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α-Blockade on Blood Pressure and on Cardiac Noradrenaline Content in SHR and DOCA-salt Rats

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Abstract—The current study was designed to explore the effects of peripherally administered α-adrenoceptor inhibiting drugs (either prazosin or yohimbine) in spontaneously hypertensive (SHR) and DOCA-salt hypertensive rats before the development of established hypertension, as well as to characterize changes induced by prazosin and/or yohimbine in the cardiac noradrenaline (NA) content that might be responsible for the development of hypertension in these strains. The α-adrenoceptor blockade can prevent hypertension in DOCA and salt-treated animals in such a way that their blood pressure stabilizes at levels significantly lower than those observed in similarly treated normotensive controls. Significantly lower cardiac NA content was observed in DOCA-salt rats under basal and experimental conditions. The blood pressure of the treated rats and the heart NA content of the SHR were unaltered by treatment. Thus, administration of the α-adrenoceptor blocking agents, prazosin and/or yohimbine, throughout the developmental stage of SHR hypertension failed to alter either the progressive rise in blood pressure or in NA content. There may be differences between the cardiac adrenergic mechanisms responsible for the development of hypertension in each of these two models of hypertension.

Many experimental studies support the view that many factors, including noradrenaline (NA), are implicated in the pathogenesis of human and experimental hypertension (Saavedra & Grobecker 1979). In addition, the blood pressure of hypertensive humans and animals is often lowered by the administration of drugs that alter the physiological disposition of NA, which indicates the importance of this neurotransmitter and of the sympathetic nervous system in the maintenance of high blood pressure.

The aim of the present study was to examine the effect of adrenoceptor blocking agents on the development of DOCA-salt hypertension in the rat. For that purpose, prazosin (α_1-blocking), yohimbine (α_2-blocking) and prazosin + yohimbine were used in this study. We also evaluated the effect of selective α_1- and α_2-adrenoceptor blockade on the development of hypertension in a genetic model of hypertension, the Okamoto-Aoki spontaneously hypertensive rat (SHR), where a role for increased sympathetic activity is less evident.

In view of the important role of the sympathetic nervous system in cardiovascular function, and since a relationship between cardiac NA content in SHR and DOCA-salt rats and the development of hypertension has been suggested (Nakamura et al 1971), it was therefore of interest to study heart NA in these models under prolonged α-blockade.

Materials and Methods

Experimental animals and treatment

Male Sprague-Dawley (S/D), SHR and Wistar Kyoto (WKY) rats were used. Sixty S/D rats were utilized at 12 to 18 weeks of age. Half of the rats were made hypertensive by subcutaneous injections of desoxycorticosterone acetate (DOCA, Sigma) (30 mg twice weekly) suspended in sesame oil, and salt was given by substitution of 1% NaCl solution for drinking water to which the animals were allowed free access. All animals received free access to Purina Lab. Chow. The normotensive and hypertensive rats were divided into the following groups: Group I: saline 0·1 mL day^{-1}; Group II: prazosin (P) (Pfizer) 0·1 mg kg^{-1} day^{-1}; Group III: yohimbine (Y) (Sigma) 0·1 mg kg^{-1} day^{-1}; and Group IV: prazosin + yohimbine (P + Y) 0·1 mg kg^{-1} day^{-1}, respectively. The drugs were injected intraperitoneally in a final volume of 0·1 mL. An identical protocol was carried out with the same drugs in SHR and WKY rats, except that studies were initiated in both these groups of rats when the animals were 5 weeks old. Since the rapid rise of blood pressure is detected normally at 5 weeks (Okamoto 1972; Nagaoka & Lovenberg 1976; Sánchez et al 1986), the period of initiation should be considered to precede 5 weeks of age (Lais et al 1977).

Blood pressure and body weight

Throughout the study, animals were housed in a room with constant temperature (22 ± 1°C) and humidity (50-60%), and were exposed to light by an automated system from 0700 to 1900 h. Systolic blood pressure (SBP) was measured between 0800-1000 h, before drug administration, and at weekly intervals by "tail cuff" plethysmography in conscious animals prewarmed to 37°C in thermostatically controlled cages. A SBP of 150 mmHg or higher was the criterion for hypertension. When the animals were killed, their body weights were determined.

Noradrenaline determination

Animals were killed by a blow on the head and the heart was quickly removed and placed in ice-cold Krebs solution. The dissection of these tissues (both left and right atria and ventricle) were rapidly blotted, weighed and transferred to a 15 mL plastic tube containing 5 mL of ice-cold 0·05 M perchloric acid. Tissues were homogenized for 30 s with a Polytron homogenizer (Kinematica Inc, Switzerland). Tissue homogenates were centrifuged and the supernatant was

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analysed for noradrenaline using Shellenberger & Gordon's method (1971). In all cases, standard solutions of NA were analysed concurrently, with recoveries ranging from 75 to 85%. Appropriate corrections for recovery and volume changes were made for each sample and NA concentrations were expressed in terms of μg^{-1} of tissue weight.

