Effects of Trimebutine Maleate (TM-906) on the Spontaneous Contraction of Isolated Guinea Pig Colon

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Abstract—Effects of trimebutine maleate (TM-906) on the spontaneous contraction of isolated guinea pig colon were investigated. TM-906 in the concentrations of $10^{-6}$ g/ml and $10^{-5}$ g/ml increased the tone without affecting the amplitude of the spontaneous contraction in the preparations with low tone, whereas it decreased the tone and the amplitude of the spontaneous contraction in the preparations with high tone. At the higher concentration ($10^{-4}$ g/ml), TM-906 decreased the tone and finally abolished the spontaneous contraction in any preparation. The increase in tone induced by TM-906 was prevented by diltiazem and exposure to Ca$^{++}$-free solution, but not by tetrodotoxin, atropine, phentolamine or propranolol, and depended on the extracellular concentration of CaCl$_2$. On the other hand, the decrease in tone and amplitude of the spontaneous contraction produced by TM-906 were not prevented by tetrodotoxin, phentolamine or propranolol. TM-906 further increased the tone increased by 10 mM KCl, while it decreased the tone increased by 30 mM KCl. From results described above, it is suggested that TM-906 possesses both a relaxing effect and an excitatory effect which seem to be due to its direct action on the smooth muscle.

Trimebutine maleate (TM-906) has been known to have a modulating effect on the motility of the stomach in clinical trials; TM-906 stimulated the spontaneous gastric motility in conditions of depressed activity and reduced it in conditions of hyperactivity (1). In conscious dogs, TM-906 evoked contractions in gastrointestinal tracts during motor quiescence in the interdigestive state, while in the digestive state, it inhibited contractions of the stomach and duodenum and potentiated contractions of the jejunum, ileum and colon (2). In in vitro experiments, TM-906 inhibited regular spontaneous contraction of guinea pig stomach, and it regularized irregular spontaneous contraction (3). The present experiments were attempted to analyze further the effects of TM-906 on the motility of smooth muscle by using isolated preparations of guinea pig colon.

Materials and Methods
Male guinea pigs weighing 300–350 g were stunned and bled. The colon (15 cm orad to anus) was isolated and emptied of its contents, and longitudinal segments, lengths of 1.0 cm, were prepared. The colon was suspended in an organ bath containing 10 ml of modified Krebs solution, and isometric contraction was recorded on an inkwriting oscillograph through a strain gauge transducer. The initial tension applied to the muscle was adjusted to approx. 0.5 g. The composition of modified Krebs solution was as follows (mM): NaCl, 141.9; KCl, 5.6; CaCl$_2$, 2.2; MgCl$_2$, 0.4; NaH$_2$PO$_4$, 0.4; NaHCO$_3$, 11.9; and glucose, 5.5. For experiments in K$^+$-rich solution, KCl was added to the modified Krebs solution. The Ca$^{++}$-free solution was prepared by omitting CaCl$_2$ and EGTA was not added. The solution was aerated with 95% O$_2$ and 5% CO$_2$. Experiments were carried out at $37 \pm 1 \, ^\circ\text{C}$. The following compounds were used: TM-906, diltiazem hydrochloride (Tanabe Seiyaku Co., Ltd.), tetrodotoxin (Sankyo Co., Ltd.).
atropine sulfate (Wako Pure Chem. Ind., Ltd.), phentolamine mesylate (CIBA-Geigy Ltd.), propranolol hydrochloride (Sumitomo Chem. Ind., Ltd.), acetylcholine chloride and papaverine hydrochloride (Nakarai Chem. Ltd.).

**Results**

1. **Effects of TM-906 and papaverine on the spontaneous contraction:** When the initial tension of 0.5 g was applied to the preparations, the tone temporally decreased, and then it gradually increased and spontaneous contraction appeared. We investigated the effects of TM-906 on the spontaneous contraction in preparations with low tone and with high tone. As shown in Figs. 1 and 2, TM-906 in the concentrations of $10^{-6}$ g/ml and $10^{-5}$ g/ml gradually increased the tone without affecting the amplitude of the spontaneous contraction in preparations with low tone, whereas it rapidly decreased the tone and the amplitude of the spontaneous contraction in preparations with high tone. However, TM-906 in the concentration of $10^{-4}$ g/ml decreased the tone and finally abolished the spontaneous contraction in any preparation. Papaverine in the concentrations of $10^{-6}$ g/ml and $10^{-5}$ g/ml decreased the tone and the amplitude of the spontaneous contraction in any preparation (not shown).

