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The Role of Cyclic AMP and Phosphodiesterase Activity in the Mechanism of Action of Tetramethylpyrazine on Human and Dog Cardiac and Dog Coronary Arterial Tissues

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Abstract—The aim of the present experiments was to explore the underlying cellular mechanisms responsible for the actions of tetramethylpyrazine (TMP) on atrial, ventricular and coronary arterial tissues. Transmembrane potentials of cardiac tissues were detected by means of the glass microelectrode technique and contractile tension by a force transducer. Tissue cyclic (c) AMP level was determined by protein binding assay. Results show that in human atrial and dog Purkinje fibres, high concentration of TMP (3 mM) induced a persistent positive inotropic effect only in the presence of adrenaline. Also, 3 mM TMP increased the cAMP level of the atrial muscle fibres, especially in the presence of adrenaline. Determination of the activity of cAMP-phosphodiesterase revealed that 0·3 and 3 mM TMP inhibited the phosphodiesterase activity of dog coronary artery and human atrial tissues in a concentration-dependent manner. When compared at the lower concentration (0·3 mM), the inhibitory effect of TMP was about 60% that of theophylline. The above findings indicate that the cardiovascular effects of TMP are related to the inhibition of phosphodiesterase activity and the subsequent elevation of the cAMP concentration.

Tetramethylpyrazine (TMP), an active ingredient isolated from a popular Chinese herbal medicine *Rhizoma ligustici wallichii*, has been shown to increase coronary blood flow, inhibit contraction of arterial smooth muscles, decrease arterial blood pressure and reduce the myocardial ischaemia caused by vasopressin in experimental animals (Dai & Bache 1985; Peking Pharmaceutical Industries Laboratory 1978). The underlying mechanisms responsible for these pharmacological effects are not clear.

In our previous experiments, we have demonstrated that TMP (0·3–3 mM) induced a sustained increase in the contractile force of dog atrial muscle fibres (Chen et al 1987) but decreased force in dog Purkinje fibres after a brief initial increase (Chen et al 1988). In depolarized human atrial fibres obtained at cardiac surgery, TMP decreased contractile force in normal physiological salt solution (PSS) but induced sustained positive inotropic effect in the presence of adrenaline (Chen et al 1987). On the other hand, TMP relaxed contraction of vascular smooth muscle induced by high-K^+ or phenylephrine (Wu et al 1989; Kwan et al 1990). One possibility is that TMP may inhibit the destruction of cellular cyclic nucleotides with a subsequent elevation of the cyclic (c) AMP and increase in contractile force. An elevated cAMP level in vascular tissues would relax vascular smooth muscle as caused by methylxanthines. This effect would increase blood flow and thus explain the beneficial effects of TMP for myocardial and cerebral ischaemic syndromes (Dai & Bache 1985; Ho et al 1989).

The aim of the present experiments was to verify the above hypothesis on the cellular mechanisms responsible for the cardiovascular actions of TMP. Effects of TMP on the tissue level of cAMP and the activity of cAMP-phosphodiesterase were determined and compared with those of theophylline in dog isolated atrium and coronary artery, as well as in human atrial tissues. In addition, electromechanical effects of TMP were tested on dog well-polarized ventricular tissues in normal PSS as well as on partially depolarized ventricular tissues in high $[K^+]_o$ PSS.

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Materials and Methods

Tissue isolation

Mongrel dogs of either sex, 6–15 kg, were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.p.) and the heart was quickly excised. Strands of atrial and ventricular muscle and Purkinje fibres with a diameter of about 1 mm were dissected from the heart. Specimens of human atrial appendage were obtained from the hearts of 17 patients as part of the routine atriotomy procedure during open-heart surgery. Institutional rules for the protection of human subjects were observed. Before surgery, informed consent was obtained. The preparations were placed in a tissue bath perfused with PSS. The composition of our PSS in mM was NaCl 137, KCl 4, $NaHCO_3$ 12, NaH_2PO_4 0·5, $MgCl_2$ 0·5, $CaCl_2$ 2·7 and dextrose 5·5. This solution was oxygenated with a gas mixture of 97% O_2–3% CO_2, yielding a pH of 7·31 at 37°C. In the high $[K^+]_o$ solution, $[K^+]_o$ was increased to 27 mM and 0·55 μM adrenaline was added. The methods used for measuring action potential characteristics and twitch force have been described previously (Lin et al 1988).

