

Ocular and cardiovascular pharmacology of tetramethylpyrazine isolated from *Ligusticum wallichii* Franch

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ABSTRACT Tetramethylpyrazine (TMP), the active principle in *Ligusticum wallichii*, *D*-timolol, and *L*-timolol were compared for their effects on retinal and choroidal blood flow (RCBF), blood pressure (BP), and heart rate (HR). TMP (10 mg · kg⁻¹) increased RCBF by 44% and did not affect systemic BP or HR. *D*-Timolol (4 mg · kg⁻¹ iv) had a tendency to increase RCBF but did not affect systemic BP and HR either. *L*-Timolol (0.4 mg · kg⁻¹ iv), on the other hand, decreased RCBF by 18% and reduced both systemic BP and HR. In isolated preparations, TMP increased coronary artery blood flow with slight vasodilation, but vasoconstriction in renal, femoral and mesenteric arteries. These results indicate that TMP could be used to prevent or alleviate certain ischemic retinal degenerations without producing significant cardiovascular side effects.

The *in vitro* vasoconstriction actions of TMP (0.2 mg · ml⁻¹) were blocked by propranolol and phenoxybenzamine, indicating that adrenergic mechanism might be involved in TMP action.

KEY WORDS eye; cardiovascular agents; tetramethylpyrazine; retinal vessels; choroid; blood flow velocity

The retina is supplied with oxygen and nutrients by two vascular systems, one within the retina itself and one in the choroid. Interruption or impairment of either system leads immediately to degeneration of the retina and ultimately to loss of vision. There are many

diseases and conditions that affect retinal and choroid circulation. These include diabetes mellitus, sickle cell retinopathy, hypertensive and atherosclerotic vascular diseases, retinal vein occlusion, and glaucoma^(1,2). Improvement in blood flow to the retina and choroid early in some of these diseases and throughout the time course of others may be the key to slowing vision loss or eliminating it altogether. Any drug, given systemically or given locally to the eye, that treats a symptom of a disease but decreases ocular circulation is actually causing more damage than it appears to be alleviating.

For example, the ultimate goal in treating glaucoma is to improve retinal and choroidal circulation by decreasing intraocular pressure (IOP). Since some patients show retinal degeneration with low IOP and many others have abnormally increased IOP with no retinal degeneration, the correlation between IOP and circulation in the retina and choroids is tenuous. A drug may lower IOP and, at the same time, decrease retinal and choroidal blood flow (RCBF), leading to the false conclusion that the glaucoma is under control while in fact the retinal and optic nerve damage continues^(3,4). It may actually be more efficacious to treat glaucoma with drugs that increase RCBF than with drugs that merely lower the IOP.

A drug that could be administered topically and/or systemically to increase RCBF would slow down certain ischemic retinal degenerations. In China, *Ligusticum wallichii* has been used to increase coronary

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blood flow, improve systemic circulation and relieve stasis⁽⁵⁻⁸⁾. They are also used for improving vision without actual knowledge of how they work. This study compares tetramethylpyrazine (TMP) with the widely used antiglaucoma drug timolol for effects on ocular circulation, especially RCBF. Some systemic effects of TMP are also investigated.

MATERIALS AND METHODS

Materials Tetramethylpyrazine HCl (TMP) was purchased from Fluka Chemika (Buchs, Switzerland). Phenoxybenzamine (Smith Kline & French Labs, PA, USA), propranolol (Ayerst Labs, NY), *D*-timolol maleate and *L*-timolol maleate (Merck Sharp and Dohme, PA) were generous gifts from the manufacturers.

Laser Doppler method The laser Doppler principle to monitor the perfusion of red blood cells in the microcirculatory bed of the retina and choroid⁽⁹⁾. Basically, the frequency of light back-scattered from moving erythrocytes is shifted (Doppler shift) in proportion to the velocity of cells. A beam of concentrated light is focused onto the retina. The reflected light is guided through an optical fiber onto a photo detector. The flow calculation is dependent on the average velocity and the density of the cells in the measured volume. The resulting photocurrent, which contain the desired information on the speed of erythrocytes, is processed by means of a frequency-to-voltage converter. The output of the processing unit can be displayed both digitally and as a record tracing.

