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Influence of repeated prazosin administration on cardiovascular responses in rats and rabbits

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Abstract. Prazosin was injected i.v. at a dose of 50 μg/kg every 2 h for 8 h in conscious rats. Its hypotensive action significantly declined. A similar effect was also observed in rabbits pretreated with prazosin (40 μg/kg, i.v.) every 1 h for 4 h. In prazosin-treated rabbits, the total peripheral resistance became less responsive to phentolamine stimulation. Repeated prazosin administration abolished its ability to block receptors in a model of anococcygue muscle contraction after noradrenaline (NA) stimulation. The α-adrenoceptors in anococcygue muscle exhibited lower pD_2 to NA and lower pA_2 to prazosin in prazosin-treated rats. The results demonstrate that repeated prazosin administration reduces the effectiveness of α-adrenoceptors blockers.

Key words. Prazosin; tolerance; blood pressure; rats; rabbits.

Patients with heart failure or hypertension may be required to have repeated prazosin administration in order to maintain an appropriate blood pressure. However, it has been shown that the effectiveness of prazosin declines after repeated use in clinical therapy[1,2,3]. As the drug has both a short half-life and duration of action[4], information about cardiovascular responses to repeated prazosin administration and its interaction with other α-adrenoceptor blockers are important. A better understanding of prazosin action could be a useful guide in therapy or research. The effects of repeated prazosin administration on cardiovascular responses were, therefore, examined in rats and rabbits in the present study.

Materials and methods

Both sexes of Sprague-Dawley rats (weighing 225 ± 15 g), and New Zealand rabbits (weighing 3.1 ± 0.5 kg) were used. Prior to the start of the experiments, rats were kept in groups of six in a rat battery whilst rabbits were kept individually. The animals were kept in an air-conditioned room (22 ± 1 °C and humidity at 65–75%) and were randomly divided into groups to be injected intravenously (i.v.) either with 0.9% (w/v) NaCl solution (saline) or with prazosin hydrochloride (Sigma).

Rats were anaesthetized by ether (Sigma). Polyethylene catheters, which were filled with heparinized saline (Novo; 500 IU/300 ml) to prevent clotting, were implanted intra-arterially (right carotid artery) and intravenously (right jugular vein); the other ends of the catheters ran subcutaneously to emerge dorsally at the neck region where they were anchored by a suture. The rats were then put into an individual steel cage (11 × 11 × 11 cm) with free access to food and water. The recovery period between the operation and the experiment with conscious rats was 24 h. The arterial catheter was connected to pressure transducers (CYX). The signals were collected and analyzed by computer. Mean arterial pressure (MAP), systolic artery pressure (SAP) and diastolic blood pressure (DBP) were recorded. Heart rate (HR) was determined from arterial pulsations. Prazosin (50 μg/kg) was administered via the intravenous catheter. The second and subsequent doses of prazosin were injected when the arterial pressure returned to its resting level.

Twenty-four rabbits were randomly divided into four groups. Two control groups received saline (2 ml/kg i.v.) and the other two groups received prazosin (40 μg/kg i.v.) four times at hourly intervals. The first two groups of saline-treated and prazosin-treated rabbits were used to study repeated prazosin administration. The other two groups of saline-treated and prazosin-treated rabbits were used to study the effect of phentolamine on systemic haemodynamic changes.

The systemic haemodynamic changes were measured 1 h after the last injection of prazosin. The measurements were carried out using a computerized analysis method as described by Elkayam et al.[3]

Rabbits were anaesthetized by intraperitoneal injection with sodium pentobarbitone (Sigma) (25 mg/kg) and urethane (Sigma) (200 mg/kg). Polyethylene catheters were cannulated in a carotid artery and an inferior cavitary vein through the femoral vein; these two

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catheters were then connected to pressure transducers (CYX). The chest was opened along the left of midline, the pericardium was incised and arcus aorta was exposed; a pulsed field electromagnetic flowmeter probe was employed to measure cardiac output. Signals from both arterial and venous pressure, through transducers or electromagnetic flowmeter (MF-27), were put into a computer for measurements of haemodynamic parameters which included arterial pressure, central venous pressure (CVP), cardiac output (CO), cardiac index (CI), stroke volume (SV), stroke volume index (SVI) and total peripheral resistance (TPR).