Statistical analysis

Data are expressed as means ± s.e. Analysis of variance was used to evaluate weekly measurement of rat blood pressures at the time of chronic experimentation. Comparisons of the effects of prazosin, yohimbine, and prazosin + yohimbine on the cardiac NA content between normotensive and hypertensive groups were also evaluated by analysis of variance. In all cases of these multiple comparisons, when a significant ($P < 0.05$) F ratio was obtained, the Newman-Keul's test was used to determine which of the comparisons was significantly different (Zar 1974).

Results

Blood pressure

Rats treated with DOCA and sodium showed a significant increase in SBP after 6 weeks. DOCA-salt rats given either prazosin, yohimbine, and prazosin + yohimbine for the same period did not develop hypertension ($P < 0.001$; $P < 0.01$ and $P < 0.001$, respectively) after 6 weeks of treatment (Fig. 1). These drugs administered to S/D normotensive rats did not lower their SBP (data not shown).

With respect to the SHR rats, the time course for the changes in blood pressure in the control period and after long term α-adrenoceptor blockade is shown in Fig. 2. The SHR used in this study were in the prehypertensive phase, and blood pressure in the control group increased during the 6 week observation period. Neither prazosin, yohimbine, nor prazosin + yohimbine treatments prevented the development of hypertension in the SHR rats.

Growth of these prazosin- and/or yohimbine-treated animals did not differ from their respective tap-water controls, and in both hypertensive models studied, heart rate was unchanged during the entire experimental period (data not shown).

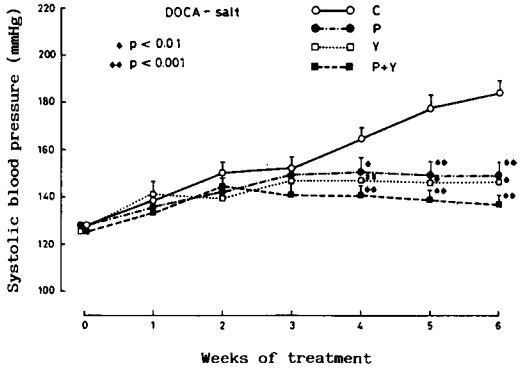

FIG. 1. Systolic blood pressure in DOCA-salt rats during 6 weeks of treatment with prazosin (P), yohimbine (Y), and prazosin + yohimbine (P + Y). The "P" value refer to comparisons with the control (C) group. Each value is the mean ± s.e. of 8 animals.

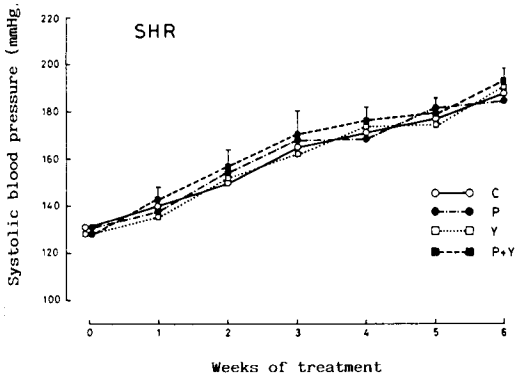

FIG. 2. Systolic blood pressure in SHR rats during 6 weeks of treatment with prazosin (P), yohimbine (Y), and prazosin + yohimbine (P + Y). Each value is the mean ± s.e. of 8 animals.

Noradrenaline content in cardiac tissues after prazosin, yohimbine, and prazosin + yohimbine

Endogenous NA levels were measured in both left and right atria and ventricle of normotensive and hypertensive rats after 6 weeks of treatment with prazosin, yohimbine, and prazosin + yohimbine.

DOCA-salt rats on saline, prazosin and/or yohimbine had lower cardiac NA content ($P < 0.001$) than normotensive controls. The concentration of endogenous noradrenaline in the left atrium of normotensive rats on P + Y was also significantly reduced when compared with their control group ($P < 0.01$) (Table 1).

As shown in Table 2, prazosin or yohimbine when injected alone, did not influence cardiac NA content of the SHR and WKY rats. However, the simultaneous administration of prazosin + yohimbine produced a significant reduction in the left atrium NA content of SHR rats when compared with WKY rats ($P < 0.05$).

Discussion

The experiments described herein were performed to obtain more insight into the physiological role of the sympathetic nervous system in genetic and non-genetic hypertension forms. In addition, the importance of an intact sympathetic nervous system was also evaluated.

DOCA-salt hypertension in the rat is characterized by an increase in peripheral sympathetic tone (De Champlain & Van Ameringen 1972; Bouvier & De Champlain 1985) and we have previously demonstrated that the long-term administration of prazosin and/or yohimbine prevents the development of hypertension in DOCA-salt rats (Sánchez et al 1985).

