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Effects of tetramethylpyrazine on heart rate and pupil diameter in rats

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ABSTRACT The α–adrenergic nature of tetramethylpyrazine (TMPZ), a commonly used cardiovascular drug in China, was studied with a combined bradycardia and mydriasis model in the pentobarbital anesthetized rat. Intravenous injections of TMPZ at 1–30 mg / kg failed to change heart rate or pupil size, whereas iv injections of both clonidine (α_2–adrenoceptor agonist, 1–30 μg / kg) and methoxamine (α_1–adrenoceptor agonist, 10–300 μg / kg) caused dose–dependent bradycardia and mydriasis. Pretreatment of rats with TMPZ (30 mg / kg) did not significantly change clonidine– or methoxamine–induced bradycardia or mydriasis. However, when TMPZ was injected 2 min after clonidine administration (30 μg / kg), while the mydriasis reached a maximum, it slightly but significantly reversed the clonidine–induced mydriasis for 15–20 s. In contrast, the α_2–adrenoceptor antagonist idazoxan (6 μg / kg) caused the same degree of reduction of the clonidine–induced mydriasis for >2 min. Our results suggested that TMPZ at the doses studied did not have α–adrenoceptor agonistic activities, but may have slight α_2–adrenoceptor antagonistic activities.

KEY WORDS tetramethylpyrazine; clonidine; methoxamine; idazoxan; heart rate; pupil

Tetramethylpyrazine (TMPZ) is an alkaloid found in *Ligusticum wallichii Franch*[1] and *jatropha podagrica*[2] as well as a metabolite from *Bacillus sultitis*[3]. TMPZ causes vasodilation[4] and reduces thrombosis[5]. These probably are the reasons why it is used for the treatment of angina pectoris[6], and to improve cerebral microcirculation[7].

In a recent study attempting to explore the mechanisms by which TMPZ causes vasodilation and reduces thrombosis, it was found that TMPZ inhibited [^{3}H]yohimbine binding in intact human platelets in a concentration–dependent manner[8]. In addition, TMPZ inhibited the cAMP increases induced by PGE_1. However, TMPZ also antagonized the inhibitory effect of *l*–epinephrine, an adrenoceptor agonist with α_2–activity, on PGE_1–induced cAMP increases. Bases on the above findings, it was concluded that TMPZ is an α_2–adrenoceptor partial agonist[8].

In this study, we used a combined bradycardia and mydriasis model in the rat to further explore the α–adrenergic nature of TMPZ. This method has been used dependably and easily to study the effects of both α_1– and α_2–adrenoceptor agonists and antagonists[9–11].

MATERIALS AND METHODS

Animal preparations Male Sprague–Dawley rats weighing 250–350 g were anesthetized with pentobarbital sodium (60 mg / kg, ip, Fort Dodge Laboratories, USA). A 27–gauge, 1.27 cm needle was placed in the tail vein and secured with adhesive tape for drug administrations. The heart rates of rats were then allowed to stabilize, generally taking 10–20 min. The heart rate measurements were taken at 1 min postinjection using Lead II of electrocardiogram and displayed on a multiple–channel recorder (Beckman R611, USA). The pupil diameter measurements were taken using a calibrated operating microscope with a green filtered external light source. All observations were made under the same low lighting conditions.

Treatment procedures When the possible dose–responses of TMPZ, methoxamine (α_1–agonist), and clonidine (α_2–agonist) were

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studied, they were administered in cumulative dosages with a 2-min interval between the doses. When TMPZ or idazoxan (α_2-antagonist)[12] was given to prevent the effects of clonidine, it was administered 5 min before the first dose of clonidine. When TMPZ or idazoxan was given to reverse the effects of clonidine, it was administered 2 min after a single dose of clonidine (30 μg / kg) with a 1-min interval between TMPZ or idazoxan doses. Controls received 1 ml / kg of NaCl 0.15 mol / L.

Drug preparations TMPZ (Fluka Chemical, FRG) was dissolved in 3 % lactic acid, whereas clonidine-HCl (Boehringer Engelheim, USA) methoxamine HCl (Burroughs Wellcome, USA), and idazoxan (Reckitt and Colman, UK) were dissolved in NaCl 0.15 mol / L. All doses were calculated on the basis of weight of drug base.

