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Effects of Trimebutine Maleate on Electrical Activities of Isolated Mammalian Cardiac Preparations

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Abstract—The effects of trimebutine maleate on electrical activity in guinea-pig isolated papillary muscles and rabbit sino-atrial nodes have been studied by means of a standard microelectrode method. In papillary muscles, trimebutine (above 10 μM) decreased the maximum rate of rise ($\dot{V}_{max}$) and the action potential duration at 90% repolarization (APD90), whereas the resting potential was not significantly altered. As to a decrease in $\dot{V}_{max}$, trimebutine produced a negative shift of the curve relating $\dot{V}_{max}$ to the resting potential along the voltage axis. Trimebutine also depressed the slow action potentials of papillary muscles produced by 27 mM K and 0·2 mM Ba. In spontaneously beating sino-atrial node preparations, trimebutine (above 10 μM) decreased the heart rate, V_{max} and the rate of diastolic depolarization. These results indicate that trimebutine maleate possesses a depressant action on the electrical activities of the fast- and slow-response fibres of the heart mainly due to inhibitions of both fast Na^+ and slow Ca^{2+} channels.

Trimebutine maleate is used in the treatment of gastrointestinal disorders including irritable colon syndrome (Moshal & Herron 1979; Luttecke 1980). According to in-vivo and in-vitro experiments (Takenaga et al 1982, 1984a), trimebutine directly affects the smooth muscles of gastrointestinal tracts. In electrophysiological studies (Furukawa & Kimoto 1984; Takenaga et al 1984b), trimebutine has been shown to reduce the influx of calcium ions across the cell membrane of smooth muscles of guinea-pig isolated stomach and gallbladder. Furthermore, trimebutine has a chemical structure similar to that of local anaesthetics such as procaine. These findings might suggest that trimebutine depresses both fast Na and slow Ca channels of excitable tissues. However, the electrophysiological effects of trimebutine have not been previously studied in isolated mammalian cardiac fibres.

Therefore, the present study was undertaken to investigate the effects of trimebutine on transmembrane action potentials of guinea-pig papillary muscles and rabbit sino-atrial node preparations by means of the conventional glass microelectrode method.

Materials and Methods

Preparations of guinea-pig papillary muscles

Guinea-pigs, 200–300 g, were killed by a blow on the neck, the heart was quickly removed and thin (less than 1 mm) papillary muscles were excised from the right ventricle. The preparation was perfused in a recording chamber with oxygenated (95% O_2, 5% CO_2), warm (36 ± 0·5°C) Tyrode solution. The composition of the normal Tyrode solution was as follows (in mM): NaCl 132·0, KCl 4·0, $MgCl_2$ 1·0, $NaHCO_3$ 12, NaN_2PO_4 0·4, $CaCl_2$ 1·8 and glucose 10·0. The pH of all solutions was 7·4. The preparation was driven at 1·0 Hz for more than 1 h after mounting in a chamber in Tyrode solution. Stimulus electrodes consisted of a pair of Ag-AgCl wires (diameter 1 mm, length 5 mm) placed near the preparation. The stimulus threshold was determined during experiments before drug perfusion and the intensity of current pulses was set 1·5 times the threshold. The effects of trimebutine on slow action potentials were studied using 0·2 mM Ba containing Tyrode solution with increasing $[K^+]$ to 27 mM.

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Preparations of rabbit sino-atrial nodes

Rabbits (1·5–2·0 kg) were stunned by a blow on the neck. The heart was quickly removed and the right atrium with sino-atrial node region was dissected in normal Tyrode solution. The sino-atrial node tissue close to the crista terminalis was cut into strands, approximately 1 mm in length and 0·3 mm in width. The pacemaking portion was ligated by two silk fibres at a distance of 0·3 mm along the length of the strand. These small specimens of sino-atrial node were prepared in the same manner as in previous studies (Noma & Irisawa 1976; Kotake et al 1985). In the present experiments, we used the central area of the node, termed the "compact" area, as previously described by Bleeker et al (1980) and Brown (1982).

The composition of the Tyrode solution was as follows (in mM): NaCl 136·9, KCl 2·7, $CaCl_2$ 1·8, $MgCl_2$ 1·0 and NaH_2PO_4 0·6. The pH was adjusted to 7·4 by adding Na_2HPO_4.

Trimebutine maleate (Tanabe Seiyaku Co. Ltd.) was dissolved in the distilled water, and then diluted in the Tyrode solution to the desired concentration.

