Effect of Propranolol on Blood Pressure and Plasma Renin Activity in the Spontaneously Hypertensive Rat

By Barr H. Forman and Patrick J. Mulrow

ABSTRACT

Spontaneously hypertensive Wistar rats have normal basal levels of plasma renin activity but do not respond to sodium depletion or the stress of ether anesthesia plus laparotomy. Both age and rat strain influence the development of this hyporesponsive renin system: normal Wistar and spontaneously hypertensive rats but not Sprague-Dawley rats develop the hyporesponsive system as they age. Therefore, these rat strains may serve as useful models for studying the effects of age and hypertension on plasma renin activity. In the present study, propranolol administration up to 18 mg/kg day\(^{-1}\) for 15 days did not lower plasma renin activity but did cause a paradoxical rise in blood pressure in spontaneously hypertensive rats. This paradoxical rise was prevented by placing the rats on a low-sodium diet prior to propranolol administration. The mechanism of the paradoxical rise is unknown, but we suggest that it could be the result of increased alpha-adrenergic activity in response to a fall in cardiac output.

KEY WORDS hypertension age hyporesponsive renin system influence of rat strain on plasma renin activity radioimmunoassay stress sodium depletion

The spontaneously hypertensive Wistar rat developed by Okamoto and Aoki (1) has been extensively studied as a model of human essential hypertension, since these rats uniformly become hypertensive by 14 weeks of age without surgical, dietary, or drug manipulation. The role of the renin-angiotensin system in the etiology and maintenance of the hypertension is unclear. deJong et al. (2) have found that plasma renin activity in the spontaneously hypertensive rat increases with age after the onset of the hypertension, although Koletsky et al. (3) have found normal levels of renin activity in plasma from the renal vein and Sokabe (4) has reported a low renal renin level. Sen et al. (5) have suggested that the renin system is important in the pathogenesis of the hypertension. They have reported high levels of plasma renin activity and renal renin in young spontaneously hypertensive rats, and they have also found that these levels decrease with age.

Recent data have indicated that the adrenergic nervous system mediates renin release (6, 7). Propranolol, a potent beta-receptor blocking agent, can acutely lower plasma renin activity and blunt the anticipated rise in activity caused by renal nerve stimulation (8), hypoglycemia (9), epinephrine (9), upright posture (7), and diuretics (7). Propranolol has also been used to treat human hypertension (10). Its mechanism of action is unclear, but acute administration lowers cardiac output (11, 12). Chronic administration, however, appears to lower blood pressure by decreasing peripheral resistance (13). Buhler and co-workers (14) have proposed that its hypotensive effect is related to the fall in plasma renin activity. The purpose of the present study was to investigate the renin-angiotensin system in the spontaneously hypertensive Wistar rat and to determine the effect of propranolol administration on this system and the hypertension in these rats.

Methods

A colony of spontaneously hypertensive Wistar rats of the Okamoto strain was bred from breeder rats obtained from the National Institutes of Health. Only male rats were used for the experiments. Nonhypertensive normal Wistar and Sprague-Dawley rats served as controls. Systolic blood pressures were measured in unanesthetized rats; a pneumatic tail pulse transducer was used, and pressures were recorded on a Narco physiograph (model DMP4A). The rats were placed in a heated clear Lucite restraining cage and allowed to rest and acclimate for 20 minutes before the recordings were made. An average of nine blood pressure measurements was made on 2 consecutive days, and the mean was recorded as the blood pressure in the data. Heart rates were determined from the pulse tracings. Plasma renin activity was measured by radioimmunoassay (15) for most experiments and in the others by bioassay (16). Both methods gave comparable results in our laboratory, but the use of
the radioimmunoassay allowed us to analyze many more samples in each assay.

Propranolol was injected intramuscularly twice a day in 0.2 ml of sterile water and supplied in the drinking water. Water consumption occurred mostly at night and averaged 30 ml/24 hours. There was no change in the amount of water or food consumed between control and treated rats. A dose of propranolol equal to the total intramuscular dose was added to each 30 ml of drinking water to provide additional medication during the night. Control rats received 0.2 ml of sterile water intramuscularly twice a day.