The development of hypertension in DOCA-salt rats correlated with decreasing cardiac NA content. The fact that the same changes occurred in the content of noradrenaline under both control and experimental conditions indicates that these changes are not due to the pharmacological effects of these drugs, but rather to pathological processes induced by DOCA-salt administration. Thus, lack of effect of any of the above treatments in altering NA content in the cardiac tissues studied, despite the prevention of hypertension, suggests that the drugs may be acting via a mechanism different from that which causes the hypertension.

Table 1. Concentrations of noradrenaline ($\mu g\ g^{-1}$) in different tissues of normotensive (S/D) and hypertensive (DOCA-salt) rats after treatment with α-adrenoceptor blockers. Results are means ± s.e. (n = 8).

	Left atrium		Right atrium		Ventricle	
Treatment	S/D	DOCA-salt	S/D	DOCA-salt	S/D	DOCA-salt
Untreated	1·89 ± 0·10	1·10 ± 0·16**	1·37 ± 0·08	0·47 ± 0·07**	0·61 ± 0·07	0·31 ± 0·03**
Prazosin (P)	1·98 ± 0·12	1·16 ± 0·05**	1·44 ± 0·01	0·83 ± 0·10**	0·61 ± 0·02	0·32 ± 0·04**
Yohimbine (Y)	1·84 ± 0·07	0·90 ± 0·09**	1·58 ± 0·14	0·50 ± 0·07**	0·61 ± 0·10	0·26 ± 0·04**
P + Y	1·33 ± 0·17+	1·00 ± 0·11*	1·26 ± 0·23	0·56 ± 0·04**	0·58 ± 0·06	0·24 ± 0·03**

Significance of differences between S/D and DOCA-salt groups: $*P < 0·05$; $**P < 0·001$.
Significance of differences from untreated group (S/D or DOCA-salt): $^{+}P < 0·01$.

Table 2. Concentrations of noradrenaline ($\mu g\ g^{-1}$) in different tissues of normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive rats (SHR) after treatment with α-adrenoceptor blockers. Results are means ± s.e. (n = 8).

	Left atrium		Right atrium		Ventricle	
Treatment	WKY	SHR	WKY	SHR	WKY	SHR
Untreated	1·34 ± 0·10	1·11 ± 0·18	1·21 ± 0·12	1·06 ± 0·13	0·35 ± 0·05	0·38 ± 0·04
Prazosin (P)	1·36 ± 0·14	1·03 ± 0·15	1·19 ± 0·09	1·21 ± 0·10	0·34 ± 0·04	0·31 ± 0·02
Yohimbine (Y)	1·26 ± 0·10	1·31 ± 0·17	1·00 ± 0·12	1·09 ± 0·17	0·39 ± 0·03	0·40 ± 0·08
P + Y	1·62 ± 0·20	1·20 ± 0·11*	1·34 ± 0·20	1·27 ± 0·18	0·46 ± 0·07	0·37 ± 0·07

Significance of differences between WKY and SHR groups: $*P < 0·05$

The results showing a decrease of NA in the heart of rats made hypertensive with DOCA-salt treatment are consistent with those of De Champlain et al (1969), who found a NA decrease in the heart of hypertensive rats. We also detected in previous studies a decrease of NA in the kidney of hypertensive rats (Sánchez et al 1986). The decrease in NA tissue may be due to an increased turnover of NA as has been demonstrated in the heart, spleen, and intestine of hypertensive rats (De Champlain et al 1969; Nakamura et al 1971). Such a decrease may also be due to a reduction in the binding and storage of the NA in the sympathetic nerves in these organs.

To further investigate the role of the sympathetic nervous system in the development of hypertension, we have also studied a genetic model of hypertension, the Okamoto-Aoki spontaneously hypertensive rat. In contrast with results reported in DOCA-salt hypertension in the rat, here we have found that long-term administration of prazosin and/or yohimbine to 5 week old SHR rats did not alter the development of hypertension in these animals. Thus, its antihypertensive action appears to be dependent on the presence of elevated sympathetic activity since it is very effective in lowering SBP in DOCA-salt hypertensive rats, in which elevated vascular sympathetic tone has been demonstrated consistently. Thus, it may be that adrenergic mechanisms responsible for the development of hypertension differ between these two models of hypertension.

Lack of effectiveness of any of the above treatments in lowering NA content in SHR and WKY rat ventricle and atria indicates two possibilities: first, none of the drugs directly affects sympathetic storage vesicles with respect to their NA content and, second, the release of NA is not sufficiently enhanced by the α-adrenoceptor blocking yohimbine to cause its reduction.

Thus, whatever alteration in peripheral adrenergic function may be involved in the pathogenesis of genetic hypertension in rats (Vanhoutte et al 1980), the elevated blood pressure in SHR cannot be related to the concentration of cardiac NA content under basal or experimental conditions. In addition, the lack in effectiveness of the α-blocking agents on SBP and on cardiac NA content suggests the non-participation of the heart, as a primary factor, in the development of hypertension in SHR rats.

These data emphasize the difficulty in relating changes in NA levels and blood pressure in genetic and non-genetic models of hypertension.

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