Recording of the membrane potential

Transmembrane potentials were recorded with conventional glass microelectrodes filled with 3 M KCl having a resistance of 10–20 MOhms. The electrodes were connected to an amplifier (Nihon Kohden, MEZ 7101). Potentials were stored on a chart recorder (Nihon Kohden, RJG 4122) and displayed on an oscilloscope (Nihon Kohden, VC 10).

The effect of trimebutine on the relationship of the maximum rate of rise ($\dot{V}_{max}$) and the resting potential (E_m) was studied in guinea-pig papillary muscles by changing $[K^+]o$ from 2·7 to 5·4, 8·1, 10·8, 13·5 and 14·9 mM. After obtaining the control records, trimebutine was added to each

perfusate and their effects were investigated. To assure that $\dot{V}_{max}$ and E_m had attained a steady-state at each K^+ concentration, measurement of these parameters was made after at least 7 min of equilibration. Data were analysed only from the records in which a single impalement was maintained throughout the procedure. The relation between $\dot{V}_{max}$ and E_m was utilized as an indication of the change of steady-state Na^+ inactivation variable at different membrane potentials. We fitted the following equation proposed by Windisch & Tritthart (1981) to the experimentally obtained $\dot{V}_{max}$-E_m relationship.

$$\dot{V}_{max} = V_s/(1+\exp[(E_m-E_f)/S])^2$$

where V_s, E_f and S denote the saturation value of $\dot{V}_{max}$, the membrane potential at which $\dot{V}_{max}$ is one-quarter of V_s, and the slope factor, respectively. These values were determined by the least squares method using a microcomputer (NEC, PC-8001). The validity of these equations to express $\dot{V}_{max}$-E_m relationship of guinea-pig ventricular muscle was verified by Hisatome et al (1985).

Electrophysiological values are expressed as mean ± s.d. Statistical analysis was performed using Student's *t*-test, and *P* values of 0·05 or less were considered significant.

Results

Papillary muscle preparations of guinea-pigs

The effects of cumulative concentrations of trimebutine maleate were examined on the action potentials of guinea-pig papillary muscle preparations generated by stimulation at 1·0 Hz. Electrophysiological measurements were made 15 min after changing to a new solution. Although perfusion with trimebutine below 1 μM has no consistent effect on the action potential, 10 μM of trimebutine caused decreases in the maximum rate of rise ($\dot{V}_{max}$), and the action potential duration (APD90) without changing the resting membrane potential (Fig. 1). At 30 μM, the agent also reduced the action potential amplitude (APA). The effects of trimebutine on various electrophysiological parameters are summarized in Table 1. At 70 μM of trimebutine, all the parameters were significantly reduced.

The action of trimebutine on slow action potentials evoked by increasing $[K^+]o$ to 27 mM with 0·2 mM Ba was studied in five papillary muscle preparations (Fig. 2). Trimebutine, at 1 μM, had no significant effect on the slow action potentials. At 10 μM, the slow action potentials became substantially depressed, and a high concentration (100 μM) abolished excitability within 15 min. The effects of increasing concentrations of trimebutine of $\dot{V}_{max}$ and on the overshoot potential (OS) of the slow action potentials are summarized in Table 2. To investigate the effect of trimebutine on the $\dot{V}_{max}$-E_m relation, $[K^+]o$ was changed from 2·7 to 5·4, 8·1, 10·8, 13·5 and 14·9 mM. $\dot{V}_{max}$ and E_m were measured during a steady-state at each K^+ concentration, and the value was normalized in Fig. 3. The smooth curves were drawn by computer according to the equation proposed by Windisch & Tritthart (1981). This graph indicates that the relation of $\dot{V}_{max}$-E_m was shifted to more negative potentials by an application of trimebutine. Similar results were obtained in three experiments.