Statistical analysis The data were analyzed by analysis of variance using a split-plot design[13]. Differences between groups at the corresponding dose of an agonist or antagonist were assessed by use of the Least Significant Difference Test[13]. In all analyses, statistical significance at the $P < 0.05$ level was accepted.

RESULTS

Lack of an effect of TMPZ on heart rate and pupil Intravenous injections of TMPZ at 1, 3, 10, and 30 mg / kg failed to change heart rate or pupil size (Tab 1). Doses of TMPZ > 30 mg / kg were lethal, and rats usually die from respiratory failure.

Tab 1. Effects of iv TMPZ (in cumulative doses a 2-min intervals) on heart rate and pupil. $n = 4$, $\bar{x} \pm SD$ $^*P > 0.05$ *vs* **baseline.**

TMPZ (mg / kg)	Heart rate (Beats / min)	Pupil diameter (mm)
Baseline	363 ± 36	0.40 ± 0.12
1	364 ± 40*	0.41 ± 0.10*
3	364 ± 46*	0.40 ± 0.08*
10	355 ± 50*	0.38 ± 0.06*
30	371 ± 34*	0.35 ± 0.06*

Lack of an antagonism of TMPZ on clonidine- and methoxamine-induced bradycardia and mydriasis. Intravenous injections of clonidine (1, 3, 10, and 30 μg / kg) elicited a dose-dependent mydriasis that was significant at 3 μg / kg or greater, and reached a maximum after a cumulative dose of 30 μg / kg (Tab 2).

Following each clonidine injection, mydriasis was initiated within 5 s, if it was seen at all. The mydriasis reached a maximum within 2 min after the clonidine administration. When clonidine was administered at 30 μg / kg, the mydriasis was sustained for > 15 min. Intravenous injections of clonidine also elicited a dose-dependent bradycardia that was significant at ≥ 3 μg / kg. When given 5 min before the first dose of clonidine, TMPZ (30 mg / kg) failed to alter the mydriatic or bradycardic effects of

Tab 2. Effects of TMPZ 30 mg / kg on clonidine-induced bradycardia and mydriasis. NaCl (0.15 mol / L) 1 ml / kg. Clonidine was given in cumulative doses at a 2-min interval. $n = 5$, $\bar{x} \pm SD$. $^*P > 0.05$, $^{**}P < 0.05$ *vs* **pretreatment.**

Clonidine (μg / kg)	Heart rate (Beats / min)		Pupil diameter (mm)	
	NaCl	TMPZ	NaCl	TMPZ
Baseline	406 ± 36	422 ± 38	0.46 ± 0.09	0.62 ± 0.16
Pretreat	394 ± 38	422 ± 51	0.46 ± 0.09	0.66 ± 0.22
1	373 ± 31*	380 ± 65*	0.60 ± 0.11*	0.70 ± 0.22*
3	310 ± 36**	348 ± 65**	1.40 ± 0.51**	1.02 ± 0.33**
10	281 ± 29**	299 ± 40**	3.10 ± 0.94**	2.80 ± 0.49**
30	270 ± 38**	286 ± 38**	3.64 ± 0.20**	3.38 ± 0.22**

Tab 3. Effects of TMPZ 30 mg / kg on methoxamine-induced bradycardia and mydriasis. Methoxamine was given iv in cumulative doses at a 2-min interval. $n=6$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$ *vs* pretreatment.

Methoxamine (μg / kg)	Heart rate (Beats / min)		Pupil diameter (mm)	
	NaCl	TMPZ	NaCl	TMPZ
Baseline	323 ± 19	342 ± 19	0.49 ± 0.10	0.53 ± 0.07
Pretreat	317 ± 24	348 ± 29	0.52 ± 0.07	0.56 ± 0.07
10	306 ± 19*	315 ± 37*	0.55 ± 0.12*	0.52 ± 0.07*
30	294 ± 19*	304 ± 19*	0.67 ± 0.12*	0.51 ± 0.10*
100	252 ± 29**	288 ± 61**	1.09 ± 0.22**	0.81 ± 0.32**
300	221 ± 34**	239 ± 59**	1.56 ± 0.37**	1.19 ± 0.49**

clonidine.

