

Effects of Thymoxamine on Ouabain-Induced Arrhythmias in Dogs

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OGURO, K., HASHIMOTO, H. and NAKASHIMA, M. *Effects of Thymoxamine on Ouabain-Induced Arrhythmias in Dogs.* Tohoku J. exp. Med., 1982, 137 (4), 361-368 — The effect of thymoxamine, a pure alpha-adrenoceptor blocking agent, on ouabain-induced arrhythmias was investigated in dogs to clarify whether thymoxamine has an arrhythmogenic or an anti-arrhythmic action. Thymoxamine, 1 mg/kg i.v., inhibited the manifestation of ventricular arrhythmias (VA) and the pressor effect produced by ouabain infusion (1 µg/kg/min i.v.) as 5 mg/kg i.v. of phentolamine did. Low doses of these drugs did not affect the VA. Simultaneous infusion of phenylephrine (5 µg/kg/min i.v.) with ouabain enhanced the manifestation of VA accompanying pressor effects, which were inhibited by thymoxamine (1 mg/kg) in a parallel manner. Infusion of isoprenaline (0.2 µg/kg/min i.v.) with ouabain enhanced the manifestation of VA, but the enhancement was to a lesser extent than with phenylephrine. Thymoxamine, 1 mg/kg, did not affect the VA. The infusion of CaCl₂ (20 mg/kg/min i.v.) with ouabain enhanced the manifestation of VA, which was not inhibited by thymoxamine. These results show the possibility that the protective effect of thymoxamine on the manifestation of VA may be mainly associated with inhibitions of pressor effects mediated through vascular alpha-adrenoceptors. — alpha blocker; thymoxamine; phentolamine; ouabain-induced arrhythmias

It has been generally recognized that many of beta-adrenoceptor blocking agents have anti-arrhythmic actions. Its possible mechanisms would be related to a blocking action on beta-adrenoceptors, an inhibition of postganglionic cardiac sympathetic neural discharges, and a direct depression of cardiac excitability in a manner similar to quindine (Lucchesi 1965; Somani and Lum 1965). On the other hand, many authors observed that alpha-adrenoceptor blocking agents also have anti-arrhythmic properties in man and laboratory animals (Leimdorfer 1952; Morris et al. 1952; Gould et al. 1969). However, its possible mechanism is not clear enough. In the present experiments, the effects of phentolamine and thymoxamine on ouabain-induced arrhythmias were examined and a possible involvement of alpha-adrenoceptors in the heart or peripheral vascular beds in manifestation of ventricular arrhythmias (VA) was discussed.

METHODS

Sixty-one mongrel dogs of both sexes, weighing 7–14 kg, were anesthetized with 30 mg/kg of sodium pentobarbital, i.v. Blood pressure (BP) was measured from the femoral artery with an electromanometer (Nihon Kohden, RP-5). Heart rate was measured with a tachometer (Nihon Kohden, RT-5) which was triggered by R waves of ECG (Lead II). These indices were recorded on an ink-writing oscillograph (Nihon Kohden, RM-45M).

Ouabain was infused intravenously (i.v.) at a constant rate of 1 μ g/kg/min to the femoral vein with an infusion pump (Truth, A-II) until the animal died from ventricular fibrillation (Morran et al. 1962). Five minutes after the administration of thymoxamine or phentolamine, ouabain infusion was started. This time was referred to *zero* min in the text and all figures.

The drugs used were as follows: ouabain octahydrate (Merck), thymoxamine hydrochloride (Fuji Zoki), phentolamine mesylate (Ciba-Geigy), phenylephrine hydrochloride (Sigma), isoprenaline hydrochloride (Tokyo Kasei), and CaCl_2 (Wako). These drugs were dissolved in 0.9% saline solution. Doses referred to salts.

The statistical significance of differences in mean values was evaluated by group comparison with the corresponding control. Differences were considered to be statistically significant when *p* values were less than 0.05.

RESULTS

Effects of phentolamine and thymoxamine on ouabain-induced arrhythmias

Basal values of BP and HR were 152 ± 6.2 mmHg (systolic BP), 100 ± 2.8 mmHg (diastolic BP) and 150 ± 8 beats/min, respectively. When ouabain was infused i.v. at a rate of 1 μ g/kg/min, both systolic and diastolic BP were gradually elevated (Fig. 1). The first ventricular extrasystole (VE) suddenly appeared at 36.5 ± 4.08 min after the start of ouabain infusion and at 41.5 ± 4.46 min VE became frequent, concealing sinus rhythm. At 61.3 ± 4.59 min, unifocal and multifocal ventricular tachycardias (VT) were observed, and ventricular fibrillation (VF) was induced at 76.1 ± 5.47 min (Table 1).