Sino-atrial node preparations of rabbits

The effects of cumulative concentrations of trimebutine maleate on the action potentials were examined in sponta-

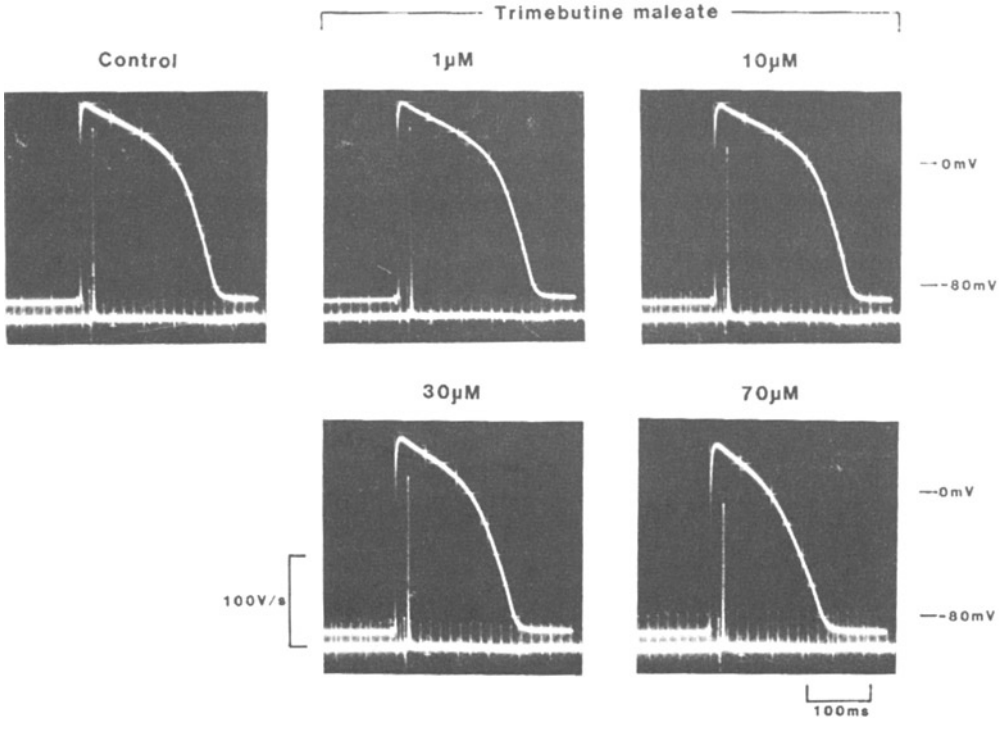

FIG. 1. Electrophysiological effects of increasing concentrations of trimebutine on guinea-pig papillary muscle. The upper trace in each panel shows the transmembrane action potential and the lower trace its first derivative.

Table 1. Effects of trimebutine on action potential parameters of guinea-pig papillary muscles. Values are means ± s.d. (n = 7). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with control values. APA: action potential amplitude, $\dot{V}_{max}$: maximum rate of rise, APD50 and APD90: action potential duration measured at 50% and 90% repolarization.

	$\dot{V}_{max}$ (Vs^{-1})	APA (mV)	APD50 (ms)	APD90 (ms)
Control	187·8 ± 47·7	126·7 ± 4·0	157·8 ± 16·4	203·6 ± 25·4
Trimebutine (μM)				
1	181·3 ± 44·6	126·4 ± 3·7	166·0 ± 38·9	201·8 ± 27·2
10	171·5 ± 45·1**	126·2 ± 3·5	158·5 ± 25·5	196·6 ± 19·6*
30	154·7 ± 55·7**	124·9 ± 3·0*	153·0 ± 22·5	194·5 ± 23·7*
70	138·6 ± 53·1***	123·5 ± 2·2**	147·0 ± 19·8**	194·3 ± 24·2*