Intravenous injections of methoxamine (10, 30, 100, and 300 μg / kg) elicited dose-dependent mydriasis and bradycardia that were significant at 100 and 30 μg / kg, respectively (Tab 3).

Following the methoxamine administration, mydriasis was initiated within 5 s, if it was seen at all. The mydriasis reached a maximum within 30 s of the methoxamine injection. The mydriatic and bradycardic effects of methoxamine lasted approximately 5 and 10 min, respectively. Methoxamine at >300 μg / kg was lethal to rats.

When given 5 min before the first dose of methoxamine, TMPZ failed to alter the mydriatic or bradycardic effect of methoxamine.

Reversal by TMPZ of the mydriatic effect of clonidine A single iv injection of clonidine (30 μg / kg) elicited profound mydriasis and bradycardia (Tab 4).

Tab 4. Reversal effects of TMPZ and idazoxan on clonidine-induced mydriasis. All injections were given iv. Post-clonidine iv were given 2 min after clonidine iv at a 20-s interval. Cumulative doses of TMPZ at 3, 10, and 30 mg / kg and those of idazoxan at 1, 3, and 6 μg / kg. $n=4$, $\bar{x} \pm$ SD. $^{*}P>0.05$, $^{**}P<0.05$ *vs* clonidine.

Postclonidine injections	Pupil diameter (mm)		
	NaCl	TMPZ	Idazoxan
Baseline	0.40 ± 0.08	0.43 ± 0.12	0.49 ± 0.18
Clonidine	3.40 ± 0.18	3.38 ± 0.28	3.44 ± 0.22
1	3.40 ± 0.18*	3.35 ± 0.40*	3.46 ± 0.50*
2	3.40 ± 0.18*	3.30 ± 0.60*	3.18 ± 0.52*
3	3.50 ± 0.20*	2.53 ± 0.72**	2.31 ± 0.58**

Two minutes after the clonidine injection while the mydriasis reached a maximum, the consecutive iv injections of TMPZ (3 and 10 mg / kg) failed to alter this effect of clonidine. TMPZ at 30 mg / kg slightly but significantly reversed the clonidine-induced mydriasis for 15-20 s. In contrast, idazoxan (6μg / kg) significantly reduced the clonidine-induced mydriasis for >2 min. This reduction for both of TMPZ and idazoxan was initiated within 10 s of the drug administration.

DISCUSSION

In this study, TMPZ 1-30 mg / kg iv failed to change heart rate or pupil diameter. In contrast, clonidine (3-30 μg / kg), an α_2-adrenoceptor agonist, and methoxamine (30-300 μg / kg), an α_1-adrenoceptor agonist, induced dose-dependent mydriasis and bradycardia. α_1-Adrenoceptor agonists induced mydriasis by increasing the contraction of the dilator muscle of the iris, whereas α_2-adrenoceptor agonists induce mydriasis by decreasing the ciliary nerve activity via a CNS mechanism[9]. Both α_1- and α_2-agonists decrease heart rate through the vasopressor effect via baroreceptor reflex. In addition, α_2-agonists decrease heart rate by inhibiting norepinephrine release from the cardiac nerve

in the anesthetized rat[14]. Thus our results suggest that TMPZ does not have detectable α–adrenoceptor agonistic activities in the rat. TMPZ inhibited [^{3}H]yohimbine binding in intact platelets with a K_i of 7.7×10^{-5} mol / L and an IC_{50} of 1.2×10^{-4} mol / L[8]. Like other α_2–adrenergic agonists, TMPZ (10^{-6} to 10^{-4} mol / L) inhibited the cAMP increases induced by PGE_1 10^{-5} mol / L[8]. Since the dosages used in our *in vivo* studies were comparable to those used in *in vitro* studies[8], our results were inconsistent with their findings. We cannot account for the discrepancies between these studies.