Phentolamine, 1 mg/kg, did not lower the BP, but at 5 mg/kg produced a fall of BP accompanied by a reflex acceleration of HR. Ouabain infusion was started 5 min after phentolamine injection. Phentolamine, 1 mg/kg, had virtually no effect on VA. Phentolamine, 5 mg/kg, however, inhibited markedly the pressor effect of ouabain (Fig. 1). Sinus rhythm was maintained for a longer period. The time required for VE to occur was significantly prolonged, while that of VF was not inhibited (Table 1).

Thymoxamine, 0.1 mg/kg, had no effect on VA or BP. However, at 1 mg/kg thymoxamine inhibited markedly the pressor effect and the manifestation of VA and VF by ouabain (Fig. 2 and Table 1). As to the inhibitory effect on the manifestation of VE thymoxamine was approximately 5 times more potent than phentolamine on a weight basis.

Effects of thymoxamine on ventricular arrhythmias produced by simultaneous infusion of phenylephrine, isoprenaline or CaCl_2 with ouabain

The infusion of phenylephrine (5 μ g/kg/min) plus ouabain caused concurrently a marked and sustained pressor effect and an enhancement of VA. Basal systolic

TABLE 1. *Effects of alpha-adrenoceptor blocking agents, thymoxamine and phentolamine, on toxic and lethal effects of ouabain produced by single infusion of 1 µg/kg/min of ouabain or by simultaneous infusion of the same dose of ouabain and various cardio-simulants in dogs*

Group	Drugs	n	Toxic effect (min)		Lethal effect (min)	
			First extrasystole	Frequent extrasystole	Ventricular tachycardia	Ventricular fibrillation
A. Ouabain single infusion						
I	Control (1 μ g/kg/min)	8	36.5 \pm 4.08	41.5 \pm 4.46	61.3 \pm 4.59	76.1 \pm 5.47
II	Phentolamine (1 mg/kg)	6	36.0 \pm 2.70n.s.	39.4 \pm 3.62n.s.	42.4 \pm 4.60	71.4 \pm 11.81n.s.
	Phentolamine (5 mg/kg)	7	59.1 \pm 6.81†	60.9 \pm 6.52†	64.9 \pm 2.45n.s.	86.6 \pm 7.39n.s.
III	Thymoxamine (0.1 mg/kg)	4	41.6 \pm 6.26n.s.	48.5 \pm 4.48n.s.	58.0 \pm 5.93n.s.	78.2 \pm 6.87n.s.
	Thymoxamine (1 mg/kg)	6	50.0 \pm 4.15*	51.8 \pm 4.17*	68.6 \pm 9.44n.s.	123.4 \pm 17.57§
B. Simultaneous infusion of ouabain and agonists						
I-a	Ouabain & Php. (5 μ g/kg/min)	5	7.7 \pm 1.47§	9.7 \pm 2.89§	15.1 \pm 2.67§	37.8 \pm 3.23§
-b	Thymoxamine (1 mg/kg)	5	23.7 \pm 5.83**	27.0 \pm 6.37**	34.6 \pm 7.40**	65.9 \pm 8.90**
II-a	Ouabain & Php. (20 μ g/kg/min)	5	3.2 \pm 0.57§	3.2 \pm 0.57§	5.3 \pm 1.76§	19.9 \pm 6.34§
-b	Thymoxamine (1 mg/kg)	6	10.5 \pm 2.23††	13.6 \pm 4.10§§	32.0 \pm 5.10§§	51.9 \pm 3.48§§
III-a	Ouabain & Iso. (0.2 μ g/kg/min)	6	30.6 \pm 2.73n.s.	35.7 \pm 1.42n.s.	36.3 \pm 1.79§	55.8 \pm 1.82§
-b	Thymoxamine (1 mg/kg)	5	42.8 \pm 5.56n.s.	45.0 \pm 4.98n.s.	49.0 \pm 5.74n.s.	71.5 \pm 6.11††
IV-a	Ouabain & CaCl ₂ (20 mg/kg/min)	4	15.4 \pm 4.50§	20.6 \pm 3.63†	21.8 \pm 3.84§	45.3 \pm 4.83§
-b	Thymoxamine (1 mg/kg)	5	22.5 \pm 1.75n.s.	23.8 \pm 1.79n.s.	27.0 \pm 2.12n.s.	37.1 \pm 4.96n.s.