To estimate the duration of beta-receptor blockade after intramuscular administration of propranolol, three spontaneously hypertensive rats were given 1.6 mg of propranolol intramuscularly and their heart rate responses to 0.4 μg/kg of isoproterenol administered intravenously through their tail veins at hourly intervals for 5 hours were measured. Three saline-injected rats served as controls; they received similar injections of isoproterenol at hourly intervals. The heart rate was estimated from the tail pulse rate before and 1 minute after the isoproterenol injection.

All rats were killed by decapitation. Blood was collected in chilled tubes containing sodium ethylenediaminetetraacetate (EDTA) and immediately centrifuged at 4°C for 20 minutes; the plasma was stored at −20°C. Determinations of blood propranolol levels were kindly performed by Ayerst Laboratories, Montreal, Canada, using the fluorometric method of Shand et al. (17). The paired t-test was used for statistical analysis.

EXPERIMENT 1
Six spontaneously hypertensive rats (6–7 months old, 365 ± 14.8 [SE] g) were given 0.2 mg of propranolol intramuscularly twice a day and 0.4 mg/30 ml drinking water (total daily dose 2 mg/kg) for 15 days. Six age-matched spontaneously hypertensive rats served as controls. Blood pressure and heart rate were measured during the control period and after 3 and 10 days of propranolol administration; the rats were killed after 15 days. Blood pressure and heart rate measurements and blood collection were carried out 1 hour after the propranolol injection. Renin was measured by bioassay. Kidneys, heart, and adrenal glands were removed, cleaned, and weighed.

EXPERIMENT 2
Seven spontaneously hypertensive rats (6–8 months old, 355 ± 14.06 g) were given 0.2 mg of propranolol intramuscularly twice a day and 0.4 mg/30 ml drinking water for 8 days and then both the intramuscular and oral doses were doubled for the next 7 days (total daily dose 5 mg/kg). On the eighth and fifteenth day, four rats received the intramuscular injection 1 hour before blood pressure and heart rate measurements and blood collection were carried out 1 hour after the propranolol injection. Renin was measured by bioassay. Kidneys, heart, and adrenal glands were removed, cleaned, and weighed.

EXPERIMENT 3
Six spontaneously hypertensive rats (6–8 months old, 350 ± 6.11 g) were placed on a sodium-free diet (18) and received 1.6 mg of propranolol intramuscularly twice a day and 3.2 mg/30 ml drinking water (total daily dose 18 mg/kg). Two rats lost weight and died; they were excluded from the data. Six littermates on the sodium-free diet served as controls. Blood pressure and heart rate were measured on the eighth and fifteenth days 1 hour after propranolol injection, and on the sixteenth day the rats were decapitated 1 hour after injection. Blood, heart, kidneys, and adrenal glands were obtained. Plasma renin activity was measured by radioimmunoassay.

EXPERIMENT 4
Sixteen spontaneously hypertensive rats (5–7 months old, 358 ± 4.62 g) were given 10 mg of propranolol intramuscularly. Four rats were killed each hour for 4 hours. Plasma propranolol levels were measured to determine the half-life of propranolol.

EXPERIMENT 5
Twelve prehypertensive spontaneously hypertensive rats (4 weeks old, 82.5 ± 2.14 g) with a mean blood pressure of 98.33 ± 17.2 mm Hg were divided into two groups. Six rats were fed the sodium-free diet and six the normal-sodium diet containing 102 mEq Na/kg. After 2 weeks all of the rats were killed. Blood was collected, and plasma renin activity was measured by radioimmunoassay. Plasma renin activity was also measured in other spontaneously hypertensive rats, normal Wistar rats, and normal Sprague-Dawley rats of various ages on normal-sodium and sodium-free diets and in spontaneously hypertensive rats 2 days after bilateral nephrectomy.