Rabbits New Zealand white ♀ rabbits weighing 3.0–3.5 kg were anesthetized with iv pentobarbital sodium 30 mg · kg⁻¹, followed 1 h later with iv urethane 0.5 g · kg⁻¹. Additional urethane was given throughout the experiment depending on the depth of anesthesia. With these anesthetics the eyes were immobilized sufficiently for measurement

of retinal blood flow using a laser Doppler (LD 5000 Capillary Perfusion Monitor, Med Pacific Corporation, Seattle WA, USA). The femoral artery and vein were cannulated for monitoring systemic blood pressure (BP) (RP-1500 Pressure Transducer and Model 1VA Physiograph, Narco Biosystems, Houston TX) and for injection of urethane and drugs. Heart rates (HR) were counted directly from the BP recordings. Drug solutions were adjusted to 1.5 ml for injection and were injected either slowly over a period of 60 s or quickly as a bolus. Control experiments were done by injecting the same volume of saline. The experiment was run for at least 60 min after drug administration and readings of RCBF, systemic BP and HR were taken at 10-min intervals. Area under curve (AUC) was computed with a Hipad Digitizer (Houston Instrument) connected to an IBM Personal Computer XT.

⁸⁵Sr-microsphere technique A radio-labelled microsphere technique^(10,11) was used to determine blood flow to various eye tissues. New Zealand white rabbits weighing 2.5–3.5 kg were anesthetized with iv pentobarbital sodium 30 mg · kg⁻¹. The left femoral artery was cannulated with PE 50 tubing; PE 90 tube was inserted into the left ventricle through the right carotid artery. Heparin (1000 IU) was injected iv to prevent clotting. The blood flow was determined by injecting microspheres labelled with ⁸⁵Sr (15.6 ± 0.8 μm sphere diameter, 3M Company, St Paul MN) into the left ventricle. The microspheres were suspended in 0.9% saline containing 1% Tween 80. The quantity of microspheres injected was approximately 1 × 10⁶ (in 0.18–0.20 ml) per rabbit. The blood was collected from the femoral artery cannula for 60 s at 5 s after the injection of microspheres. The rabbit was killed by iv 5 ml saturated KCl solution. The eyes were removed quickly and the iris, ciliary body,

retina, and choroid isolated. All tissues were weighed and their radioactivities determined with a Packard Auto-Gamma 500 (Packard Instruments Co., Downers Grove IL). The blood flow in the tissues was calculated using the rate of blood flow through the femoral artery and its radioactivity as reference: $F = Q \times d'/d$ where, F = blood flow of the tissue; Q = blood flow of femoral artery; d' = dpm/mg of tissue; d = dpm/ μ l of blood collected from the femoral artery. The blood flow rate was expressed as μ l \cdot min⁻¹ \cdot (mg tissue)⁻¹. Drug solutions were injected as described in previous section.

Systemic BP and HR Rats of either sex weighing 290–360 g were anesthetized with iv urethane 1 g \cdot kg⁻¹. The left carotid artery was cannulated for BP measurement. The right jugular vein was cannulated for drug injection.

Female albino rabbits weighing 2.3–3.2 kg were anesthetized with iv urethane 1 g \cdot kg⁻¹. The left femoral artery was cannulated for measurement of BP. The left femoral vein was cannulated for drug injection. HR were taken from the BP recording.

Langendorff heart preparation Guinea pigs of either sex weighing 240–350 g were stunned by a blow on the head. The heart with aortic arch was excised quickly and hung on the Langendorff apparatus following the method of Trzeciakowski and Levi⁽¹²⁾. The heart was perfused with Ringer-Locke solution (NaCl 154; KCl 5.6; CaCl₂ 2.1; NaHCO₃ 6.0; glucose 5.6 nmol \cdot L⁻¹) maintained at 37°C and oxygenated with 95% O₂+5% CO₂. The perfusion pressure was set at 5.07–5.33 kPa (38–40 mm Hg). A polyethylene cannula was introduced into the left ventricle for monitoring left ventricle systolic pressure with a Statham P23 pressure transducer.

Isolated vasculatures Female albino rabbits weighing 2.2–3.0 kg were stunned and

exsanguinated. The heart was removed and the interventricular branches of the left epicardial coronary artery (with outside diameter of 0.5–0.8 mm) isolated. Renal, mesenteric, and femoral arteries were cut into ring segments 4 mm long and suspended individually in organ baths. Each vessel was bathed in 30 ml Krebs' solution (NaCl 119; KCl 4.7; NaHCO₃ 23; NaHPO₄ 1.2; CaCl₂ 1.8; MgCl₂ 1.2, and dextrose 7.9 mmol \cdot L⁻¹) oxygenated with 95% O₂+5% CO₂ and maintained at 37°C. The initial tension was adjusted to 1.0 g and each preparation was equilibrated in the organ bath for 60–80 min before initiation of experiments. During the equilibration period, Krebs' solution was replaced every 15–20 min. Muscle contractility was recorded with a Gould VC2 force-displacement transducer on a Gould 2400 S recorder.