Coronary artery experiments

The dog left coronary artery was transversely cut into rings about 3 mm wide and suspended on L-shaped hooks in a 10-mL organ bath containing oxygenated PSS. An initial tension of 2 g was applied to each arterial ring. The rings were allowed to equilibrate for 2 h before drugs were added to the bath. Changes in tension were recorded with a Grass FT 03C transducer connected to a Grass 7B polygraph as described previously (Lin et al 1982). Sustained tonic contraction was

induced by $PGF_{2\alpha}$ (1-6 μM) and the relaxant effect of compounds was evaluated by cumulative addition.

Determination of cyclic nucleotide content

To determine effects of TMP (Aldrich Chemical, USA) on cellular content of cyclic nucleotides in cardiac tissues, preparations (10–30 mg) were divided randomly into control and experimental groups and incubated in beakers containing 50 mL PSS with or without 0·3 μM adrenaline. The period of drug exposure was 10 min and then the preparation was frozen with dry ice within 10 s and kept at −70°C.

Methods for the measurement of cyclic nucleotides in tissue have been previously described in detail (Lin et al 1989). cAMP and cGMP were assayed using Amersham's cAMP assay kit and Amersham's cGMP ^{125}I assay kit, based on a protein binding assay for cAMP and radioimmunoassay for cGMP (Gilman 1970; Steiner et al 1972).

Determination of cAMP-phosphodiesterase activity

To determine effects of TMP on the activity of cAMP-phosphodiesterase, the atrial and coronary arterial preparations were homogenized in ice-cold solution containing 40 mM Tris HCl (pH = 7·5), 0·25 M sucrose and 0·1 mM EDTA, and then diluted to a concentration of 0·5–0·7 mg protein mL^{-1}. The atrial and coronary arterial preparations from the same subject were randomly divided into control and experimental groups. In the experimental groups, 0·3 or 3 mM of either TMP or theophylline were added into the assay mixture. cAMP-phosphodiesterase activity was assayed using the two-step procedure of Thompson et al (1974) with batch-wise application of the anionic exchange resin Dowex 1 × 2 in twice-distilled water. The assay mixture contained 40 mM Tris HCl (pH = 8·0), 3·75 mM 2-mercaptoethanol, 5 mM $MgCl_2$ and enzyme preparation (100–140 μg protein). The reaction was initiated by addition of the substrate [^{3}H]cAMP (~200 000 counts min^{-1}) and unlabelled cAMP (0·125 μM) to make a total reaction volume of 500 μL, and incubated for 10 min at 30°C. The reaction was terminated by immersing the reaction tubes in a dry ice–acetone bath until frozen (12 s), followed by subsequent immersion into a boiling water bath for exactly 45 s. The 5′-AMP product was then converted to the corresponding nucleoside by a second incubation with 0·1 mL of 5′-nucleotidase of snake venom (*Ophiaphagus hannah*) (1 mg mL^{-1}) for 10 min at 30°C. The anionic exchange resin (Dowex 1 × 2) was used to isolate [^{3}H]adenosine. Protein content was determined according to Lowry et al (1951), using bovine serum albumin as standard. Data were means of triplicate determinations. Values obtained in the absence of experimental drugs were equated to 1 and those obtained in the presence of TMP or theophylline were expressed as a fraction (mean ± s.e.) of the control values. Statistical analysis was performed with Student's *t*-test.

Results

Effects of TMP on dog ventricular tissues

In dog ventricular muscle fibres, 1–10 mM TMP increased significantly the force of contraction but shortened action potential duration (APD). In six experiments, APD at 50% repolarization (APD50) was reduced from 191 ± 4 to 154 ± 7 ms (−37 ± 6 ms, $P < 0·01$) in the presence of 10 mM TMP. The steady-state increments in force induced by 1, 3 and 10 mM TMP were 25 ± 6, 42 ± 10 and 150 ± 27% ($P < 0·01$), respectively, in seven experiments.