![Fig. 1. Effects of TM-906 on the spontaneous contraction in preparations with low tone and with high tone.](image)

![Fig. 2. Relationship between the effects of TM-906 on the tone and control values of the tone. The values were obtained at 20 min after addition of TM-906.](image)

2. **Effects of TM-906 in the presence of tetrodotoxin, atropine, phentolamine or propranolol:** Figure 3A demonstrates the effects of TM-906 in the presence of tetrodotoxin ($10^{-6}$ g/ml). When tetrodotoxin was added, the spontaneous contraction was markedly inhibited with decrease in tone, while TM-906 at the concentration of $10^{-6}$ g/ml increased the tone. However, TM-906 at the concentration of $10^{-4}$ g/ml produced a reduction of the tone and a temporary potentiation, followed by a reduction of the
amplitude of spontaneous contraction in the presence of tetrodotoxin. TM-906 (10^{-5} \text{ g/ml}) also increased the tone which was markedly decreased by atropine (10^{-6} \text{ g/ml}) as shown in Fig. 3B. Figure 3C shows the effects of TM-906 (10^{-5} \text{ g/ml}) on the spontaneous contraction in the presence of both phentolamine (10^{-6} \text{ g/ml}) and propranolol (10^{-7} \text{ g/ml}), which had no significant influence on the spontaneous contraction. In the presence of both phentolamine and propranolol, TM-906 reduced the tone and the amplitude of the spontaneous contraction in the preparations with high tone, while it increased the tone in the preparations with low tone.

3. Effects of diltiazem and different extracellular concentration of CaCl_2: The following experiments were performed in the bathing solution containing tetrodotoxin (10^{-6} \text{ g/ml}). When diltiazem (3\times10^{-6} \text{ g/ml}) was added, the spontaneous contraction was remarkably inhibited, and TM-906 (10^{-5} \text{ g/ml}) did not increase the tone (Fig. 4A). Figure 4B demonstrates the relationship between the effect of TM-906 on the tone and the extracellular concentration of CaCl_2. When the preparation was exposed to the Ca^{2+}-free solution, the spontaneous contraction was abolished, but acetylcholine (10^{-7} \text{ g/ml}) elicited a contraction. After washing out the preparation with normal solution (2.2 mM CaCl_2), the extracellular CaCl_2 was again removed and TM-906 (10^{-5} \text{ g/ml}) was added. TM-906 did not increase the tone. However, TM-906 increased the tone and the amplitude of the spontaneous contraction with increase in the extracellular concentration of CaCl_2.

4. Effects of TM-906 in the K^+-rich solution: A transient phasic contraction and a sustained tonic contraction were elicited by increasing the extracellular concentration of KCl. Figure 5 shows the effects of TM-906 on the sustained tonic contraction (defined

Fig. 3. Effects of TM-906 (10^{-5} \text{ g/ml}) on the spontaneous contraction in the presence of tetrodotoxin (10^{-6} \text{ g/ml}, TTX), atropine (10^{-6} \text{ g/ml, Atr}), phentolamine (10^{-5} \text{ g/ml, Phen}) or propranolol (10^{-7} \text{ g/ml, Prop}).

Fig. 4. Effects of TM-906 (10^{-5} \text{ g/ml}) on the spontaneous contraction in the presence of diltiazem (3\times10^{-6} \text{ g/ml}). (A) and relationship between the effects of TM-906 on the tone and extracellular concentration of CaCl_2. (B) under pretreatment with tetrodotoxin (10^{-6} \text{ g/ml}).
as tone) induced by KCl in the presence of tetrodotoxin (10^{-6} \text{ g/ml}). TM-906 at the concentration of 10^{-5} \text{ g/ml} further increased the tone increased by 10 mM KCl, while it produced a slight increase followed by a slight reduction of the tone increased by 20 mM KCl. However, TM-906 remarkably decreased the tone increased by 30 mM KCl.

