Verapamil-Sensitive and Less Sensitive Contractions in the Intestinal Smooth Muscle of the Guinea-Pig Taenia Caeci

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Abstract—Effect of verapamil, a Ca^{2+} channel blocker, on the contractions in the intestinal smooth muscle of taenia isolated from guinea-pig caecum was examined. In normal medium, 10 mM–80 mM KCl or 10^{-7} M– 10^{-5} M carbachol induced a transient contraction followed by a sustained one. When the muscle strips were treated with a Ca^{2+} -free solution for 2 min, these stimulants failed to induce contraction except an initial transient contraction induced by 10^{-6} M– 10^{-5} M carbachol. Cumulative application of verapamil during the sustained contraction induced muscle relaxation. Higher concentration of verapamil was needed to inhibit the sustained contraction induced by 10 mM or 20 mM K^+ than that induced by 40 mM or 80 mM K^+ . Similarly, the sensitivity to verapamil of the sustained contraction induced by 10^{-7} M carbachol was lower than that induced by 10^{-6} M or 10^{-5} M carbachol. In the presence of verapamil, addition of either high K^+ or carbachol induced an initial transient contraction, although the sustained contraction was strongly inhibited. These results suggest that high K^+ and carbachol activate two types of Ca^{2+} channels; lower concentrations of the stimulants open the channel which is less sensitive to verapamil, and higher concentrations open the channel which is more sensitive to verapamil.

It has become recognized that a larger portion of the smooth muscle contraction is attributable to Ca^{2+} influx through Ca^{2+} channels and that Ca^{2+} channel blockers such as verapamil inhibit the smooth muscle contraction by inhibiting Ca^{2+} channels (1–3). In the intestinal smooth muscle of taenia isolated from guinea-pig caecum, contractions induced by high K^+ -depolarization or by receptor agonists are composed of initial transient contraction followed by a sustained contraction. As for the mechanisms for these contractions, Urakawa and Holland (4) proposed that the transient contraction is due to release of cellular Ca^{2+} , whereas the sustained contraction is due to Ca^{2+} influx. However, Imai and Takeda (5) showed that both of these contractions are dependent on the existence of external Ca^{2+} and suggested that both of these contractions are due to Ca^{2+} influx. Recently, it has been shown that although verapamil strongly inhibits the sustained con-

traction, it has relatively weak inhibitory effect on the transient contraction (1, 6). In the present experiments, we further examined the effect of verapamil on the contractions in the taenia in order to clarify the mechanisms of these contractions in the intestinal smooth muscle of taenia. A part of the result has been briefly reported (7).

Materials and Methods

Male white guinea-pigs, weighing about 300 g, were killed by a blow on the neck and bled. Strips of taenia were removed from the caecum and cut into small pieces, 5–10 mm in length.

The normal physiological salt solution (PSS) contained (mM): NaCl, 136.9; KCl, 5.4; glucose, 5.5; NaHCO_3 , 23.8; CaCl_2 , 1.5; MgCl_2 , 1.0; and ethylenediamine tetraacetic acid (EDTA), 0.01. Ca^{2+} -free PSS was made by omitting CaCl_2 and adding 0.5 mM ethyleneglycol bis(beta-aminoethylether)-

Fig. 1. Contractions induced by high K^+ (left) and carbachol (right) in the absence and in the presence of verapamil. Stimulant was added at the triangle. Verapamil was added 10 min before the addition of the stimulants. Scale indicates 5 min and 5 g tension. Traced from typical experimental data.

N,N,N',N'-tetraacetic acid (EGTA) to the PSS. High K^+ solution was made by adding 10 mM–80 mM KCl to the PSS. These solutions were aerated with a mixture of 95% O_2 and 5% CO_2 at $37^\circ C$ (pH 7.4).

Muscle tension was recorded isometrically with a force-displacement transducer connected to a polygraph (Nihon Kohden). Passive tension of 0.2 g was initially applied, and the tissue was allowed to equilibrate for 60 min before starting the experimental period. Verapamil was cumulatively applied when the contractile tension induced by stimulants reached a steady level. In some experiments, verapamil was added 10 min before the addition of the stimulants unless otherwise stated.

Results of the experiments were expressed as the mean $\pm$ S.E.M. Values were considered to be significantly different when the P value was less than 0.05 using Student's t -test.

($\pm$) Verapamil hydrochloride (Sigma), nifedipine hydrochloride (Sigma) and carbamyl choline chloride (carbachol, Sigma) were used.