In this study, TMPZ (30 mg / kg) failed to prevent the effects of clonidine and methoxamine. Prazosin (1 mg / kg) and idazoxan (0.2 mg / kg) prevented the effects of methoxamine and clonidine, respectively (unpublished data). However, when TMPZ (30 mg / kg) was given after the clonidine administration (30 μg / kg), it caused a slight and transient reversal of clonidine–induced mydriasis. Nevertheless, the α_2–antagonistic activity of idazoxan was much greater than that of TMPZ, since idazoxan 6 μg / kg caused a greater and more persistent reversal of clonidine–induced mydriasis than TMPZ 30mg / kg. We do not know whether TMPZ can reverse methoxamine–induced mydriasis if it is given after methoxamine administration. However, it would be impossible to demonstrate this phenomenon with our model, since methoxamine–induced mydriasis is too short–lived for such reversal studies. In summary, our results showed that TMPZ (1–30 mg / kg) did not have detectable α–adrenoceptor agonistic activities, but might have slight α_2–antagonistic activities. Thus, TMPZ–induced vasodilation is probably not dependent upon the activation of α_2–adrenoceptors.

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猪缺血心肌嗜中性白细胞浸润及维拉帕米的保护作用[1]

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Neutrophil infiltration in ischemic porcine myocardium and protective effect of verapamil[1]

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ABSTRACT Neutrophil infiltration in the ischemic porcine hearts was analyzed by spectrophotometrical assay of the neutrophil specific enzyme-myeloperoxidase (MPO). The results showed that neutrophil infiltration in the ischemic myocardium was increased with time. An increase of MPO activity per 1 g tissue from 1.3± 0.8 to 4± 3 IU was observed as early as 10 min after occlusion of the coronary artery. Three h after occlusion MPO activity increased to 8.4± 1.3 IU, 7 times greater than that of normal tissue. one-h occlusion followed by 2-h reperfusion caused even higher neutrophil accumulation. MPO activity increased to 14± 3 IU, 11 times greater than normal tissue. Pretreatment with verapamil to reperfusion hearts decreased MPO activity to 4.7± 0.7 IU. In addition, verapamil completely eliminated the first episode of arrhythmia 5-10 min after occlusion. Our studies demonstrate that neutrophils can rapidly accumulate into the ischemic myocardium and suggest that the protective action of verapamil may in part due to its inhibition of neutrophil infiltration in the ischemic myocardium.

KEY WORDS myocardium; ischemia; myocardial reperfusion injury; myeloperoxidase; neutrophils; calcium channel blockers; verapamil; swine

提要 结扎猪冠脉左前降支造成心肌缺血再灌模型，以髓过氧化酶法定量测定浸润于缺血和再灌注组织中的中性白细胞. 结果表明在心肌缺血 10 min 即可见白细胞浸润，缺血 3h 心肌中浸润的白细胞为正常的 7 倍，缺血 1 h 再灌 2 h 白细胞的浸润更多. 维拉帕米明显降低白细胞的浸润，推测其抗心肌缺血作用，部分与降低白细胞的浸润有关.

关键词 心肌; 缺血; 心肌再灌注损伤; 髓过氧化酶类; 嗜中性白细胞; 钙通道阻滞剂; 维拉帕米; 猪

中性白细胞的浸润可直接影响缺血再灌时注心肌组织损伤和修复[1]. 白细胞一方面具有吞噬和清除坏死组织的能力，另一方面在吞噬过程中释放蛋白溶酶、氧自由基和花生四烯酸代谢产物使缺血和再灌损伤加重. 应用抗炎药和降低白细胞数可使损伤减轻[2]，但也有人认为，白细胞浸润与缺血再灌注损伤关系不大[3]. 钙拮抗剂抗心肌缺血再灌损伤可归因于抑制细胞的 Ca^{2+} 内流从而防止 Ca^{2+} 超载[4]. 它还有降低白细胞活性的作用[5]，后者可能与抗缺血再灌损伤有关. 本工作选用猪造成缺血再灌损伤，应用髓过氧化酶法来观察白细胞的浸润及钙拮抗剂的作用.

MATERIALS AND METHODS

猪心肌缺血再灌模型的建立 猪，体重

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