The reference marks indicate the statistical significance obtained by intergroup comparison to the corresponding control. The single marks indicate the significance between ouabain control (I) and treatment with alpha blockers (II, III) or between ouabain control (A-I) and each group of simultaneous infusion of ouabain and agonists (B-a). The double marks indicate the significance between each control of simultaneous infusion of ouabain and agonists (a) and treatment with thymoxamine (b). *, ** $p < 0.05$, †, †† $p < 0.02$, ‡, †† $p < 0.01$, §, §§ $p < 0.001$. n shows the number of animals. Frequent extrasystole indicates that ventricular extrasystoles continue more than 10 beats/min. n.s., not significant; Php., phenylephrine; Iso., isoprenaline.

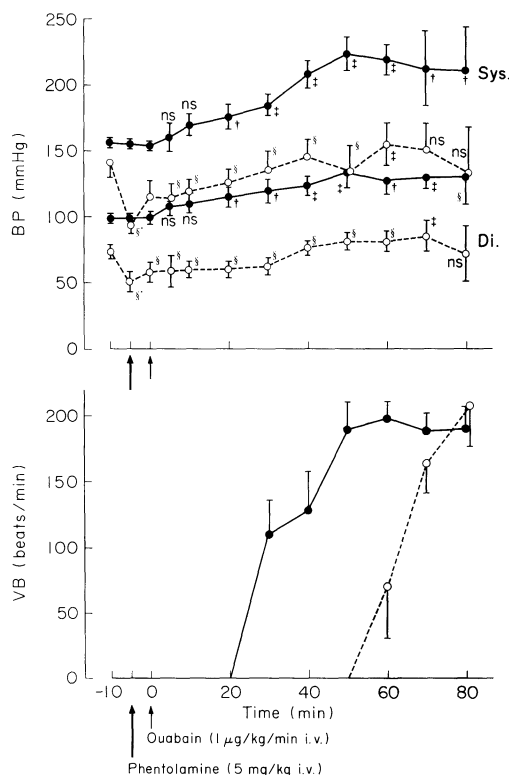


Fig. 1. Inhibitory effect of phentolamine (5 mg/kg i.v.) on ouabain-induced pressor effects and the number of ventricular beats.

●—●, control (ouabain single infusion); ○---○, phentolamine-treatment. Each circle indicates the mean value obtained from 6 to 8 animals. The bar indicates the standard error of mean. Phentolamine-induced hypotension is shown as §'. * $p < 0.05$; † $p < 0.02$; ‡ $p < 0.01$; § $p < 0.001$ against basal values. ** $p < 0.05$; †† $p < 0.02$; ‡‡ $p < 0.01$; §§ $p < 0.001$ against ouabain control. ns, not significant; BP, blood pressure; Sys. or Di., systolic or diastolic blood pressure; VB, ventricular beats. These abbreviations are the same to those in Figs. 2–5.

and diastolic BP (159 ± 12.1 mmHg and 93 ± 8.7 mmHg) were elevated to 302 ± 25.6 mmHg and 163 ± 11.1 mmHg, respectively, at 5.2 ± 0.2 min. This pressor effect was maintained for 30 min (Fig. 3). VE appeared at 7.7 ± 1.47 min. Sinus rhythm decreased and VA gradually increased. VT developed to VF at 37.8 ± 3.23 min (Table 1). Thymoxamine, 1 mg/kg, inhibited the pressor effect of ouabain plus phenylephrine for 20 min and the manifestation of VA.

On the other hand, the infusion of isoprenaline ($0.2 \mu\text{g/kg/min}$) with ouabain enhanced the manifestation of VA accompanying abrupt sinus tachycardia without elevation of BP (Fig. 4, Table 1). Thymoxamine, 1 mg/kg, did not affect the manifestation of VA.

Infusion of CaCl_2 (20 mg/kg/min) with ouabain increased slightly the BP but enhanced markedly the manifestation of VA (Fig. 5, Table 1). Sinus rhythm

