2$ 1, HEPES 0.1, tetraethylammonium (TEA)-Cl 20, EGTA 5, pH 7.4.

RESULTS

Effects of noradrenaline on the slow waves

The antral circular muscle of guinea-pig stomach exhibited very regular electrical slow waves. Their frequency was about 4~5/min, and the membrane potential was −60~−65 mV. Figure 1 shows the effects of noradrenaline on the spontaneous contractions and slow waves recorded simultaneously in the antral circular muscle strips. Noradrenaline (10^{-6} M) caused dramatic changes in slow waves consisting of hyperpolarization of the membrane potential, an increase in the amplitude of slow waves and generation of spikes on the peak of slow waves. The amplitudes of phasic contrac-

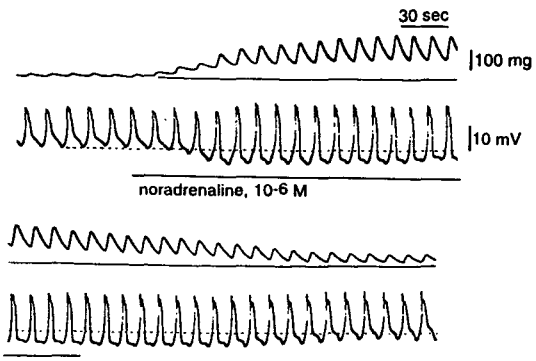

Fig. 1. The effects of noradrenaline on spontaneous contractions and slow waves from a strip of antral circular muscle in the guinea-pig. The observed changes in slow waves after the administration of noradrenaline (10^{-6} M) were the hyperpolarization of membrane potential, the increase of amplitude, and the generation of spikes in the peak on the slow waves.

Note the sequential changes in spontaneous contractions and slow waves: prior to the start of hyperpolarization, an increase in tonic contraction was developed.

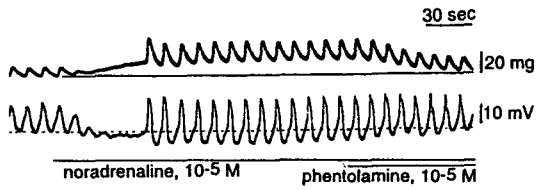

Fig. 2. Antagonistic influences of noradrenaline and phentolamine upon the slow waves recorded from a strip of the antral circular muscle in the guinea-pig. The characteristic noradrenaline-induced changes in slow waves were completely antagonized by the administration of phentolamine (10^{-5} M).

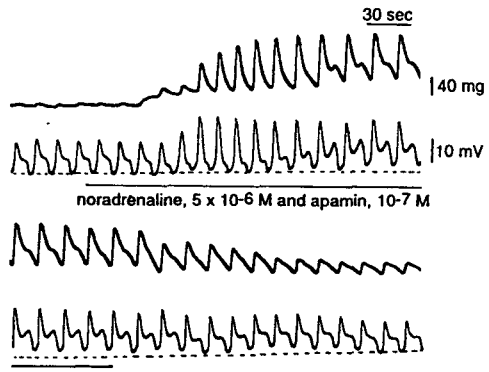

Fig. 3. Effects of apamin on the characteristic noradrenaline-induced changes in slow waves and spontaneous contractions recorded from a strip of antral circular muscle in the guinea-pig.

Noradrenaline (5×10^{-6} M) with apamin (10^{-7} M) revealed the excitatory contractile responses similar to those of noradrenaline only. However, the hyperpolarization of membrane potential was blocked completely by the presence of apamin.

tions were also increased. Noradrenaline-induced changes in slow waves and contractions were antagonized by the administration of phentolamine (10^{-5} M) (Fig. 2).

Figure 3 shows the effects of apamin which is Known to block a kind of Ca^{2+}-dependent K^+ channel, on noradrenaline-induced changes in slow waves and contractions. The application of noradrenaline (5×10^{-6} M) with apamine (10^{-7} M) revealed excitatory contractile responses similar to those of noradrenaline alone except that the characteristic hyperpolarization did not occur.

