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Influence of Adrenergic Nervous System on Vasodilator-Induced Renin Release in the Conscious Rat (41119)

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Abstract. The influence of the adrenergic nervous system on vasodilator-induced renin secretion was studied in conscious rats with chronically implanted jugular and carotid cannulae. Hydralazine (0.1, 0.5, and 1 mg/kg body wt) was administered iv to rats and the dose–response relationship to plasma renin activity was calculated using linear regression analysis. These data were compared to results obtained in the same rats when similar doses of hydralazine were administered 15 min after pretreatment with iv propranolol (1 mg/kg body wt). A highly significant dose–response relationship was demonstrated between hydralazine dose and plasma renin activity ($r = 0.83$; $P < 0.0005$). In the presence of propranolol the increase in plasma renin activity was approximately 50% of the increase noted in studies without propranolol. The dose–response relationship remained highly significant ($r = 0.64$; $P < 0.0005$) in the presence of β-adrenergic blockade, which was confirmed by the failure to increase heart rate despite a significant reduction in blood pressure. These results show that in the conscious rat renin secretion following vasodilatation is only partially controlled through the adrenergic nervous system and that other mechanisms appear to play a prominent role in this response.

Drug-induced peripheral vasodilatation is known to act as a potent stimulus for renin secretion in humans (1, 2) and animals (3, 4). Previous studies in rats, in which approximately 90–95% of the increase in plasma renin activity observed following administration of hydralazine was inhibited by pretreatment with propranolol (5), have suggested that this response is mediated almost entirely via the adrenergic nervous system.

Recently it has been shown that chemical sympathectomy does not limit the capacity of the renin–angiotensin system to respond maximally to vasodilatation (3). Mature rats treated from birth with 6-hydroxydopamine hydrobromide had an increase in plasma renin activity after intravenous hydralazine that was equal to the increases noted in nonsympathectomized control rats. These data suggested that renin secretion in response to vasodilatation is effected to a greater degree than formerly recognized by nonadrenergic stimuli.

The present study was designed to evaluate the dependence of vasodilator-induced renin secretion on activation of renal β-adrenergic receptors. Conscious rats with chronically implanted venous and arterial catheters were used to make direct paired comparisons of dose–response relationships between hydralazine and plasma renin activity in the presence and absence of β-adrenergic blockade.

Methods. Male Sprague–Dawley rats (175–200 g) were purchased from Bio-Lab, White Bear Lake, Minnesota, and housed in a controlled light and temperature environment. The rats were maintained on tap water *ad lib* and regular rat chow throughout the experimental period.

Cannulae (PE-50) were placed into the left jugular vein and right carotid artery under pentobarbital anesthesia (30 mg/kg). The catheters were passed subcutaneously and exteriorized posterior to the ear at the midline. Patency of the catheters was maintained with heparin.

Experiments were performed 24–48 hr after the rats had recovered from the catheter placement. Rats were placed in a plexiglass rat holder (Narco Bio-Systems No. 709-0204) and the arterial line attached to a blood pressure transducer (Bell and Howell, Type 4-327-I) for continuous recording of blood pressure and heart rate on a Beckman Type RB dynograph. The rats were then left undisturbed until steady-

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state hemodynamic conditions had been achieved.

All drug doses were administered into the venous catheter in a volume of 0.1 ml. The doses of hydralazine used were 0.1, 0.5 and 1 mg/kg body wt. Propranolol was used in a dose of 1 mg/kg body wt. At a given dose of hydralazine the same rats were used for experiments performed in the presence or absence of propranolol and this sequence was randomly selected. In some but not all cases rats were studied with more than one dose of hydralazine. In no case did a given rat receive more than a single injection of hydralazine (with or without propranolol) within a 24-hr period.

Blood samples (0.1 ml) were collected from the arterial catheter prior to and 15 min after administration of each drug. Samples were collected in tubes containing EDTA, centrifuged at 4°, and the plasma was stored at −20° for 1−2 weeks until assayed for plasma renin activity.

Rat plasma (0.05 ml) was incubated with 0.1 ml maleate buffer (pH 6), 0.01 ml 2,3-dimercapto-1-propanol (BAL) (1.7%) and 0.01 ml 8-OH-quinoline (6.6%) at 37° for 3 hr. Plasma renin activity was determined by radioimmunoassay as previously described (3). The intraassay and interassay coefficients of variation for this assay are 7.2 and 13.3% respectively.

Analysis of data was performed using Student's *t* test (paired). Regression coefficients, representing the dose−response relationship between hydralazine administration and plasma renin activity, were calculated by the method of least squares; significance of these relationships and differences between treatment regimens were analyzed by Student's t test. A P value of less than 0.05 was considered to be significant.

Results. A highly significant dose−response relationship was demonstrated between hydralazine dosage (0.1−1.0 mg/kg) and plasma renin activity ($y = 4.78 + 25.54x$; $r = 0.83$; $P < 0.0005$) (Table I, Fig. 1). A similar relationship was also noted between hydralazine dosage and heart rate ($P < 0.0005$) (Table II). Blood pressure did not decrease significantly at any point following administration of the lowest dose of hydralazine (0.1 mg/kg); however, a significant reduction in blood pressure occurred after hydralazine doses of 0.5 mg/kg and 1 mg/kg ($P < 0.0025$), and the levels recorded at these two doses were not significantly different.