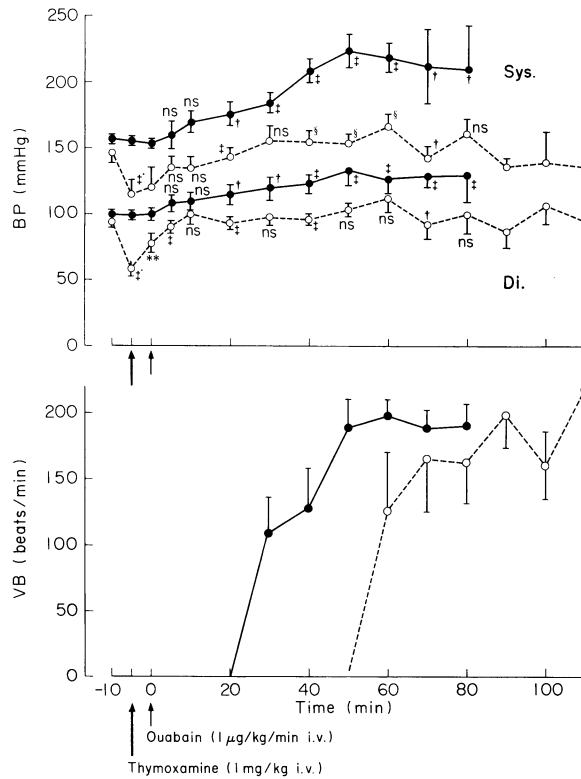


Fig. 2. Inhibitory effects of thymoxamine (1 mg/kg i.v.) on ouabain-induced pressor effects and the number of ventricular beats. ●—●, control; ○---○, thymoxamine-treatment. Each circle indicates the mean value obtained from 7 animals.

disappeared 20 min after the start of infusion. VE appeared at 15.4 ± 4.50 min and progressed the VF at 45.3 ± 4.83 min (Table 1). Thymoxamine, 1 mg/kg, did not inhibit the manifestation of enhanced VA.

DISCUSSION

In the present experiments, alpha-adrenoceptor blocking agents, phentolamine and thymoxamine, showed anti-arrhythmic properties against the ouabain-induced arrhythmias. In inhibiting the manifestation of VA thymoxamine was roughly 5 times as effective as phentolamine. When the alpha-adrenoceptor blocking activity was evaluated from the 50% inhibition (ID 50) for the NE-induced pressor effects in pithed dogs, the ID 50 was 1 mg/kg for phentolamine and 2 mg/kg for thymoxamine (Oguro et al. 1970). Thus, it appears the anti-arrhythmic properties of these drugs might be related to alpha-adrenoceptor blocking properties.

Hashimoto et al. (1973) demonstrated that catecholamines released from sympathetic nerves and adrenal glands by ouabain influence the manifestation of