Statistical analysis of data Every value was expressed as $\bar{x} \pm$ SD. All data were analyzed with *t* test or analysis of variance.

RESULTS

When retinal blood flow was measured with the laser Doppler technique, the control reading with saline injection were very stable for up to 60 min. Longer exposure to the laser beam coupled with no blinking tended to dehydrate the cornea. Therefore, continuous measurement of RCBF was limited to 1 h only. RCBF was increased markedly ($44 \pm 38\%$, $n = 5$) by TMP (10 mg \cdot kg⁻¹). *D*-Timolol had a tendency to increase RCBF ($P > 0.05$). *L*-Timolol actually decreased RCBF significantly ($-18 \pm 4\%$, $n = 5$). The amount of increase in RCBF caused by TMP is remarkable. The RCBF reached a maximum plateau 20 min after TMP administration and remained there throughout the experiment.

The blood flow to various eye tissues was measured with a ⁸⁵Sr-microsphere technique

(Fig 1). TMP increased blood flow significantly to iris (112.3%), ciliary body (55.5%), retina (60.0%), and choroid (36.1%).

L-Timolol (0.4 mg · kg⁻¹ iv) reduced systemic BP (Tab 1) and HR significantly (Tab 1). TMP, when administered slowly over a period of 1 min, did not affect BP or HR, indicating that the increase in RCBF is independent of these two factors.

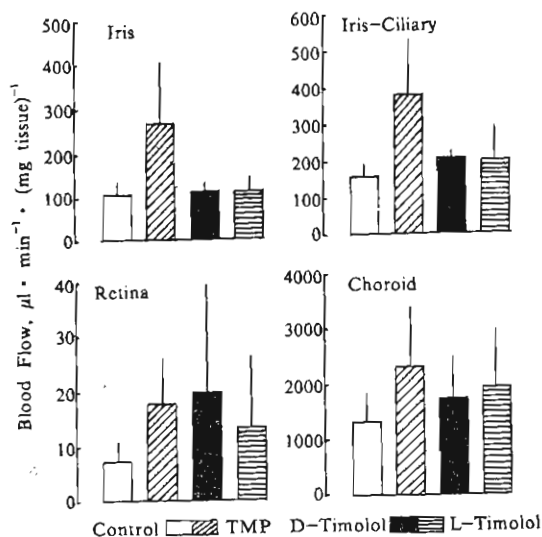


Fig 1. Effects of TMP, *D*-timolol, and *L*-timolol on blood flow in the eye measured with microsphere technique. *n* = 5 experiments, $\bar{x} \pm SD$. **P* > 0.05, ***P* < 0.05.

Tab 1. Effects of TMP, *D*-timolol and *L*-timolol on blood pressure, heart rate and retinal and choroidal blood flow (RCBF). *n* = 5. $\bar{x} \pm SD$. **P* > 0.05, ***P* < 0.05 vs controls.

Drug	Blood pressure (kPa)		Heart rate (Beat/min)		RCBF Changes, %
	Before	After	Before	After	
TMP (10 mg · kg ⁻¹ iv)	13.7 ± 4.0 10.0 ± 2.0	13.5 ± 3.3 9.7 ± 2.7	290 ± 45	296 ± 43*	+52%**
<i>D</i> -Timolol (4 mg · kg ⁻¹ iv)	12.9 ± 2.0 10.1 ± 2.4	11.9 ± 1.7 10.0 ± 2.4	311 ± 25	270 ± 52*	+9%*
<i>L</i> -Timolol (0.4 mg · kg ⁻¹ iv)	12.5 ± 2.7 10.1 ± 2.4	10.3 ± 2.9 8.1 ± 2.0**	377 ± 48	243 ± 50**	-17%**

Fig 2 shows effects of TMP on isolated perfused heart of the guinea pig. TMP (5 mg/injection) increased the coronary blood flow significantly for 3 min after the bolus injection. No obvious changes in HR and contractility were noted throughout the experiment.

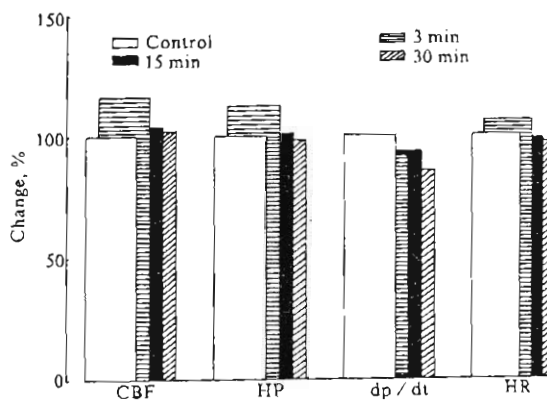


Fig 2. Effects of TMP on isolated guinea pig heart. CBF = coronary blood flow; HP = intraventricular pressure; HR = heart rate. *n* = 5, ***P* < 0.05.