Effects of noradrenaline on voltage-dependent Ca^{2+} currents

For recording inward currents alone, we used cesium-aspartate solution in the pipette. Depolarizing voltage steps from a holding potential of −60 mV elicited inward currents. The inward currents proved to be Ca^{2+} currents because the currents were completely blocked by removal of Ca^{2+} in the bath solution or by the application of nifedipine or verapamil Rhee et al, 1993). Also, the amplitudes and shapes of current responses were not changed in the presence of tetrodotoxin (10^{-7} M, data not shown).

Figure 4 shows the enhancing effects of noradrenaline on the Ca^{2+} current elicited by a voltage clamp pulse to 0 mV from a holding potential of −60 mV with a duration of 350 ms in the antral circular myocytes. The current-voltage relationship for the peak Ca^{2+} currents before and after the application of noradrenaline is also shown. The enhancing effect of noradrenaline was reversed by treatment with phentolamine. In the control condition Ca^{2+} currents were activated at −40 mV and peaked at around 0 mV. On the application of noradrenaline, the Ca^{2+} currents were increased over the whole test voltage range. However, there was no left or right shift of the current-voltage relationship.

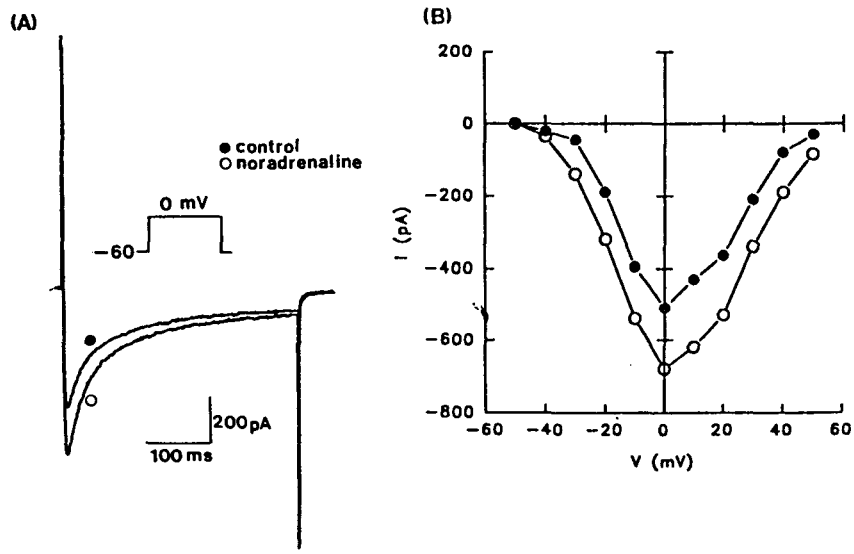

Fig. 4. Effects of noradrenaline on voltage-dependent Ca^{2+} currents in isolated single antral myocytes. (A) shows Ca^{2+} current when voltage clamped to 0 mV from a holding potential −60 mV in the control and after treatment with noradrenaline (10^{-5} M). (B) shows the current-voltage relationship for the Ca^{2+} current. Noradrenaline increased Ca^{2+} current over the whole range of test voltages. (C) shows the antagonistic effect of phentolamine on Ca-current enhanced by noradrenaline. The holding potential was −60 mV and the test potential was 20 mV.