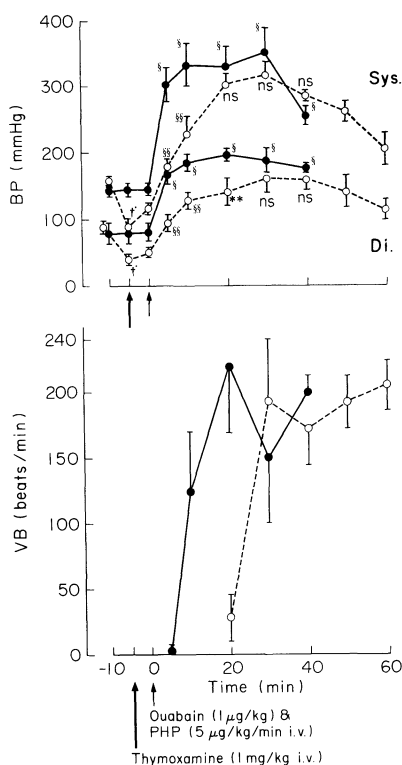


Fig. 3

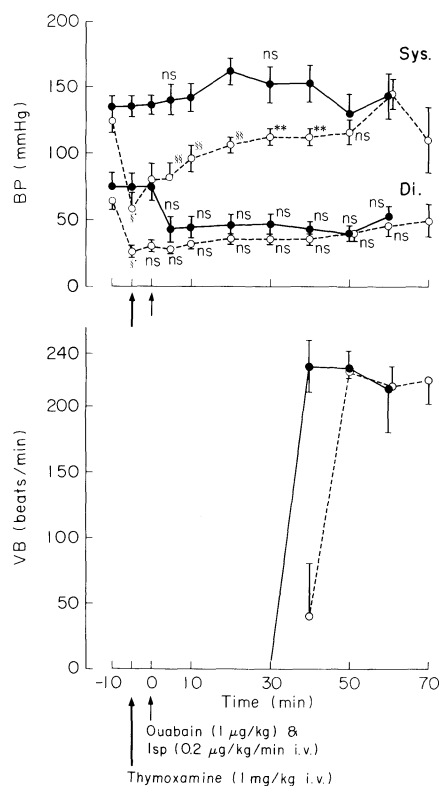


Fig. 4

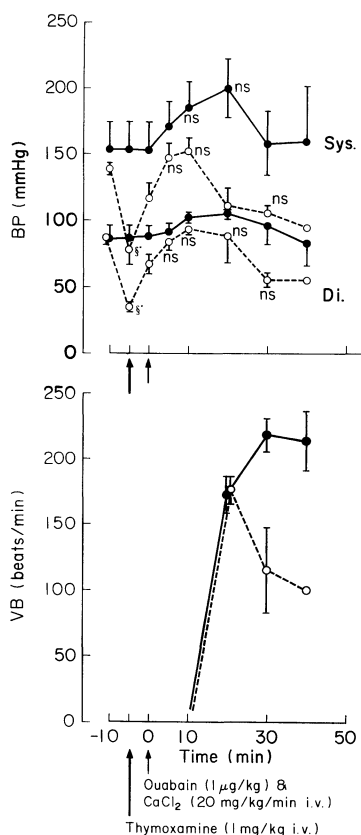
Fig. 3. Inhibitory effects of thymoxamine on pressor effects and the number of ventricular beats produced by simultaneous infusion of phenylephrine and ouabain. ●—●, control (simultaneous infusion of phenylephrine and ouabain); ○---○, thymoxamine-treatment. Each circle indicates the mean value obtained from 5 to 6 animals.

Fig. 4. Absence of the inhibitory effect of thymoxamine on the number of ventricular beats produced by simultaneous infusion of isoprenaline and ouabain. ●—●, control (simultaneous infusion of isoprenaline and ouabain); ○---○, thymoxamine-treatment. Each circle indicates the mean value obtained from 5 to 6 animals.

the ouabain-induced VA in dogs. However, it is still unclear whether the released catecholamines influence the beta-adrenoceptors or the alpha-adrenoceptors in inducing ouabain arrhythmias. Therefore, in the present experiments phenylephrine or isoprenaline was infused simultaneously with ouabain for selective stimulation of alpha- or beta-adrenoceptors. The simultaneous infusion of phenylephrine with ouabain enhanced the manifestation of VA, which was markedly inhibited by thymoxamine. On the other hand, although the infusion of isoprenaline with ouabain enhanced the manifestation of VA, the enhancement is to a lesser extent than with phenylephrine. Therefore, the manifestation of VA by phenylephrine is probably not due to stimulation of beta-adrenoceptors but alpha-adrenoceptors.

Ferrier and Moe (1973) and Moe and Farah (1975) suggested that the

Fig. 5. Absence of the inhibitory effects of thymoxamine on pressor effects and ventricular beats produced by simultaneous infusion of CaCl_2 and ouabain. ●—●, control (simultaneous infusion of CaCl_2 and ouabain); ○—○, thymoxamine-treatment. Each circle indicates the mean value obtained from 4 to 5 animals.



abnormal metabolism of Ca^{++} in the heart muscle is involved in the ouabain-induced VA. The simultaneous infusion of Ca^{++} with ouabain markedly enhanced the manifestation of VA, supporting the results obtained by Ferrier and Moe (1973) that an addition of suitable concentration of CaCl_2 to a solution of ouabain caused an enhancement of the manifestation of VA. Thymoxamine, however, did not significantly inhibit the VA, suggesting that thymoxamine might not directly affect the abnormal Ca^{++} metabolism induced by ouabain.

Endoh et al. (1978) and Chiba (1977) showed using the blood perfused canine sinus node and papillary muscle preparations that there were no alpha-adrenoceptors in the canine heart. But it may be possible that there are alpha-adrenoceptors related to prolongation of refractory period of heart as to anti-arrhythmic effect of alpha-adrenoceptor blocking agent. Because, Rosen et al. (1971) have shown that the anti-arrhythmic effect of phentolamine may be ascribed to a decrease in V_{max} , and membrane responsiveness, an increase in conduction time, and a decrease in automaticity. Thus, further experiments would be required to clarify the relation between the manifestation of ouabain-induced VA and anti-arrhythmic effect of alpha-adrenoceptor blocking agent.

On the other hand, Varrier et al. (1974) showed that a rapid injection of

phenylephrine enhanced the manifestation of VA induced by electrical stimulation of the ventricle and the pressor effect by phenylephrine might be a trigger in manifestation of VA. In the present experiments, phentolamine and thymoxamine inhibited the pressor effect induced by ouabain infusion or by simultaneous infusion of phenylephrine with ouabain. Therefore, the event that thymoxamine inhibited the manifestation of those VA would be due to the antagonism of the pressor effect mediated through vascular alpha-adrenoceptors. From these results, it is possible that the protective effect of thymoxamine on the manifestation of VA would be mainly related to inhibition of alpha-adrenoceptors of the peripheral vascular beds.

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