The isolated anococcygeus muscle model is now accepted as a simple and reliable method to examine the effects of drugs on receptors[5]. We used this preparation to evaluate the influences of prazosin on the α-adrenoceptor so as to understand the cardiovascular response. The isolated anococcygeus muscle was prepared according to Gillespie's method[5]. Twelve male rats were randomly divided into two groups which received i.v. injections of either saline (as controls) or prazosin (50 μg/kg) at 15 min intervals. Fifteen mins after the administration of the fifth dose, the rats were killed by a sharp blow to the base of the skull. Contractions of the anococcygeus muscle were recorded on a physiograph (Narco Bio-Systems). The preparations were allowed to equilibrate for 45 min before making experimental observations. During this period, the Krebs solution was replaced every 15 min. The resting tension of the muscle was repeatedly adjusted to five g. The basal dose-response curve was obtained by cumulative doses of noradrenaline (NA); then the tissue was washed three times with Krebs solution; after 1 h of equilibration, another dose-response curve to NA in the presence of a fixed dose of prazosin (0.1 μM) was obtained. The procedures were repeated three times and a total of four dose-response curves to NA were obtained. The pD_2 values were calculated as negative $\log_{10}$ molar concentration of the agonist producing 50% of the maximal effect. pA_2 values are negative $\log_{10}$ of the molar concentration of antagonist required to cause a 50% depression of the maximal response to the agonist. The Emax values indicate the tension of maximal contraction. The pD_2 and pA_2 values were calculated by Logistic mode[6].

The results were expressed as mean ±S.D. and the statistical significance was analyzed by Student's t-test or trend test[7].

Results

The arterial pressure was significantly decreased in response to the first dose of prazosin to conscious rats. The second dose of the drug was administered at 1–2 h intervals when the arterial pressure returned to normal level. The latter procedure was repeated for the third and fourth injections of prazosin. Prazosin showed a marked reduction in its hypotensive action (table 1, $p < 0.01$; trend test).

In anaesthetized rabbits, prazosin at a dose of 20 μg/kg produced an increase in HR, CVR and CO, but a decrease in MAP, SAP and DAP (table 2). The net fall in MAP, SAP and DAP in response to the acute dose of prazosin in the prazosin-treated animals was significantly less than that of the control group. The percentage net fall in blood pressure between pre- and post-drug administration was significantly less in those animals pretreated with prazosin (table 2, $p < 0.01$). The increase in HR induced by the acute dose of prazosin was also significantly less in the prazosin-treated group than in the saline-treated controls ($p < 0.05$). Although prazosin enhanced both CVP and CO, the increased percentages in both saline- and prazosin-treated rabbits were comparable.

Table 1. Effects of repeated prazosin (50 μg/kg, i.v., four applications) on blood pressure in conscious rats.

Sequence of injection		Prazosin (50 μg/kg i.v.)			
		1st	2nd	3rd	4th
Net fall in blood pressure (kPa)					
MAP	pre	13.8 ± 1.3	13.5 ± 1.0	13.4 ± 0.9	13.3 ± 1.1
	post	10.4 ± 0.5	11.7 ± 0.7	12.2 ± 0.8	12.4 ± 0.8
	▲%***	24.1 ± 3.5	13.0 ± 2.3	9.3 ± 1.2	6.7 ± 2.5
SAP	pre	15.6 ± 1.5	15.5 ± 1.1	15.5 ± 1.0	15.4 ± 1.1
	post	12.8 ± 1.5	13.9 ± 1.1	14.3 ± 1.1	14.4 ± 1.2
	▲%***	17,8 ± 4.4	10.0 ± 3.1	7.6 ± 1.8	8.4 ± 3.8
DAP	pre	12.5 ± 0.8	12.3 ± 1.2	12.4 ± 1.1	12.3 ± 1.4
	post	9.1 ± 0.4	10.4 ± 0.9	11.1 ± 1.0	11.3 ± 1.2
	▲%***	26.7 ± 4.4	15.2 ± 3.9	10.5 ± 1.4	8.1 ± 3.4

The values are means ±S.D. of eight rats.
*** $p < 0.01$ by trend test in same horizontal group.
▲%: the precentage net fall in blood pressure when comparing pre- and post-prazosin treatment.
MAP: mean arterial pressure; SAP: systolic arterial pressure; DAP: diastolic arterial pressure.

Table 2. Effects of prazosin on systemic haemodynamic changes in open-chest saline-treated and prazosin-treated rabbits.