**Discussion**

The effects of TM-906 on the spontaneous contraction of the isolated colon were modulating ones: namely, it gradually increased the tone in the preparations with low tone, while it reduced the tone and the amplitude of spontaneous contraction in the preparations with high tone. These effects of TM-906 were different from that of papaverine which decreased the tone and the amplitude of spontaneous contraction in any preparation. The increase in tone produced by TM-906 was not abolished in the presence of tetrodotoxin, atropine, phenolamine or propranolol, indicating that the effect of TM-906 was not due to nervous systems, cholinergic or adrenergic \(\alpha\)- or \(\beta\)-mechanisms. Under the condition of treatment with tetrodotoxin, the increasing effect in tone by TM-906 was prevented by diltiazem, a calcium channel blocker (4–7), and by exposure to Ca^{2+}-free solution and depended on the extracellular concentration of CaCl_{2}. These results suggested that the increase in tone induced by TM-906 was due to direct action on the smooth muscle and was ascribed to increasing the amount of free Ca^{2+} available to the contractile elements.

On the other hand, the decrease in tone and amplitude of spontaneous contraction produced by TM-906 was not prevented by phentolamine or propranolol, indicating that the effects of TM-906 were not due to adrenergic \(\alpha\)- or \(\beta\)-mechanisms. Similar phenomena have been demonstrated in the guinea pig stomach (3). The relaxing effect of TM-906 at the concentration of 10^{-6} \text{ g/ml} was never detected in the presence of tetrodotoxin, since tetrodotoxin itself produced a remarkable reduction of the tone and amplitude of spontaneous contraction (Fig. 3A). TM-906 at the concentration of 10^{-4} \text{ g/ml}, however, decreased the tone of the preparation, as well as its amplitude of spontaneous contraction, even in the presence of tetrodotoxin. Furthermore, TM-906 at the concentration of 10^{-5} \text{ g/ml} decreased the tone increased by 30 mM KCl in the presence of tetrodotoxin. These results suggested that the relaxing effect of TM-906 was due to direct action on the smooth muscle. It has been suggested that TM-906 may inhibit the spontaneous contraction by interfering with the transmembrane influx of calcium ion in the isolated guinea pig stomach (3). Whether this hypothesis can be also applied to the colon remains to be examined.

TM-906 at the concentration of 10^{-4} \text{ g/ml} reduced the tone and finally abolished the spontaneous contraction in most preparations. Under the condition that the tone of the preparation and the amplitude of spontaneous contraction were markedly decreased by tetrodotoxin, however, a transient augmentation of the amplitude of spontaneous contraction preceding its reduction was produced by TM-906 at the same concentration (Fig. 3A). These observations suggested that TM-906 at the concentration of 10^{-4} \text{ g/ml}, as well as at 10^{-6} \text{ g/ml} and 10^{-5} \text{ g/ml},...
g/ml, may have both a relaxing effect and an excitatory effect.

That the modulating effect of TM-906 was ascribed to the direct action on the smooth muscle was also demonstrated by the present experiments in which the tone was deliberately changed by KCl as shown in Fig. 5. Since the increasing effect in tone by TM-906 was converted into the decreasing effect in tone by increasing the degree of depolarization with KCl (8, 9), it is assumed that the effects of TM-906 on the tone changed by KCl may be associated with membrane potential. However, the mechanism by which TM-906 modulated the spontaneous contraction remains to be investigated. We could not rule out the possibility that the effects of TM-906 on the spontaneous contraction in a longitudinal direction observed in the present experiments were partially influenced by those in a circular direction, since we did not separate a longitudinal muscle from a circular muscle.

From these evidences, it is suggested that TM-906 possesses both a relaxing effect and an excitatory effect which seem to be direct actions on the smooth muscle.

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