Results

In the isolated taenia strips, high K^+ or carbachol induced a transient contraction which was followed by a sustained contraction. As shown in Fig. 1, the sustained contraction reached a steady level 5 to 10 min after the addition of the stimulants. Magnitudes of the transient and sustained contractions induced by KCl or carbachol are shown in Fig. 2. Concentration-dependent contractions were obtained when 10, 20 and 40

Fig. 2. Magnitude of contractions induced by KCl and carbachol. Open column represents initial transient contraction. Shaded column represents the steady level of the sustained contraction which was measured 5 to 10 min after the addition of stimulant. Magnitude of the sustained contraction induced by 40 mM KCl was taken as 100%. Values of the mean $\pm$ S.E.M. of 8 to 14 experiments are shown. *: Significantly different ($P < 0.05$) from the corresponding value obtained in the presence of 10 mM KCl (left) or 10^{-7} M carbachol (right).

mM KCl were added. However, the contractions induced by 80 mM KCl were almost the same as or slightly smaller than those induced by 40 mM KCl. Carbachol, 10^{-7} M, induced a transient contraction which was smaller than that induced by 10^{-6} M or 10^{-5} M carbachol, and a sustained contraction which was greater than that induced by 10^{-5} M carbachol.

In order to know the Ca^{2+} dependency of these contractions, muscle strips were treated with a Ca^{2+} -free solution for 2 min, and then

high K^+ or carbachol was added. Neither high K^+ nor carbachol induced contraction in the absence of Ca^{2+} except the initial transient contraction induced by higher concentrations (10^{-6} – 10^{-5} M) of carbachol, as has been reported (8).

The inhibitory effect of verapamil added cumulatively during the sustained contraction induced by high K^+ or carbachol is shown in Fig. 3. It is demonstrated that verapamil inhibited the sustained contractions and that the sensitivity to verapamil was dependent on the concentration of stimulant. Sustained contraction induced by 20 mM K^+ was less sensitive than that induced by 40 mM K^+ . Similarly, sustained contraction induced by

10^{-7} M carbachol was less sensitive than that induced by 10^{-6} or 10^{-5} M carbachol.

Effects of verapamil on transient and sustained contractions induced by high K^+ or carbachol are shown in Fig. 1. When added 10 min after the addition of verapamil, these stimulants induced a transient contraction followed by a sustained contraction, and the latter contraction was more strongly inhibited than the former. Magnitudes of the sustained contractions measured in the presence of verapamil are shown in Fig. 4. This figure again shows that the contraction induced by 10 or 20 mM K^+ was less sensitive to verapamil than that induced by 40 or 80 mM K^+ , and that the contraction induced by 10^{-7}

Fig. 3. Effect of verapamil on the sustained contractions induced by 20 mM KCl (20 K) and 40 mM KCl (40 K) (left) and carbachol (right). Verapamil was cumulatively applied during the sustained contraction. Values of the mean $\pm$ S.E.M. of 4 to 6 experiments are shown. *: Significantly different ($P < 0.05$) from the corresponding value obtained in the presence of 20 mM KCl (left) or 10^{-7} M carbachol (right).

Fig. 4. Effects of verapamil on the sustained contractions induced by high K^+ (left) and carbachol (right). Experiment was done as shown in Fig. 1, and the steady level of the sustained contraction in the absence of verapamil is taken as 100%. Values of the mean $\pm$ S.E.M. of 4 to 6 experiments are shown. *: Significantly different ($P < 0.05$) from the corresponding value obtained in the presence of 10 mM KCl (left) or 10^{-7} M carbachol (right).

Fig. 5. Effects of verapamil on the transient and sustained contractions induced by 40 mM KCl (40K, left) and 10^{-6} M carbachol (right). Experiment was done as shown in Fig. 1, and the level of the contractions in the absence of verapamil is taken as 100%. Values of the mean $\pm$ S.E.M. of 4 to 6 experiments are shown. *: Significantly different ($P < 0.05$) from the transient contraction.

M carbachol was less sensitive to verapamil than that induced by 10^{-6} M or 10^{-5} M carbachol.

Since Ca^{2+} channel blockers have a "use-dependent" effect (9), the above experiment was repeated in which the effect of verapamil was expected to be maximally developed. In this experiment, verapamil was added during the 40 mM K^+ -induced contraction; and when the full inhibitory effect of verapamil was obtained, the muscle was washed with PSS containing the same concentration of verapamil, and then high K^+ or carbachol was added. Results of this experiments were almost the same as that in the above experiment, suggesting that the "use-dependent" nature of verapamil is not so clear in the guinea-pig taenia strips.

The concentration-inhibition curves for verapamil on the transient and sustained contractions, calculated from the experiments similar to that in Fig. 1, are shown in Fig. 5. It was shown that the transient contraction is less sensitive to verapamil than the sustained contraction induced by either 40 mM KCl or 10^{-6} M carbachol. This figure also shows that the transient contraction induced by 10^{-6} M carbachol was slightly less sensitive to verapamil than that induced by 40 mM KCl.