Results

PROPRANOLOL EFFECT ON BLOOD PRESSURE, HEART RATE, AND ORGAN WEIGHT

Figures 1 and 2 show that propranolol-treated rats had lower heart rates 1 hour after intramuscular administration of the drug (P < 0.01–0.05) but not 16 hours after administration. In rats on a normal-sodium diet, propranolol produced a paradoxical rise in blood pressure 1 hour after injection (P < 0.01). The low-sodium diet appeared to prevent the hypertensive effects of propranolol but not the reduction in heart rate (P < 0.01) (Fig. 3).

The plasma disappearance rate of propranolol in the spontaneously hypertensive rat is shown in Figure 4; the half-life is 78 minutes. The observed fall in heart rate 1 hour but not 16 hours after propranolol injection is consistent with the short half-life and suggests that the oral intake of propranolol overnight had little physiological effect. However, in the three rats given propranolol intramuscularly 16 hours prior to death, plasma propranolol levels were 230, 660, and 0 ng/ml. Levels high enough to be considered within the pharmacologic range were obtained in two rats. There was a slight but not significant fall in heart rate. In the four rats receiving propranolol intra-
Effect of 0.2 mg of propranolol administered intramuscularly twice a day and 0.4 mg/day taken orally on heart rate and blood pressure. The fall in heart rate (HR) and the rise in blood pressure (BP) are statistically significant in the propranolol-treated group. Means ± se are shown. Days indicate number of days of propranolol treatment.

Effect of 0.2 mg of propranolol administered intramuscularly twice a day and 0.4 mg/day taken orally for 8 days and 0.4 mg intramuscularly twice a day and 0.8 mg/day orally for 7 more days. Propranolol injected 1 hour but not 16 hours before measurements lowered heart rate (HR) and raised blood pressure (BP). The P value is the significance between the bars with asterisks.

Effect of 1.6 mg of propranolol administered intramuscularly twice a day and 3.2 mg/day taken orally on blood pressure (BP) and heart rate (HR) in spontaneously hypertensive rats fed a diet containing no sodium. Heart rates but not blood pressure are significantly different in the treated group (P < 0.01).

Muscularly 1 hour before death, the plasma levels were 395, 459, 520, and 915 ng/ml.

To estimate the duration of beta-receptor blockade, three spontaneously hypertensive rats were given 1.6 mg of propranolol intramuscularly, and their heart rate responses to intravenously administered isoproterenol were measured at hourly intervals for 7.5 hours. Three saline-injected rats served as controls. As can be seen in Table 1, basal heart rate and the response to isoproterenol remained suppressed for at least 5 hours in the propranolol-treated rats. At 7.5 hours, one of the two rats successfully injected with isoproterenol still had a suppressed response.

Plasma propranolol levels after 10 mg of propranolol was administered intramuscularly to 16 spontaneously hypertensive rats. Four rats were killed each hour for 4 hours. T½ = half-life.
Duration of Beta-Receptor Blockade following Intramuscular Administration of Propranolol in Spontaneously Hypertensive Rats

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Propranolol-treated rats received 1.6 mg of propranolol intramuscularly after 0-time measurements. Estimation of beta-receptor blockade was made from the heart rate response to 0.4 μg isoproterenol/kg body weight. Control rats received saline rather than propranolol. The two values given for each time indicate heart rate before and 1 minute after the isoproterenol injection.

* Prolonged manipulation of this rat before successful isoproterenol injection.

Propranolol had no effect on adrenal, heart, or kidney weight.

PLASMA RENIN ACTIVITY IN THE SPONTANEOUSLY HYPERTENSIVE RAT

Propranolol did not lower the plasma renin activity significantly in any experiment, although in experiments 2 and 3 there was a trend toward lower activity after propranolol administration (Fig. 5). Of particular interest, however, is the fact that plasma renin activity was not stimulated in the 6-8-month-old spontaneously hypertensive rats by ether anesthesia and laparotomy (experiment 2) or sodium depletion (experiment 3). Therefore, the blocking effect of propranolol on renin release could not be evaluated.

Basal plasma renin activity (0.9–3.5 ng/ml hour⁻¹) measured in spontaneously hypertensive rats on a normal-sodium diet and without prior stress remained normal and relatively constant and did not change with age (Fig. 6). Postnephrectomy, plasma renin activity measured by radioimmunoassay (0.35 ± 0.2 ng/ml hour⁻¹) was very

![Graph](image_url)

**FIGURE 5**

Plasma renin activity (PRA) in spontaneously hypertensive rats (experiments 1, 2, and 3). Experiments 1, 2, and 3 indicate the same rats described in Figures 1, 2, and 3, respectively.