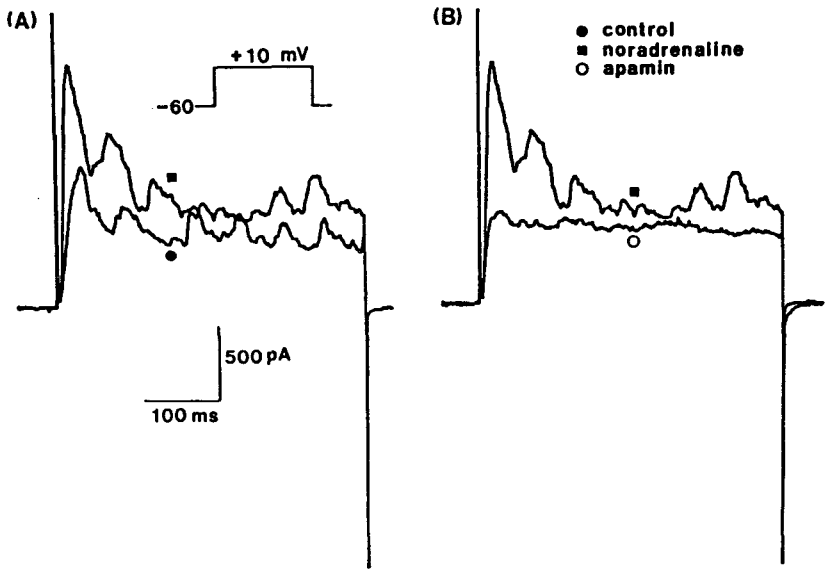

Fig. 5. Effects of noradrenaline and apamin on Ca^{2+}-dependent K^+ currents. Transient and spontaneous oscillatory outward currents were evoked by a depolarizaing pulse to +10 mV from a holding potential of −60 mV. The application of noradrenaline (10^{-5} M) increased transient and oscillatory outward currents, and these currents were abolished by the application of apamin (10^{-7} M).

Effects of noradrenaline on the outward currents

We recorded outward currents in the antral circular myocytes with K^+-aspartate solution containing 0.1 mM EGTA in the pipette.

Depolarizing voltage steps from a holding potential of −60 mV elicited outward currents of 2 types: transient and spontaneous oscillatory outward currents, and sustained outward currents. Transient and spontaneous outward currents were Ca^{2+}-dependent because they were suppressed by the removal of Ca^{2+} or by the application of Ca^{2+} antagonists and were completely abolished by the addition of 10 mM EGTA in the pipette (Rhee et al, 1993).

Figure 5 shows the effects of noradrenaline on the Ca^{2+}-dependent outward currents by a voltage clamp to 10 mV from a holding potential of −60 mV with a duration of 400 ms. On the application of noradenaline (10^{-6} M), Ca^{2+}-dependent outward currents were increased. The currents enhanced by noradrenaline were abolised by the application of 10^{-7} M of apamin.

DISCUSSION

Spontaneous contractions of gastrointestinal smooth muscle were initiated by an electrical slow waves. It has been reported that electrical slow waves are composed of two components (voltage-independent and voltage-dependent components) with spike potentials on the peak of each slow wave in guinea-pig antral circular muscle (Ohba, et al, 1987). The precise ionic mechanisms of gastric slow waves are not known. However, it has been suggested that the upper voltage-dependent component of slow wave is due to activation of voltage-dependent Ca^{2+} channels, because the amplitude of slow waves are decreased or abolished by Ca^{2+} channel antagonists and by removal of Ca^{2+} from the extracellular solution (Ohba et al, 1987; Rhee & Kim, 1987).

In the present experiments, noradrenaline

produced prominent changes in electrial activity: hyperpolarization of membrane potential, and increase in the amplitude and spike potentials at the peaks of slow waves (Fig. 1). As the upper component of slow waves and spike potentials could be enhanced by blocking outward current with tetraethylammonium (Kim, 1993) or by enhancing Ca-inward current with increased $[Ca^{2+}]_{ext}$ (Rhee & Kim, 1987), each possibility should be considered. The effects of noradrenaline on slow waves seem to be mediated by an increase in calcium influx through voltage-dependent Ca^{2+} channels because noradrenaline increased the outward current instead of decreasing it.

In gastric smooth muscles, it has been reported that catecholamines produce contraction through α-receptors, and relaxation throught α- or β-receprots (Baily, 1971; Bülbring & Tomita, 1987). Our experiment also confirms that the excitatory action of noradrenaline is mediated through α-adrenoceptors because noradrenaline-induced changes in slow waves and mechanical contraction are completely antagonized by phentolamine, an α-adrenoceptor blocker (Fig. 2).

As shown by recordings of simultaneous spontaneous contractions and slow waves, the contractile tone increased prior to changes in slow waves in response to noradrenaline, and then hyperpolarization occurred and followed large phasic contractions accompanied by an increased amplitude and spikes of slow waves (Fig. 1, 2). These results suggest that the increased intracellular Ca^{2+} by noradrenaline activates Ca^{2+}-dependent K^+ channels and produces hyperpolarization. This hypothesis is supported by the fact that the noradrenaline-induced hyperpolarization was completely blocked by the presence of apamin (Fig. 3). Apamin is a component of bee venom, which is known to block a class of Ca^{2+}-dependnet K^+ channels (Bankes et al, 1987; Bülbring & Tomita, 1987; Haylet & Zenkinson, 1990). A similar apamin-sensitive hyperpolarization was reported in the intestinal smooth muscle of guinea-pig (Den Hertog, 1981; Bülbring & Tomita, 1987).