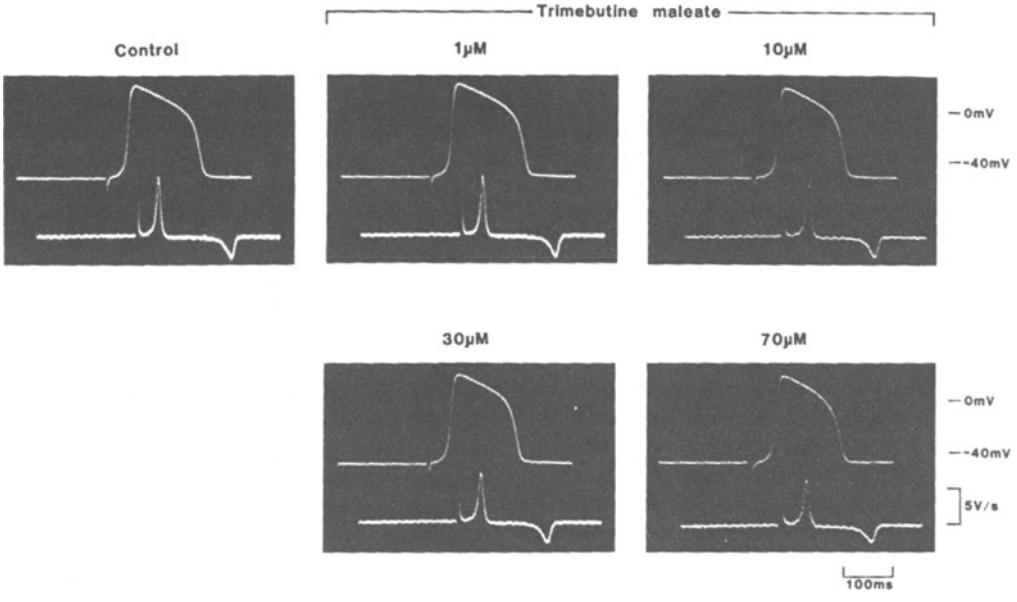

FIG. 2. Effects of trimebutine on slow action potentials of guinea-pig papillary muscles induced by 27 mM K and 0·2 mM Ba containing Tyrode solution. In each panel, the upper trace represents the action potential and the bottom shows its first derivative.

Table 2. Effects of trimebutine on slow action potential characteristics of guinea-pig papillary muscles. $\dot{V}_{max}$: maximum rate of rise, OS: overshoot potential. (Means ± s.d. n = 5.) $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

	$\dot{V}_{max}$ (Vs^{-1})	OS (mV)
Control	9·4 ± 1·9	22·3 ± 7·4
Trimebutine (μM)		
1	9·2 ± 1·9	21·5 ± 7·3
10	7·4 ± 1·9**	18·1 ± 7·8*
30	6·4 ± 1·9**	15·0 ± 7·6**
70	5·2 ± 2·1**	11·4 ± 8·0**

neously beating preparations of rabbits. As shown in Fig. 4, although 0·1–1 μM of trimebutine had no significant effect on the action potential, 10 μM of trimebutine decreased the heart rate, $\dot{V}_{max}$ and the rate of diastolic depolarization (RDD) significantly. At 30 μM, trimebutine also decreased the APA. The effects of trimebutine on action potential characteristics of sino-atrial node preparations are summarized in Table 3.

Discussion

The results of the present study showed that trimebutine maleate (above 10 μM) depressed electrical activities of fast and slow action potentials obtained from guinea-pigs and rabbits. In papillary muscle fibres, trimebutine depressed $\dot{V}_{max}$ due to a negative shift of the curve relating $\dot{V}_{max}$ to the resting potential (E_m) along the voltage axis, and depressed $\dot{V}_{max}$ remarkably in more depolarized membrane potential. This finding suggests that trimebutine could depress the "residual (partially inactivated) sodium channel" (Arita et al 1983) and hence suppress conduction in depolarized ventricular muscle. Furthermore, trimebutine produced decreases in APA and APD90 without a significant change in the resting membrane potential.

In sino-atrial node preparations, trimebutine decreased the heart rate, $\dot{V}_{max}$, APA and RDD in a dose-dependent manner, whereas APD50 was not significantly altered by the drug. In slow action potentials of guinea-pig papillary muscles elicited by 27 mM K and 0·2 mM Ba containing Tyrode solution, trimebutine also depressed $\dot{V}_{max}$ and OS

Table 3. Effects of trimebutine on action potential parameters of sino-atrial node preparations. (means ± s.d., n = 7.) $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. SCL: spontaneous cycle length, $\dot{V}_{max}$: maximum rate of rise, APA: action potential amplitude, APD50: action potential duration at 50% repolarization, RDD: rate of diastolic depolarization.

	SCL (ms)	$\dot{V}_{max}$ (Vs^{-1})	APA (mV)	APD50 (ms)	RDD ($mV\ s^{-1}$)
Control	356·1 ± 34·7	8·5 ± 1·3	98·4 ± 5·3	87·3 ± 10·1	106·3 ± 11·7
Trimebutine (μM)					
0·1	363·1 ± 36·9	8·5 ± 1·3	98·7 ± 5·0	87·4 ± 10·1	104·9 ± 11·5
1	373·9 ± 42·5	8·5 ± 1·4	99·6 ± 4·6	88·4 ± 11·9	100·5 ± 13·9
10	419·7 ± 60·5**	6·8 ± 1·2***	96·1 ± 3·2	88·1 ± 15·0	88·8 ± 13·3**
30	473·7 ± 68·2***	5·0 ± 1·2***	92·1 ± 5·0*	88·4 ± 13·0	81·1 ± 13·1***