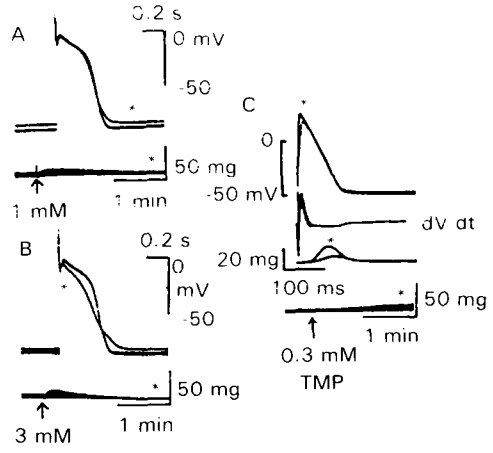

FIG. 1. Effects of TMP on action potential and twitch tension of a Purkinje fibre preparation in 4 mM $[K^+]_o$ PSS (A and B) and in 27 mM $[K^+]_o$ PSS containing 0·55 μM adrenaline (C). In each panel, the bottom trace is the slow-speed chart recording of contractions. TMP was added at the arrow. In A and B, action potentials before and during the third minute of TMP exposure (asterisks, 1 and 3 mM, respectively) are superimposed. In C, action potential, its first derivative and twitch curve before and at the end of the second minute of TMP exposure (asterisks) are superimposed.

In contrast, TMP induced biphasic inotropic responses in dog Purkinje fibres as reported previously (Chen et al 1988). Fig. 1A and B show the effects of TMP on the fast action potential and twitch force of a dog Purkinje fibre preparation driven at 1 Hz in normal $[K^+]_o$ (4 mM) PSS. TMP depressed the amplitude and the plateau of action potential and shortened APD50, but prolonged APD90 as the force declined after a brief initial increase. The concentration-response data for percentage changes in action potential characteristics induced by 0·03–3 mM TMP in 14 preparations is summarized in Fig. 2A.

When the Purkinje fibre was depolarized (maximum diastolic potential (MDP) near −40 mV) in 27 mM $[K^+]_o$ in the presence of 0·55 μM adrenaline, the upstroke velocity and the

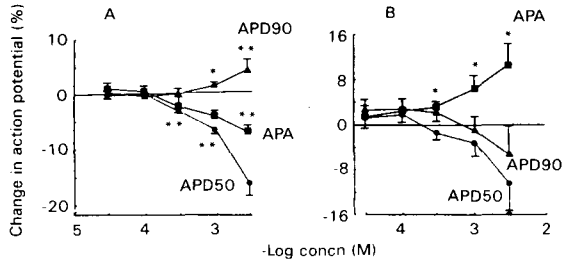

FIG. 2. Effects of TMP (0·03–3 mM) on the action potential characteristics of dog Purkinje fibres in 4 mM $[K^+]_o$ PSS (A) and in 27 mM $[K^+]_o$ PSS containing 0·55 μM adrenaline (B). The abscissa shows the logarithmic concentration of TMP. Data (mean ± s.e.) were expressed as % change from the control values in the absence of TMP. APA, action potential amplitude. APD50 and APD90, action potential duration at 50 and 90% repolarization respectively. $*P < 0·05$, $**P < 0·01$ compared with control values. The average control maximum diastolic potential of 14 preparations in A was −89·3 ± 3·7 mV, that of 13 preparations in B was −40·2 ± 3·4 mV.