Pretreatment	**Saline** (2 ml/kg, i.v. 4 times)			**Prazosin** (40 μg/kg, i.v. 4 times)		
Treatment	**Prazosin** (20 μg/kg, i.v.)					
Parameter	pre	post	▲%	pre	post	▲%
HR (bpm)	259 ± 40	293 ± 30	15 ± 8	233 ± 28	241 ± 23	4 ± 2*
MAP (kPa)	14.1 ± 1.5	10.3 ± 1.2	↓27 ± 5	13.4 ± 1.6	11.5 ± 1.3	↓14 ± 5**
SAP (kPa)	20.5 ± 1.3	16.2 ± 1.1	↓21 ± 4	19.3 ± 1.7	17.1 ± 1.9	↓11 ± 5**
DAP (kPa)	11.0 ± 1.7	7.4 ± 1.4	↓32 ± 8	10.5 ± 1.6	8.8 ± 1.1	↓16 ± 5**
CVP (Pa)	206 ± 39	225 ± 49	9 ± 14	216 ± 20	235 ± 49	10 ± 20
CO (ml)	377 ± 43	424 ± 33	13 ± 5	385 ± 31	485 ± 41	11 ± 6

All values are means ± S.D. of six rabbits.
*$p < 0.05$, ** $p < 0.01$ when compared to the same parameters in saline-injected control.
▲%: The percentage changes between pre- and post-prazosin (20 μg/kg, i.v.) injection.
↓ indicates negative changes in percentage of response to pre- and post-prazosin administration. HR: heart rate; CVP: central venous pressure; CO: cardiac output; MAP, SAP and DAP: same as in table 1.

Table 3. Effects of phentolamine on systemic haemodynamic changes in open-chest saline-treated and prazosin-treated rabbits.

Pretreatment	**Saline** (2 ml/kg, i.v. 4 times)			**Prazosin** (40 μg/kg, i.v. 4 times)		
Treatment	**Phentolamine** (250 μg/kg, i.v.)					
Parameter	pre	post	▲%	pre	post	▲%
HR (bpm)	239 ± 23	276 ± 24	16 ± 11	221 ± 26	234 ± 22	7 ± 7*
MAP (kPa)	14.0 ± 2.1	8.7 ± 1.5	↓38 ± 5	12.6 ± 2	10.6 ± 1.8	↓15 ± 3**
SAP (kPa)	20.0 ± 2.8	13.7 ± 3.1	↓32 ± 9	18.1 ± 1.9	15.6 ± 2.3	↓14 ± 5**
DAP (kPa)	11.2 ± 1.9	6.3 ± 0.8	↓43 ± 4	9.9 ± 2.0	8.2 ± 1.6	↓17 ± 3**
CVP (Pa)	203 ± 37	221 ± 62	7 ± 14	193 ± 44	209 ± 11	10 ± 16
CO (ml)	366 ± 55	405 ± 6	11 ± 5	33 ± 42	384 ± 52	14 ± 7
SV (ml)	1.5 ± 0.2	1.5 ± 0.1	3 ± 9	1.5 ± 0.2	1.6 ± 0.3	9 ± 13
SVI (ml/m^2)	19.7 ± 1.7	19.9 ± 1.9	1 ± 7	19 ± 3	20 ± 4	9 ± 13
CI ($L.min/m^2$)	4.6 ± 0.6	5.1 ± 0.5	11 ± 5	4.2 ± 0.7	4.7 ± 0.7	13 ± 7
TPR ($dyn.s.cm^{-5}$)	3060 ± 459	1719 ± 296	↓41 ± 7	2982 ± 473	2208 ± 55	↓25 ± 6**

All values are means ± S.D. of six rabbits.
*$p < 0.05$, **$p < 0.001$ when compared to the same parameters in saline-injected control.
▲%: the percentage change between pre- and post-phentolamine injection.
↓ indicates negative changes in percentage of response to pre- and post-phenotolamine administration. SV: stroke volume; SVI: stroke volume index; CI: cardiac index; TPR: total peripheral resistance. HR, MAP, SAP, DAP, CO and CVP: same as in tables 1 and 2.

Phentolamine, 250 μg/kg i.v., elevated HR, CVP and CO, and reduced MAP, SAP, DAP and TRP; however, the drug did not affect CI, SV and SVI in open-chest anaesthetized rabbits (table 3). In prazosin-treated animals, the haemodynamic changes in HR, MAP, SAP, DAP and TPR induced by phentolamine were markedly less than those of the saline-treated controls ($p < 0.05$).