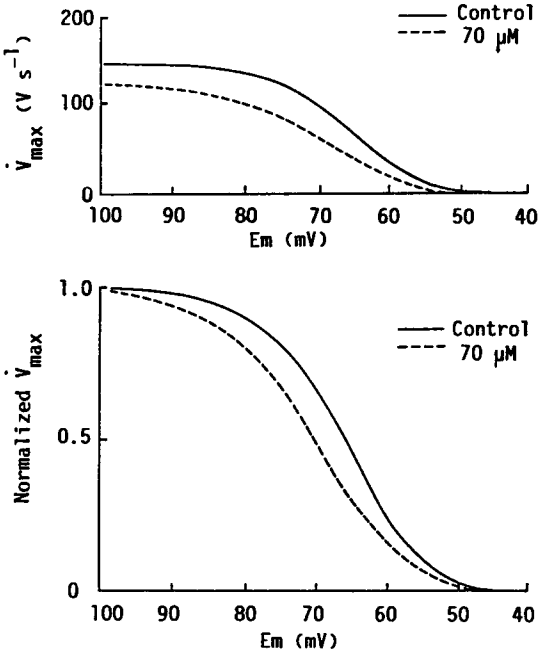

FIG. 3. Effects of trimebutine (70 μM) on the $\dot{V}_{max}$-E_m relation of guinea-pig papillary muscle action potentials. Upper panel: effects of trimebutine on the relationship between $\dot{V}_{max}$ and E_m. Lower panel: normalized curve of upper panel in which 1·0 in the ordinate corresponds to the maximum $\dot{V}_{max}$ measured in each condition.

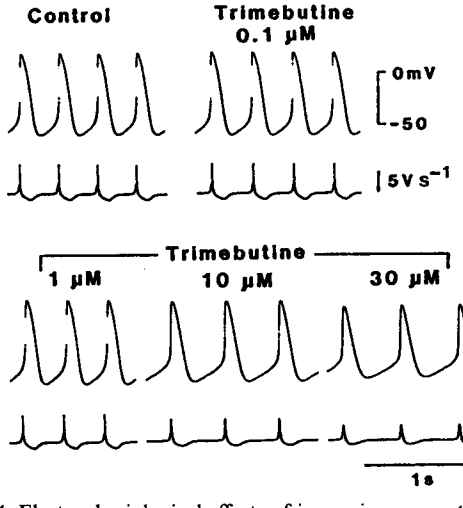

FIG. 4. Electrophysiological effects of increasing concentrations of trimebutine on the action potentials of rabbit sino-atrial node. Upper trace shows action potential and lower shows its first derivative.

significantly. These electrophysiological observations indicate that trimebutine possesses inhibitory actions not only on the fast Na^+ channel but also on the slow Ca^{2+} channel.

In isolated smooth muscle preparations of guinea-pig gall bladder, Takenaga et al (1984b) showed that trimebutine inhibited the contractile responses to cholinergic nerve stimulation and to acetylcholine. Takenaga et al (1984a) also demonstrated that trimebutine caused an inhibition of the spontaneous contraction in preparations of duodenum, ileum and colon from guinea-pigs and rabbits which exhibited marked contractile activity. The mechanism of trimebutine-induced effects on these preparations is probably associated with a decrease in the influx of calcium ion across the cell membrane and an inhibition of Ca-release from storage site (Furukawa & Kimoto 1984; Takenaga et al 1984a). In the present experiments, we have shown that trimebutine depressed the electrical activity of rabbit sino-atrial nodes and the slow action potentials of guinea-pig papillary muscles. Although any changes in intracellular calcium ions after perfusion of trimebutine remain unclear, it is at least indicated that the agent produces an inhibition of transmembrane influx of Ca^{2+} in myocardial cells.

Trimebutine has a chemical structure similar to that of local anaesthetics like procaine. However, no previous study on the effect of trimebutine on fast Na^+ channels has been carried out. Our results on fast action potentials of guinea-pig papillary muscles showed that trimebutine also depressed the fast Na^+ inward current and that this effect was similar to that of the so-called class I antiarrhythmic agents (Vaughan Williams 1970).

In summary, these observations suggest that trimebutine maleate modifies the electrophysiological properties of myocardial cells probably due to an inhibition of both fast Na^+ and slow Ca^{2+} channels. Although such a depressant effect of trimebutine on the heart may be secondary, this effect should be considered when using trimebutine experimentally in the clinic.

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