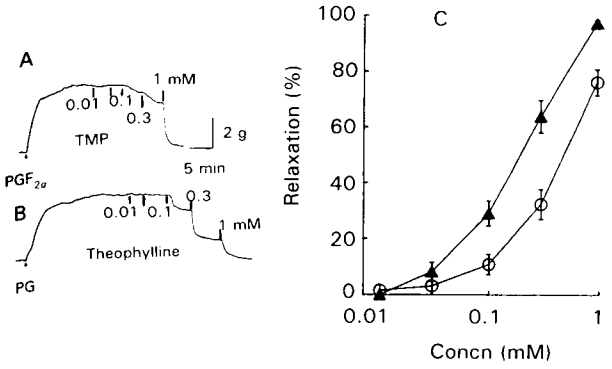

FIG. 3. Relaxant effects of cumulative additions of TMP (A) and theophylline (B) on contractions of a dog coronary arterial ring. At the arrowhead, contraction was induced by 2 μM $PGF_{2\alpha}$. C shows the cumulative concentration-response curves for the relaxant effect of TMP (○, n = 8) and theophylline (△, n = 5). Data (mean ± s.e.) were expressed as % of maximum relaxation. *$P < 0.01$ by Student's t-test between groups (TMP vs theophylline).

amplitude of slow-response action potential and the force were progressively increased by 0·3 mM TMP (Fig. 1C). In 13 preparations, the amplitude of slow-response action potential was significantly increased in the presence of 0·3–3 mM TMP, while the APD50 was reduced only at the highest concentration (3 mM) of TMP (Fig. 2B). The concentration-dependent increase in the amplitude of slow-response action potential indicates an enhanced transmembrane Ca influx (Sperelakis 1985) induced by TMP.

Relaxant effect of TMP on dog coronary artery

Relaxant effect of TMP (0·01–1 mM) on the contraction of coronary arterial ring induced by $PGF_{2\alpha}$ was studied and compared with that of theophylline. As shown in Fig. 3, the relaxant effect of TMP was less potent than that of theophylline. In eight preparations, the average concentration for TMP required for half maximum relaxation (ED50) of a contraction induced by $PGF_{2\alpha}$ was 0·491 ± 0·062 mM. This value was significantly ($P < 0.05$) higher than the average ED50 for theophylline (0·206 ± 0·039 mM, n = 5).

Effects of TMP on tissue levels of cyclic nucleotides

Since the effects of TMP on cardiac and vascular tissues were similar to those of methylxanthines (Lin et al 1982, 1987), it is important to determine whether TMP produced a similar change in the cellular contents of cyclic nucleotides (Beavo et al 1970). As shown in Table 1, 3 mM TMP significantly increased the cAMP content of the human and dog atrial muscle fibres, especially in the presence of 0·3 μM adrenaline. However, no significant change in cGMP content was observed, in contrast to the effects of 0·3 mM theophylline (Lin et al 1989). Also, as compared with human atria, the increase in cAMP content induced by 3 mM TMP (+31%) was significantly less than that induced by 0·3 mM theophylline (Lin et al 1989).

Effects of TMP on the activity of cAMP-phosphodiesterase

As shown in Table 2, TMP inhibited the activity of cAMP-phosphodiesterase of dog coronary artery and human atrial tissues in a concentration-dependent manner. When compared at the lower concentration (0·3 mM), the inhibitory effect of TMP was about 60% that of theophylline. TMP also inhibited the cAMP-phosphodiesterase activity of dog atria although the inhibition was smaller than those observed in human atria and dog coronary artery.

Discussion

Mechanism of TMP action

Results of determinations of cAMP content and phosphodiesterase activity support our hypothesis that the positive inotropic and chronotropic effects of TMP observed in spontaneously depolarized (MDP around −50 mV) human atrial tissues in the presence of adrenaline (Chen et al 1987) are consequences of an elevation of cAMP level secondary to the inhibition of phosphodiesterase activity. The Ca^{2+}-dependent slow-response action potential developed at a reduced MDP (around −40 mV) is regulated by intracellular cAMP (Sperelakis 1985). Thus, the enhanced slow-response action potential and the sustained positive inotropic effect induced by TMP on high $[K^+]_o$-depolarized Purkinje fibres in the presence of adrenaline also indicates an elevation of cAMP level induced by TMP. The relaxant effect of TMP on dog coronary artery could likewise be due to the same mechanism, as an elevation of cAMP has been related to the relaxing effects of β-adrenergic agents and phosphodiesterase inhibitors (Kramer & Hardman 1980).

