Inhibitory Action of Propranolol on the Contractions Induced by Nerve Stimulations or Calcium in the Smooth Muscle of Rat Vas Deferens

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Accepted December 9, 1985

Abstract—Inhibitory effects of propranolol on the contractions to various treatments were investigated in the epididymal half of the rat vas deferens. Reportedly, $10^{-6} - 3 \times 10^{-4}$ M propranolol inhibited 150 mM K-induced contractions dose-dependently; $3 \times 10^{-4}$ M propranolol abolished the contractions. The present results showed that propranolol at concentrations up to $10^{-4}$ M did not inhibit the maximal contractions to $10^{-3}$ M norepinephrine (NE) or $10^{-2}$ M methacholine (MCh). Propranolol at $3 \times 10^{-4}$ M slightly inhibited contractions to NE and MCh by 11% and 12%, respectively. In contrast, propranolol inhibited twitch components of the contractions induced by nerve stimulations at similar doses to those reported for high K contractions. Propranolol also inhibited contractions to Ca in high K-containing solution and shifted the dose-response curve to the right. Propranolol did not affect the depolarizations by high K measured by microelectrodes. Propranolol at concentrations of $10^{-5} - 3 \times 10^{-5}$ M diminished the magnitude of spikes dose-dependently. Spikes were rarely observed in the presence of $10^{-4}$ M propranolol in spite of generation of e.j.p.s with amplitudes that would be sufficient to induce spikes in the absence of propranolol. These results suggest that propranolol inhibits contractions by decreasing Ca-influx through the potential-operated Ca-channels in the smooth muscle cells of rat vas deferens.

Possible Ca-antagonistic action of propranolol has been demonstrated in smooth muscles. Sakanashi and Nishi (1) and Rokutanda et al. (2) reported that propranolol inhibited contractions induced by high K, but not by La, in the coronary artery and colon of the dog. Increasing the concentration of external Ca counteracted the inhibition by propranolol. These effects of propranolol were similar to those of nifedipine in the two smooth muscles of dog. In the guinea pig taenia coli, propranolol inhibited contractions induced by Ca in 40 mM K-containing solution, and it shifted the dose-response curves to the right (3).

We previously reported that $10^{-6} - 3 \times 10^{-4}$ M propranolol inhibited high K-induced contractions dose-dependently in the vas deferens of rats (4). The present study was undertaken to characterize the inhibitory action of propranolol in the rat vas deferens by investigating the effects on the contractions induced by various treatments and also on the membrane potentials.

Materials and Methods

Vasa deferentia were removed from male Wistar rats (250–350 g) and cleaned of adhering fat and connective tissues in Krebs solution of the following composition (mM): NaCl, 120; KCl, 6; CaCl₂, 2.5; MgCl₂, 1.2; NaHCO₃, 25; NaH₂PO₄, 1.2; glucose, 14. For the experiments to measure contractions, the epididymal half of vas deferens was mounted with a resting tension of 0.5 g in an organ bath containing 30 ml Krebs solution,
and it was equilibrated for 60 min. Krebs solution in the bath was continuously bubbled with 95% $O_2$ and 5% $CO_2$. Temperature of the solution was maintained at 37°C. Vasa deferentia used for nerve stimulations or Ca-contractions were treated with 10^{-7} M dibenamine for 15 min during the equilibration period. Treatments with dibenamine did not affect the contractions induced by nerve stimulations. Isometrical contractions were measured with a force-displacement transducer (SB 1T, Nihon Kohden, Japan). Dose-response curves to norepinephrine (NE) and methacholine (MCh) were determined in the same way as previously reported (5). Various concentrations of agonist drugs, from lowest to highest, were applied non-cumulatively with an interval period of 12–15 min. Stimulation of intramural nerves were made with a force-displacement transducer (SB 1T, Nihon Kohden, Japan). Dose-response curves to Ca were determined in 48 mM K solution of the following composition (mM): NaCl, 80; KCl, 48; MgCl$_2$, 1.2; NaHCO$_3$, 25; glucose, 14. Contractions were induced by adding CaCl$_2$ cumulatively to the high K solution. For the experiments to measure the membrane potentials, 3-5 mm muscle sections taken from the middle portion of the vas deferens were fixed on the bottom of an organ bath with a capacity of 10 ml. Sections were treated with 10^{-7} M dibenamine for 15 min during the 60 min equilibration period. Krebs solution in the bath was bubbled with 95% $O_2$ and 5% $CO_2$ and periodically replaced with fresh solution warmed to 37°C. To stimulate intramural nerves, a pair of platinum electrodes (3 mm in length) were placed along the muscle section. Square pulses of 0.05 msec duration were delivered to the electrodes from an electric stimulator. Stimulations were done at 0.8 Hz in most experiments. During the train of stimulation, voltages were raised little by little until excitatory junction potentials (e.j.p.s.) reached the threshold potential and triggered the generation of spikes (6). Threshold potentials were determined by measuring the crest level of the last e.j.p.s of which the next stimulation induced the spikes. In some cells, determinations were made by measuring the potential levels of e.j.p.s from where spikes arose. High K solutions were made by replacing NaCl with equimolar KCl in the Krebs solution. Glass microelectrodes were made of quick-fill glass capillaries (o.d., 2 mm; Narishige Kagaku, Japan) and had a resistance of 40–70 MΩ when filled with 3 M KCl. Impalements were made into the smooth muscle cells exposed after removing the artery attached. Signals from electrodes were fed to a microelectrode amplifier (MEZ-8101, Nihon Kohden, Japan) and displayed on an oscilloscope (VC-9, Nihon Kohden, Japan). Recordings of traces on the oscilloscope were made with a continuous recording camera (RLG-6201, Nihon Kohden, Japan). Impalements were considered successful when the following conditions were fulfilled: (a) sharp drop from zero level, (b) stable recordings lasting for more than 1 min, (c) occurrence of spontaneous miniature e.j.p.s (6).