The dose−response relationship between hydralazine dosage and plasma renin activity was also highly significant in the presence of β-adrenergic blockade with propranolol ($y = 5.17 + 0.56x$; $r = 0.64$; $P < 0.0005$) (Table I, Fig. 1). However, treatment of rats with propranolol significantly

TABLE I. RESPONSE OF PLASMA RENIN ACTIVITY (PRA) (ng ANGIO I/ml/hr) TO iv HYDRALAZINE ADMINISTERED IN THE PRESENCE AND ABSENCE OF PROPRANOLOL (1 mg/kg)

Treatment	*n*	Control	15 min after propranolol	15 min after hydralazine	
Hydralazine (0.1 mg/kg)	5[a]	4.5 ± 0.5		6.8 ± 0.7[b]	$P < 0.05$
+ Propranolol		7.5 ± 1.1	6.3 ± 0.8	4.8 ± 0.8	
Hydralazine (0.5 mg/kg)	9	4.3 ± 0.6		18.5 ± 2.2[b]	$P < 0.01$
+ Propranolol		4.7 ± 1.0	5.9 ± 1.1	10.2 ± 1.9[c]	
Hydralazine (1 mg/kg)	5	5.7 ± .07		30.0 ± 5.6[b]	$P < 0.025$
+ Propranolol		7.5 ± 1.0	3.8 ± 0.9	14.3 ± 2.2[c]	

[a] The same rats were used for studies with and without propranolol at each hydralazine dose with randomization of this sequence.
[b] $P < 0.025$ cf. control.
[c] $P < 0.05$ cf. control.

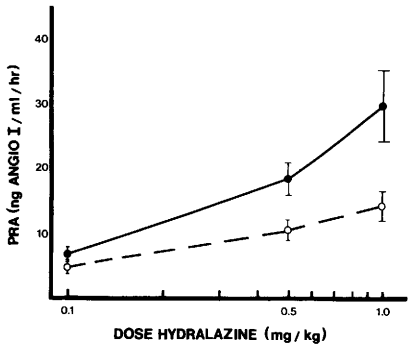

FIG. 1. Dose–response relationship between iv hydralazine (0.1, 0.5, and 1 mg/kg body wt) and plasma renin activity (PRA) in rats studied in the presence (○– – –○) or absence (●——●) of β-adrenergic blockade with propranolol (1 mg/kg body wt iv). Each point represents the mean ± SEM of five, nine, and five rats, respectively. Using linear regression analysis, a significant dose–response relationship was demonstrated both in the presence ($y = 5.17 + 0.56x$; $r = 0.64$; $P < 0.0005$) and absence ($y = 4.78 + 25.54x$; $r = 0.83$; $P < 0.0005$) of propranolol. The slopes of these two regression lines were also significantly different from each other ($P < 0.025$).

reduced plasma renin activity as demonstrated by statistical comparison of the slopes of the two regression lines ($P < 0.025$). The reduction in plasma renin activity by β-adrenergic blockade was approximately 30% at a hydralazine dose of 0.1 mg/kg ($P < 0.05$); and approximately 50% at hydralazine doses of 0.5 mg/kg ($P < 0.01$) and 1 mg/kg ($P < 0.025$). The addition of propranolol caused a significant reduction in heart rate at all hydralazine doses (Table II). However, only at a hydralazine dose of 0.1 mg/kg was blood pressure in the presence of propranolol significantly lower ($P < 0.01$) than without propranolol.

The effectiveness of propranolol in blocking β-adrenergic neuronal activity was confirmed by measurement of changes in heart rate following administration of hydralazine. In each group of rats receiving propranolol, heart rate declined significantly immediately after drug administration. The heart rate did not increase significantly in response to any of the three hydralazine doses in the propranolol-treated rats, regardless of the degree of hypotensive response observed.

At each hydralazine dose measurements of renin secretion in the presence and absence of propranolol were made in the same rats. Therefore, it was possible to assess the independent effect of adrenergic input on renin secretion by determining the difference in plasma renin activity between the two treatment regimens. When this difference was plotted against hydralazine dose, regression analysis confirmed a positive and highly significant dose–response

TABLE II. RESPONSE OF BLOOD PRESSURE (BP) (mm Hg) AND HEART RATE (HR) (BEATS/min) TO iv HYDRALAZINE ADMINISTERED IN THE PRESENCE AND ABSENCE OF PROPRANOLOL (1 mg/kg)