In order to clarify the underlying ionic nature of the noradrenaline-induced changes in slow waves, we carried out whole cell patch clamp recording in isolated gastric antral myocytes. Depolarization steps to -40 mV and more positive potentials activated voltage-dependent Ca^{2+} currents, which were completely blocked by the Ca^{2+} channel antagonist, nifedipine. The activation was initiated at -30 mV and the peak current appeared around 0 mV (Fig. 4). Two types of Ca^{2+} channels have been reported in gastrointestinal smooth muscle cells and vascular smooth muscle cells (Been, 1989; Vivadow et al, 1988), but only the L-type Ca^{2+} channel was shown in guinea-pig antral circular muscle cells, because the Ca^{2+} channel kinetics were not altered in the presence of Bay K 8644 or by the change of holding potentials from -60 mV to -80 mV and also these currents were very sensitive to nifedipine (Kim et al, 1993). Similar results have been reported in various gastrointestinal smooth muscle cells (Mitra & Morad, 1985; Ganitkevichi et al, 1986; Ohya et al, 1987; Cole & Sanders, 1989; Katzka & Morad, 1989; Sims, 1992).

Noradrenaline increases voltage-dependent Ca^{2+} currents in some vascular smooth muscle cells and cardiac myocytes (Benham & Tsien, 1988). In this experiment noradrenaline increased volatge-dependent Ca^{2+} currents over the whole test voltage range, indicating that excitatory responses to noradrenaline were due to the activation of these currents. However, it has been reported that epinephrine has no effect on the Ca^{2+} currents in guinea-pig gastric myocytes of corpus region (Mitra & Morad, 1985). This difference may be due to the difference in experimental region, because electrical activities vary depending on the gastric region.

Transient and spontaneous oscillatory outward currents as well as sustained outward currents were recorded in the isolated myocytes with an internal solution of K-aspartate containing 0.1 mM EGTA. These transient and oscillatory outward currents are abolished by the application of caffeine or nifedipine in the bath or by the addition of 10 mM EGTA in the pi-

pette (Mitra & Morad, 1985; Ohya et al, 1986; Katzka & Morad, 1989; Carl et al, 1990). These currents are recorded in other visceral smooth muscle cells and they are considered to be due to the activity of Ca^{2+}-activated K^+ channels (Rusco et al, 1990; Takeda et al, 1991; Kim et al, 1993). Noradrenaline increased these Ca^{2+}-activated K^+ currents. This effect was blocked by apamin (Fig. 5). Noradrenaline had no effect on the voltage-operated sustained outward current recorded when a high concentration of EGTA (10 mM) was included in the pipette solution (data not shown). These results, considering together the results shown in Fig. 3, suggest that apamin-sensitive K^+ channels produced the hyperpolarization induced by noradrenaline. We did not identify this channel, but as apamin is known to be a relatively specific blocker of the small conductance Ca-activated K^+ channel (SK_{Ca}), SK_{Ca} might be responsible for the noradrenaline-induced hyperpolarization. Similar results have been obtained in the hepatocyte, intestinal smooth muscle, and neurons (Bülbring & Tomita, 1987; Haylett & Zenkinson, 1990).

Dual effects of noradrenaline on the inward and outward ionic currents in this smooth muscle cell described above look somewhat contradictory. We can not clearly explain the precise contribution from each effect on ionic current, and further studies on the basic mechanisms of slow waves will be necessary for the explanation.

In conclusion, our results suggest that noradrenaline-induced tall, steep slow waves and spikes on their peaks are associated with the activation of voltage-dependent Ca^{2+} channels through α-adrenoceptors, which causes an excitatory response and that noradenaline-induced hyperpolarization of the membrane potential was associated with the activation of apamin-sensitive Ca^{2+}-dependent K^+ channels.

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