TMP could also induce effects through mechanisms other than that on phosphodiesterase as suggested in our previous observations in dog Purkinje fibres (Chen et al 1988). In

Table 1. Effect of TMP on cAMP and cGMP content (pmol (mg protein)$^{-1}$) of cardiac tissues in the absence and presence of adrenaline.

	cAMP		cGMP	
Dog atria	Without adrenaline	With adrenaline (0·3 μM)	Without adrenaline	With adrenaline (0·3 μM)
n	9	10	5	5
Control	6·28 ± 0·40	7·70 ± 0·59	0·514 ± 0·127	0·678 ± 0·165
TMP (3 mM)	6·80 ± 0·45	10·77 ± 0·86*	0·489 ± 0·108	0·650 ± 0·142
Human atria†				
n	12	11	7	7
Control	5·86 ± 0·75	6·78 ± 1·03	0·351 ± 0·063	0·349 ± 0·026
TMP (3 mM)	7·69 ± 0·69*	9·16 ± 1·26*	0·387 ± 0·056	0·373 ± 0·037

n, number of preparations; values are mean ± s.e.; $P < 0.01$ by Student's t-test between groups (control vs TMP). † In seven preparations, 3 mM TMP did not significantly change the force of contraction in physiological salt solution but increased the force by 87 ± 33% ($P < 0.05$) in the presence of 0·3 μM adrenaline.

Table 2. Effects of TMP and theophylline on cAMP-phosphodiesterase activity of isolated atrial and coronary arterial tissues.

		TMP		Theophylline	
Tissues	n	0·3 mM	3 mM	0·3 mM	3 mM
Human atria	5	0·743±0·036	0·394±0·021	0·566±0·028	0·253±0·015
Dog atria	12	0·932±0·011	0·765±0·043	0·859±0·022	0·476±0·039
Dog coronary artery	6	0·755±0·025	0·412±0·011	0·567±0·010	0·327±0·036

[^{3}H]cAMP (0·034 mM) and cAMP (1·36 μM) were used as substrates. n, number of preparations. Values obtained in the absence of TMP or theophylline were equated to 1. Those obtained in the presence of drugs were expressed as a fraction (mean±s.e.) of the control values. $P<0·01$ by Student's t-test for all values in the experimental groups.

normal [K^+] PSS, TMP has been shown to enhance initially the release of Ca^{2+} from intracellular stores as did methylxanthines (Beavo et al 1970), and then progressively decrease the intracellular Na^+ activity, thus inducing biphasic inotropic effects (Chen et al 1988). When the Purkinje fibres were depolarized in high $[K^+]_o$ plus adrenaline, Na^+ channels would be inactivated at a maximum diastolic potential near −40 mV (Noble 1979) and presumably no further decrease in intracellular Na^+ was induced by TMP exposure. The inhibition of cAMP-phosphodiesterase activity would potentiate the effects of adrenaline and induce sustained positive inotropy. Whether TMP induced similar effects on the intracellular Ca^{2+} store and intracellular Na^+ activity in arterial smooth muscle awaits further experiments.

Clinical implications

The present results show that TMP induces a relaxing effect on dog coronary artery with an ED50 about 2·4 times that of theophylline. Measurements of cAMP-phosphodiesterase activity also show that TMP was about 60% less potent than theophylline as an inhibitor of phosphodiesterase. In human atrial fibres showing slow-response action potential, however, TMP could induce a positive inotropic effect and accelerate automatic rhythms, but only at high concentrations (0·3–3 mM) and in the presence of 0·1 μM adrenaline (Chen et al 1987). These stimulatory effects were also less potent than those of theophylline (Lin et al 1987, 1989). Nevertheless, when TMP is used for the treatment of myocardial or cerebral ischaemic syndromes, its serum concentration should be kept below 0·3 mM to avoid the possible hazardous effects on the heart. It would appear that although the mechanism of action of TMP is appropriate, its potency falls short of clinical usefulness.

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