Results

Effects of propranolol on contractions to NE and MCh: In the present study, d,l-propranolol was used. Figure 1 shows effects of $10^{-5}$~$10^{-3}$ M propranolol on the maximal contractions induced by $10^{-3}$ M NE and $10^{-2}$ M MCh in the epididymal half of rat vas deferens. Propranolol in the concentrations up to $10^{-4}$ M did not affect the maximal contractions to NE and MCh. Higher concentrations of propranolol inhibited contractions to both agonists. At $10^{-3}$ M propranolol, contractions to NE were reduced to 67% and those to MCh reduced to 55% of control responses. However, the degrees of inhibition were much less than those obtained for high K-induced contractions. While 150 mM K-induced contractions were almost abolished by $3\times10^{-4}$ M propranolol (4), contractions to NE or MCh were reduced only by 11% and 12% by $3\times10^{-4}$ M propranolol, respectively.
Fig. 1. Effects of various concentrations of propranolol on the maximal contractions to $10^{-9}$ M nor-epinephrine (NE, left) and $10^{-2}$ M methacholine (MCh) in the epididymal half of rat vas deferens. Constant contractions were elicited when agonist drugs were applied at an interval of 20 min. Tensions of the control response were 2.2±0.1 g for NE and 1.5±0.2 g for MCh. Increasing concentrations of propranolol were applied 5 min prior to the applications of agonists. Bars represent standard errors (n=5 for NE and MCh).

Effects of $10^{-4}$ M propranolol on the dose-response curves to NE and MCh are shown in Fig. 2. Propranolol did not affect the maximal response to NE, but potentiated the contractions to submaximal doses, causing a leftward shift of the dose-response curve. Contractions to MCh were also slightly potentiated by $10^{-4}$ M propranolol. Propranolol at concentrations of $10^{-4}$ to $3\times10^{-4}$ M occasioned rhythmic phasic contractions (less than 0.3 g). The phasic contractions were abolished by $3\times10^{-6}$ M phentolamine, or were not observed in the vas deferens pretreated with dibenamine, indicating that these contractions were induced by NE released from nerve endings. Potentiating effects of propranolol on NE- and MCh-induced contractions may be partly due to the effects of released NE. Therefore, in the following experiments, vasa deferentia were treated with dibenamine to eliminate the indirect action of propranolol (see Materials and Methods).

Effects of propranolol on nerve-stimulated contractions: When intramural nerves were stimulated with single pulses, biphasic contractions, i.e., initial twitch and slowly developing tonic component, were produced in the epididymal half of rat vas deferens (7). Illustrated in Fig. 3 are the effects of propranolol at various concentrations on the peak values of twitch components elicited at 0.03 Hz. Contractions were eliminated by $10^{-6}$ M tetrodotoxin. Less than $3\times10^{-6}$ M propranolol did not affect twitch components.
Propranolol in the concentrations greater than $10^{-5}$ M inhibited twitches dose-dependently; twitches were almost abolished by $3 \times 10^{-4}$ M propranolol.

**Effects of propranolol and D-600 on Ca-induced contractions:** Figure 4 shows effects of $10^{-4}$ M propranolol and $10^{-6}$ M D-600 on the dose-response curves to Ca obtained in the partially depolarized vas deferens in 48 mM K solution. Propranolol at $10^{-4}$ M shifted the dose-response curve to Ca to the right. D-600 at $10^{-6}$ M decreased the slope of the dose-response curve and reduced the maximal response to Ca.