		Control		15 min after propranolol		15 min after hydralazine	
Treatment	*n*	BP	HR	BP	HR	BP	HR
Hydralazine (0.1 mg/kg)	5[a]	128 ± 8	421 ± 20			126 ± 7[d]	445 ± 26[d]
Hydralazine + propranolol		125 ± 6	444 ± 11	123 ± 6	336 ± 22[b]	110 ± 4[c]	348 ± 20
Hydralazine (0.5 mg/kg)	9	113 ± 7	396 ± 16			85 ± 5[b]	463 ± 12[c,e]
Hydralazine + propranolol		112 ± 9	423 ± 16	119 ± 10	348 ± 7[b]	90 ± 6[b]	351 ± 13
Hydralazine (1 mg/kg)	5	115 ± 4	375 ± 15			90 ± 4[b]	480 ± 20[b,e]
Hydralazine + propranolol		124 ± 6	390 ± 13	120 ± 7	360 ± 8[c]	93 ± 9[b]	386 ± 12

[a] The same rats were used for studies with and without propranolol at each hydralazine dose with randomization of this sequence.
[b] $P < 0.0025$ cf. control.
[c] $P < 0.01$ cf. control.
[d] $P < 0.01$ cf. experiments with propranolol.
[e] $P < 0.0025$ cf. experiments with propranolol.

relationship ($y = 0.68 + 14.79x$; $r = 0.64$, $P < 0.0005$).

Discussion. Currently available data suggest that virtually the entire stimulus to renin secretion following administration of vasodilating drugs (4, 5) and certain other hypotensive agents (6) results from activation of the sympathetic nervous system. However, the present study has shown that vasodilator-induced renin secretion is reduced by only 50% in propranolol-treated rats and that a dose–response relationship between dose of hydralazine and plasma renin activity occurs both in the presence and absence of β-adrenergic blockade. Therefore, nonadrenergic mechanisms appear to play a significant role in stimulation of renin secretion by vasodilating agents.

Administration of hydralazine causes a number of hemodynamic alterations that may effect renin secretion (7). In addition to a reduction in mean arterial blood pressure, there is a reduction in vascular resistance in the renal arterial bed (8), an increase in total renal plasma flow (8), and an increase in salt and water reabsorption in the presence of normal glomerular filtration, believed to be secondary to a redistribution of intrarenal blood flow (9). Although the mechanisms responsible for these physiologic adjustments may, in part, be related to sympathetic activation, they also appear to be regulated by the prostaglandin system, since inhibition of prostaglandin synthesis with indomethacin in hydralazine-treated dogs has been reported to reduce total renal blood flow and increase renal vascular resistance (8).

Prostaglandins also directly stimulate renin secretion as shown in studies utilizing prostaglandin injection into the renal artery (10, 11) and superfusion of rat kidney cortical slices (12). Recently, evidence has been presented to suggest that the mechanism of hydralazine-induced renin secretion is dependent upon prostaglandin synthesis, since increased plasma renin activity was completely inhibited in rats given indomethacin prior to administration of hydralazine (13). Those findings imply that prostaglandin activation is a critical intermediary step in the biochemical pathway between the β-adrenergic receptor and renin secretion from the juxtaglomerular cell. However, renal nerve stimulation has been shown to modulate renin secretion mediated via other stimuli (14) thought to be dependent, in part, on the prostaglandin system, e.g., furosemide administration (11) and reduction in renal perfusion pressure (10). Although prostaglandins are likely to be operative in hydralazine-induced renin secretion, their degree of involvement, particularly with reference to an interrelationship with the adrenergic nervous system, requires clarification.

It is not clear why the results of this study differ from previous studies (4–6) demonstrating that propranolol causes a greater degree of inhibition of vasodilator-induced renin release. However, a major difference in the protocol used herein was administration of drugs by the intravenous route rather than intraperitoneally. It seems likely that plasma drug levels following intravenous injection would be considerably higher; equivalent doses given intraperitoneally may, therefore, result in a lesser stimulus to renin secretion. The result would be similar to that seen in the present study when 0.1 and 0.5 mg/kg doses were used. At the lower dose an increase in renin secretion following hydralazine was completely blocked by propranolol, whereas at the higher dose plasma renin activity increased significantly even in the presence of propranolol.

It has also been shown that the percentage inhibition of vasodilator-induced renin secretion declines when high doses of propranolol are administered (15 mg/kg, subcutaneously), resulting in high drug levels (750 ng/ml) (5). Although plasma propranolol levels were not measured in the present study, it seems unlikely that renin secretion was stimulated by a dose of 1 mg/kg. Plasma renin activity 15 min after propranolol was not significantly different from control values and the dose of propranolol administered was the same for each hydralazine dosage.

Hypotensive response following hydralazine does not appear to be the major determinant to renin secretion, since plasma renin activity was not directly correlated with blood pressure. The reduction in blood pressure in response to 0.5 and 1

mg/kg doses of hydralazine was similar, but renin secretion was significantly greater at the higher dose.

This study has shown that induction of renin secretion following vasodilatation is only partially controlled through the adrenergic nervous system and that other mechanisms appear to play a prominent role in this response. Identification of these mechanisms may help clarify the complex interrelationship between physiologic systems controlling renin secretion and lead to development of more effective therapeutic agents and regimens for the treatment of hypertension.

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