The above experiments were repeated using a dihydropyridine Ca^{2+} channel blocker, nicardipine, instead of verapamil. This blocker, at the concentration of $3-5 \times 10^{-10}$ M, more strongly inhibited the sustained contraction

than the transient contraction induced by 40 mM K^+ or 10^{-6} M carbachol. Further, 5×10^{-10} M nicardipine more strongly inhibited the sustained contraction induced by 10^{-5} M carbachol (to $36.0 \pm 6.5\%$, $n=4$) than that induced by 10^{-7} M carbachol (to $64.1 \pm 5.9\%$, $n=6$, $P < 0.01$). In contrast to this, inhibitory effects of nicardipine on the sustained contractions induced by 20 mM K^+ and 80 mM K^+ were not statistically significant.

Discussion

In the guinea-pig taenia strips, sustained contraction induced by 10^{-7} M carbachol was more resistant to verapamil and nicardipine than those induced by 10^{-6} – 10^{-5} M carbachol. Similarly, sustained contraction induced by 10–20 mM KCl was more resistant to verapamil than that induced by 40–80 mM KCl, although the results with nicardipine were ambiguous. We did not expect such a result because opposite results have generally been obtained. For example, sustained contraction induced in vascular smooth muscle of rabbit aorta by 15 mM KCl was 10-fold more sensitive than that induced by 140 mM KCl, and that induced by 10^{-8} M norepinephrine was 10,000-fold sensitive than that induced by 10^{-5} M norepinephrine to a Ca^{2+} channel blocker, diltiazem (10). Similarly, sodium nitroprusside and forskolin more strongly inhibited the contraction induced by 10^{-8} M norepinephrine than that induced by 10^{-6} M norepinephrine in rat aorta (11).

We also confirmed previous findings that the high K^+ - or carbachol-induced transient contractions in the taenia are more resistant to Ca^{2+} channel blockers than sustained contraction (1, 6, 8, 12). It has been shown that in the absence of external Ca^{2+} , high concentrations of carbachol release Ca^{2+} from the cellular store to induce a transient contraction, and Ca^{2+} channel blockers do not inhibit this contraction (8, 13). However, contractions induced by 10–80 mM K^+ or 10^{-7} M carbachol were rapidly inhibited in the absence of external Ca^{2+} , although transient contractions induced by 10^{-6} – 10^{-5} M carbachol were more resistant to Ca^{2+} removal. These results support the suggestion (5) that both transient and sustained contractions induced by high K^+ and by 10^{-7} M carbachol are triggered by Ca^{2+} influx. Only the transient contraction induced by 10^{-6} – 10^{-5} M carbachol may partly be due to a mechanism which is not triggered by Ca^{2+} influx, and this may be the reason why the transient contraction induced by 10^{-6} M carbachol was more resistant to verapamil than that induced by high K^+ (Fig. 5).

In electrophysiological studies, it has been demonstrated that high K^+ and carbachol induce an increase in spike discharges. If the concentration of these stimulants are sufficiently high, the cell membrane is gradually depolarized to inhibit spike discharges. The concentration of KCl to inhibit spike discharges is approximately 40 mM (14) and that of carbachol is approximately 10^{-6} M (15). Thus, the initial transient contraction may be due to spike discharges (14–16). Further, sustained contractions induced by 10–20 mM KCl or 10^{-7} M carbachol may also be due to spike discharges, whereas the sustained contraction induced by 40–80 mM KCl or 10^{-6} – 10^{-5} M carbachol may be due to spike-free depolarization (14–16).

In smooth muscle, spike discharges are due to Ca^{2+} entry through Ca^{2+} channels. Further, membrane depolarization also opens Ca^{2+} channels (for review see 1). Recently, it has been shown that the membrane depolarization opens two types of Ca^{2+} channels: T (for transient) channel, which is inactivated very rapidly at the polarized membrane potential, and L (for long) channel, which is in-

activated very slowly even at the depolarized membrane potential (17, 18). The T channel is much less sensitive to Ca^{2+} channel blockers than the L channel (17, 18). In cardiac muscle, there are similarly two types of Ca^{2+} channels (19), and Fabiato (20) proposed that Ca^{2+} current through the T channel, but not the L channel, releases Ca^{2+} from the storage site by activating the Ca^{2+} -induced Ca^{2+} release mechanism. This hypothesis also explains the present results: Lower concentrations of the stimulants increase spike discharges possibly by activating T channels, trigger Ca^{2+} release and induce a transient contraction. Summation of such transient contractions may result in a sustained contraction which is less sensitive to Ca^{2+} channel blockers. In contrast to this, higher concentrations of the stimulants increase spike discharges to induce a transient contraction followed by a spike-free depolarization which opens the Ca^{2+} channel blocker-sensitive L channel to induce a sustained contraction. Only higher concentrations of carbachol may release the Ca^{2+} store in the absence of triggering Ca^{2+} currents (8).

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