**Electrophysiological effects of propranolol:** Resting membrane potential of smooth muscle cells of dibenamine treated vas deferens was $-60.2 \pm 1.1$ mV (mean±S.E., n=65), which was not statistically different from $-60.1 \pm 0.8$ mV obtained in intact muscles (n=60). Effects of propranolol on the resting membrane potentials were assessed by measuring the potentials in different cells with repeated impalements for 1 hr after the application of propranolol. Propranolol at $10^{-5} - 3 \times 10^{-5}$ M did not affect the resting membrane potential: $-60.0 \pm 1.2$ mV at $10^{-5}$ M propranolol (n=34) and $-59.8 \pm 1.0$ mV at $3 \times 10^{-5}$ M propranolol (n=40). Propranolol at $10^{-4}$ M depolarized the membrane by approximately 3 mV ($-57.3 \pm 1.2$ mV, n=35). However, $10^{-4}$ M propranolol did not affect the magnitudes of depolarizations induced by high K as shown in Fig. 5 which depicts the relationship
between membrane potentials and concentrations of external K.

Figure 6A shows excitatory junction potentials (e.j.p.s) and spikes evoked by intramural nerve stimulations in the control vas deferens. E.j.p.s were elicited in all smooth muscle cells tested. When stimulations were repetitively applied with the same duration and voltage at a frequency less than 1 Hz, amplitudes of e.j.p.s were almost constant; facilitation was not so eminent as in the vas deferens of guinea pig and mouse (8, 9). Amplitudes of e.j.p.s were increased as the stimulation voltages were raised. When depolarization of e.j.p.s reached the threshold potentials, spikes were induced (Fig. 6A). Figure 6B and C illustrate the effects of $10^{-5}$ M and $3 \times 10^{-5}$ M propranolol on e.j.p.s and spikes, respectively. In the presence of propranolol, higher stimulation voltages were required to induce e.j.p.s, possibly, due to the local anesthetic action of propranolol, but amplitudes of e.j.p.s could be increased to the threshold level for generating spikes. Prominent effect of propranolol was seen in spikes as shown in the figure. Overshoot potentials were reduced by propranolol in a dose-dependent manner. Table 1 summarizes the effects of $10^{-5}$ to $3 \times 10^{-5}$ M propranolol on the electrophysiological parameters of the action potentials. Overshoot potentials were reduced from 7.8 mV to 6.3 and to 1.5 mV by $10^{-5}$ and $3 \times 10^{-5}$ M propranolol, respectively. Threshold potentials for spike generation were not affected by these concentrations of propranolol. At $10^{-4}$ M propranolol, e.j.p.s were evoked in most of the cells; however, spikes were rarely induced (Fig. 6D). Amplitudes of e.j.p.s evoked in $10^{-4}$ M propranolol seemed to be sufficient to induce spikes as shown in Fig. 7B. In this cell, a spike was elicited by the e.j.p. at the second stimulation in the presence of $10^{-4}$ M propranolol. However, amplitudes of spikes were quickly diminished and abolished with subsequent stimulations.

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Table 1. Effects of propranolol on the electrophysiological parameters of action potentials in the smooth muscle cells of rat vas deferens

|               | Control (n=9) | $10^{-5}$ M (n=25) | Propranolol $3 \times 10^{-5}$ M (n=18) | $10^{-4}$ M (n=35) |
|---------------|---------------|-------------------|--------------------------------------|-------------------|
| Resting membrane potential (mV) | $-60.7 \pm 0.8$ | $-60.4 \pm 0.5$ | $-59.2 \pm 0.9$ | $-57.3 \pm 1.2$ |
| Overshoot potential (mV)      | $7.8 \pm 1.2$  | $6.3 \pm 0.7$    | $1.5 \pm 1.1$           |                   |
| Threshold potential (mV)      | $-28.2 \pm 0.9$| $-29.4 \pm 0.7$ | $-29.0 \pm 0.7$         |                   |

Values are the mean±standard errors.
During the stimulations, amplitudes of e.j.p.s were constant, indicating that abolishment of spikes was not due to the decrease in the depolarization of e.j.p.s. In the control cell, repetitive spikes were generated with slight decrement responding to each stimulation (Fig. 7A).

Fig. 7. Effects of propranolol on generation of e.j.p.s and spikes induced by repetitive stimulations (10 Hz). Higher stimulation voltage was used in the presence of propranolol. Muscle preparations were pretreated with 10^{-7} M dibenamine for 15 min before the experiments. A, control response; B, test response in the presence of 10^{-4} M propranolol. Calibration bars represent 100 msec (horizontal) and 60 mV (vertical). Recordings were made from different cells in the same vas deferens.