After pretreatment with prazosin (50 μg/kg i.v. 15 min for five injections), the contraction of isolated anococcygeus muscle induced by NA stimulation was not significantly different between saline- and prazosin-treated rats (cf. figure). In the saline-treated group, the pD_2 and Emax of the responses to NA were 6.93 ± 0.15 and 7.3 ± 1.3 g, respectively. The pA_2 value of prazosin was found to be 8.73 ± 0.3. However, in prazosin-treated rats, the pD_2 and Emax of NA and the pA_2 of prazosin were 5.53 ± 0.81, 8.2 ± 2.9 g and 7.43 ± 0.5, respectively. All values are mean ± S.D. Both pD_2 and

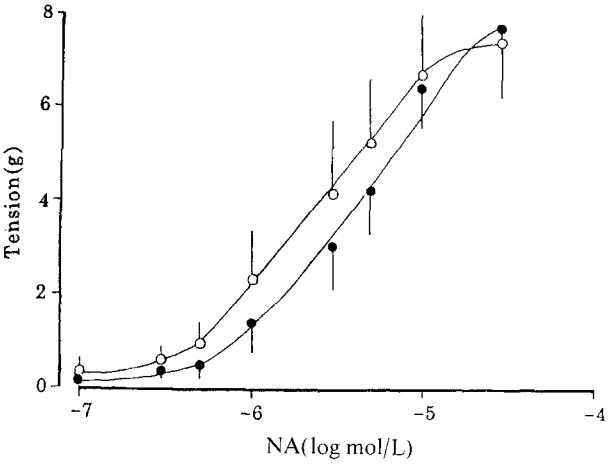

Figure. A cumulative concentration-response curve of isolated anococcygeus muscle contraction to noradrenaline (NA). ○ Saline (2 ml/kg, i.v. 15 min intervals, five times); ● pretreatment with prazosin (50 μg/kg, i.v. 15 min intervals, five times). All values are means ± SD of six rats.

pA_2 values in prazosin-treated animals were significantly less than those in vehicle-treated controls ($p < 0.05$), whilst Emax values showed no significant difference.

Discussion

The major effect of prazosin is the blockade of α_1-adrenoceptors in the arterioles and veins. This leads to a fall in both the peripheral vascular resistance and venous return to the heart. Previous studies using prazosin in congestive heart failure patients[8] or hypertension emergencies[4] have been generally disappointing. In the present study, repeated prazosin administration in conscious rats produced an acute tolerance to its hypotensive action. A similar tolerance in the systemic haemodynamics also developed when prazosin was repeatedly used in rabbits.

The initial dose of prazosin produced a marked increase in cardiac index associated with a substantial decrease in left ventricular filling pressure and total systemic vascular resistance. However, when the drug was repeatedly used, the effects of prazosin on systemic haemodynamic changes were significantly decreased. It is noticed that the drug influenced only blood pressure and TPR whilst other cardiac functions, such as CVP, CO, SV, SVI and CI, were not affected.

A tachycardiac effect was observed after the administration of the first dose of prazosin, then the increase in HR declined following the subsequent doses of the drug. The first administration of prazosin induces a marked hypotension[9]; such an exaggerated hypotensive response is due to prazosin's action as a potent α_1-adrenoceptor blocker. The second dose of the drug produces less response, which could be attributed to its acute tolerance. With the development of tolerance to the drug, the effects of phentolamine on systemic haemodynamic influences were also reduced although phentolamine is a nonselective α-adrenoceptor blocker. This phenomenon was also observed using other α-adrenoceptor blockers (e.g. terazosin and phenoxybenzamine) after prazosin pretreatment; however, pretreatment with phentolamine did not reduce the effect of prazosin (unpublished data). It is possible that repeated prazosin administration changed α-adrenoceptor properties which led to a reduction in the effectiveness of α-adrenoceptor blockers, whereas phentolamine blocked the α-adrenoceptor without further changing it. It is known that some drugs may change receptor properties. Repeated treatment of intact human astrocytoma cells and rat glioma cells with β-adrenergic agents was reported to cause lower affinities to β-receptors, which subsequently led to desensitization of β-adrenoceptors[10]. Chronic treatment with β-adrenoceptors antagonists has also been shown to lead to upregulation of the receptors[11].

The present findings point to the possibility that prazosin may change α-adrenoceptor responses to its blockers. Indeed, Kersting et al.[12] have shown that chronic prazosin administration destroyed its ability as an α-adrenoceptor blocker and reversed the effects of dobutamine on pulmonary artery pressure; they further suggested that chronic prazosin administration may induce α-adrenoceptor upregulation. The present results showed that repeated prazosin administration reduced the effectiveness of either the drug itself or other α-adrenoceptor blockers. However, since the anococcygeus muscle model showed that pretreatment with prazosin did not increase the contraction after NA stimulation, receptor upregulation is unlikely in this situation.

In the present study, the Emax values in the control and prazosin-treated groups were comparable, but pA_2 and pD_2 values were significantly lower in animals that received repeated prazosin treatment. Although specific strudies are needed, the findings suggest that repeated prazosin administration abolished its blockade properties, which may be due to changes in α-adrenoceptor affinity.

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