TMP (0.2 mg · ml⁻¹) induced a slight vasodilation in the coronary artery but constricted renal, mesenteric, and femoral arteries in rabbit isolated vasculatures (Tab 2). TMP-induced vasodilation of the coronary artery was attenuated by propranolol (1 µg · ml⁻¹); vasoconstriction of the other vessels

Tab 2. Adrenergic effects of TMP on various isolated blood vessels. ** $P < 0.05$ vs controls.

TMP (0.2 mg · ml ⁻¹) plus	n	Tension of isolated vessels (mg)			
		Coronary artery	Renal artery	Mesenteric artery	Femoral artery
-	5	-88 ± 24	511 ± 65	141 ± 35	717 ± 59
+ phenoxybenzamine (1 µg · ml ⁻¹)	5	-	-106 ± 37**	-41 ± 12**	-306 ± 77**
+ propranolol (1 µg · ml ⁻¹)	5	-11 ± 2**	-	-	-

was reversed by phenoxybenzamine (1 µg · ml⁻¹) (Tab 2). These results indicate that TMP acts at least partially through activation of adrenergic mechanisms.

DISCUSSION

TMP is one of the active principles isolated from *L. wallichii*^(5,6). The sustained increase of RCBF caused by TMP could be very important for treating ischemic retinal degeneration^(1,2). While other drugs, such as epinephrine, increase RCBF markedly, the effect is short and the increase seems to depend on an increase in systemic BP, an undesirable side effect⁽¹³⁾. TMP given systemically does not seem to affect BP or HR.

L-Timolol affects circulation to both the eye and the heart. The decrease in RCBF caused by this drug, coupled with its definite side effects on BP and HR⁽¹⁴⁾, makes its actual efficacy in treating glaucoma questionable. It is possible that IOP can be reduced to a "normal range" by *L*-timolol yet the RCBF is also reduced. As a result, patient and doctor could develop an impression that the disease is well under control yet the glaucoma could actually be exacerbated.

TMP would appear to be worthy of continued study as a useful drug for treating retinopathies resulting from reduced blood flow in the eye. Several aspects to study are its safety and therapeutic indices, and its mechanism of action.

In guinea pig isolated heart preparations, a bolus injection of TMP caused a significant increase in coronary flow within 3 min. The intraventricular pressure, dp/dt and heart rate were not significantly affected by TMP at any dose tested. These results indicate that TMP increases coronary blood flow temporarily without affecting other cardiac functions.

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l-Tetrahydropalmatine increases leucine enkephalin levels in corpus striatum of rats¹

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ABSTRACT The effect of chronic *l*-tetrahydropalmatine (*l*-THP) administration on the level of leucine enkephalin (Leu-Enk) in rat corpus striatum was studied. After *l*-THP sc injection once daily for 2 wk, the striatal Leu-Enk level was elevated dose-dependently. However, a single injection of *l*-THP failed to change the Leu-Enk level. When rats received sc Sch-23390, a selective D₁ antagonist, 15 nmol · kg⁻¹ tid for 2 wk, striatal content of Leu-Enk increased from 0.17 ± SD 0.03 ng · mg⁻¹ tissue in control group to 0.23 ± SD 0.05 ng · mg⁻¹ tissue in Sch-23390 group (*n* = 8, *P* < 0.05). Sulpiride (Sul), a selective D₂ antagonist, 140 μmol · kg⁻¹ sc given bid for 2 wk had no significant effect on the striatal Leu-Enk content. The results

suggested that the blockade of D₁ receptors by *l*-THP might be responsible for the increase of the striatal Leu-Enk content in rat.

KEY WORDS *l*-tetrahydropalmatine; Sch-23390; sulpiride; dopamine receptor blockers; leucine enkephalin; corpus striatum

Repeated administration of dopamine (DA) antagonists, endowed with major tranquilizing activity, results in increases in striatal proenkephalin mRNA and enkephalins, indicating a functional relation between the DA and enkephalin system⁽¹⁾. *l*-Tetrahydropalmatine (*l*-THP) is a potent sedative-tranquilizing agent which has been used in China in clinical alleviation of pain and anxious insomnia⁽²⁾. Recently *l*-THP and its

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