Discussion

Previously, we reported that 10^{-5}-3 \times 10^{-4} M propranolol inhibited contractions induced by high K in the rat vas deferens (4). Maximal contractions induced by 150 mM K were almost abolished by 3 \times 10^{-4} M propranolol. The present results have shown that propranolol in the same concentrations inhibited twitch contractions induced by transmural nerve stimulations as strongly as high K-induced contractions. Ca-induced contractions were also inhibited by propranolol. However, 10^{-5}-10^{-4} M propranolol did not inhibit, but rather slightly enhanced, the contractions induced by NE and MCh. Propranolol of higher concentrations inhibited contractions induced by these agonist drugs, but degrees of inhibition of these contractions were much less than those observed for high K-induced contractions or twitch contractions.

Evidence has accumulated to indicate the existence of two kinds of Ca-channels, namely potential-operated Ca-channels and receptor-operated Ca-channels, in the smooth muscle cells (see 10, 11 for review). Different sensitivity of contractions to inhibition by Ca-antagonists has been taken as evidence for the presence of the two channels. It has been reported in the rat vas deferens that sensitivity of high K-induced contractions to inhibition by verapamil and nifedipine was higher than that of NE-induced contractions (12, 13). Furthermore, nitroprusside which did not affect K-contractions inhibited rhythmic contractions induced by an \alpha-receptor agonist, methoxamine (14). These results suggest the presence of potential- and receptor-operated Ca channels in the rat vas deferens. It is generally accepted that contractions produced by high K or Ca in high K solutions are due to the influx of Ca through potential-operated Ca channels in the smooth muscles. Contractions to nerve stimulations are dependent on generation of Ca spikes which are believed to be caused by Ca-influx through potential operated Ca channels (see below). Since propranolol inhibited these contractions to a much greater extent than those by NE or MCh, it seems likely that propranolol inhibits the Ca-influx through potential-operated Ca channels in the rat vas deferens.

Propranolol did not affect the depolarization by high K (Fig. 5). It has been reported that organic Ca-antagonists did not modify the depolarization by high K in several smooth muscles (15, 16). Therefore, it is most likely that the inhibition of Ca-influx by propranolol or D-600 is not due to reducing the depolarization, but due to the actions on the Ca channels.

Furness and Burnstock (17) showed that contractions of vas deferens to nerve stimulations were caused by spikes, which are considered to be carried by Ca influx as in other smooth muscles (18, 19). In the guinea pig vas deferens, 10^{-6}-3 \times 10^{-6} M nifedipine was reported to abolish the spikes of action potentials and twitch contractions induced by hypogastric nerve stimulations (20). These results suggest that spikes are generated by Ca influx through the potential-operated Ca-channels in the vas deferens. The present results revealed that 10^{-3}-3 \times 10^{-3} M propranolol decreased overshoot potentials dose-dependently without affecting the threshold potential. Spike generation was
strongly inhibited by $10^{-4}$ M propranolol. Propranolol reduced the amplitudes of e.j.p.s, probably due to its local anesthetic action. However, this may not mainly contribute to the inhibition of spikes or twitch contractions by propranolol. Although rare, spikes were induced in the presence of $10^{-4}$ M propranolol as demonstrated in Fig. 7B, indicating that sufficient amount of depolarization to induce spikes was evoked even in the presence of $10^{-4}$ M propranolol. Therefore, it can be expected that amplitudes of e.j.p.s reach the threshold potentials in the experiments where effects of propranolol on the nerve-induced contractions were examined, since supra-maximal voltages were used in these experiments. Accordingly, it seems conceivable that propranolol impeded spike generation and thus reduced the twitch contractions by inhibiting potential-operated Ca-channels rather than by decreasing the amplitudes of e.j.p.s.

The mechanism of inhibition by propranolol of potential-operated Ca-channels is not clear from the present study, but should be discussed briefly. It has been reported that there is no difference between the d- and l-isomer of propranolol regarding the potency to inhibit high K- or Ca-induced contractions in the coronary artery of the dog and taenia coli of the guinea pig (3, 21). On the other hand, significant stereoselectivity has been shown to exist for the inhibitory effects of organic Ca-antagonists on the Ca-channels (22). These results may suggest that propranolol inhibits Ca channels due to non-specific actions on the membrane rather than the direct blocking actions of Ca channels as organic Ca-antagonists do. However, inhibition of Ca channels by propranolol may be caused by a mechanism other than one which involves local anesthetic action since inhibition of contractions by local anesthetics was much weaker in the vas deferens and coronary artery (4, 21).

In conclusion, effects of propranolol on mechanical and electrophysiological activity of rat vas deferens may be explained on the basis that potential-operated and receptor-operated Ca-channels are present in the smooth muscle cells of rat vas deferens. Propranolol at the proper concentration, e.g., $10^{-4}$ M, may inhibit Ca-influx through potential-operated Ca-channels by impairing the proper functions of these channels.

Acknowledgement: Authors would like to thank Dr. Y. Gomi for his constructive criticism in preparing this manuscript.

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