![Graph](image_url)

**FIGURE 6**

Basal plasma renin activity (PRA) in spontaneously hypertensive rats of various ages. No significant differences were seen at any age. Plasma renin activity 2 days after nephrectomy (Nepex) was significantly lower than that for the age-matched controls (P < 0.01). The double dagger indicates that plasma renin activity was measured by bioassay.

*Circulation Research, Vol. 35, August 1974*
low. The young prehypertensive spontaneously hypertensive rats 4 weeks of age had a marked response to sodium depletion, but they failed to respond to sodium restriction or the combined stress of ether anesthesia and laparotomy after the onset of hypertension (Fig. 7). Wistar and Sprague-Dawley rats 6-8 months of age (mean weight 400 ± 27 g) responded to the low-sodium diet, but the response of the Wistar rats was blunted. At 12-14 months of age, normotensive Wistar rats did not respond at all to sodium depletion. A stepwise decrease in response to sodium depletion with age was seen in the spontaneously hypertensive rats and the Wistar rats but not in the Sprague-Dawley rats. Since the spontaneously hypertensive rats were bred from the Wistar strain, the hyporesponsive renin system appears to be a feature of the rat strain. Furthermore, when the basal plasma renin activities of Wistar and Sprague-Dawley rats 6-8 months of age, studied simultaneously on a normal-sodium diet, were compared, the plasma renin activity of the Wistar rats was significantly lower than that of the Sprague-Dawley rats (1.20 ± 0.30 vs. 2.04 ± 0.33 ng/ml hour⁻¹, P < 0.05).

**Discussion**

Propranolol administration to spontaneously hypertensive rats for up to 15 days did not lower blood pressure. A paradoxical rise occurred in the treated rats on a normal-sodium diet 1 hour after intramuscular injection at a time when heart rates were significantly lowered, indicating physiological beta-receptor blockade. This increase in blood pressure could be compensatory and related to increased alpha-adrenergic activity in response to a fall in cardiac output. Although we did not measure cardiac output, propranolol does lower it (13). The rats fed a low-sodium diet did not manifest this increase in blood pressure, possibly because the alpha-adrenergic system was maximally stimulated by volume depletion.

Failure to develop a sustained chronic hypotensive effect and slowing of the heart rate could be related to propranolol's short half-life in the rat. Although plasma levels can be dissociated from propranolol's effect on exercise or isoproterenol-induced tachycardia, adequate blood levels must be attained to observe blockade of beta receptors (19-21). If the hypotensive action observed in man requires sustained inhibition of the beta-adrenergic system, then the dose schedule we used may not have been sufficient. However, plasma propranolol levels of 572 ± 136 ng/ml 1 hour after the intramuscular injection of 0.4 mg of propranolol were measured and are certainly above the effective range of 30-50 ng/ml in man (21, 22). Data on propranolol's effective plasma level in spontaneously hypertensive rats is not available; however, on a per weight basis (up to 18 mg/kg) the doses we used were large, yet they did not cause a hypotensive effect. Acute oral administration of much larger doses of beta-receptor blocking agents, including propranolol, in 4-month-old spontaneously hypertensive rats has recently been shown (23) to lower blood pressure. If propranolol's chronic hypotensive effect is due to the lowering of peripheral resistance, as has been shown in man (13), and not just to a fall in cardiac output, then it is possible that this fall in peripheral resistance does not occur in the spontaneously hypertensive rat, so that these rats remain hypertensive.

If renin is important in the hypertension of the spontaneously hypertensive rat, then the failure of propranolol to lower plasma renin activity could explain its failure to lower blood pressure. However, there is no evidence to indicate that the renin-angiotensin system is necessary for the maintenance of the hypertension. Hypertension persists after nephrectomy (4) and after infusion of an angiotensin II inhibitor (Mulrow and Forman, unpublished data). There is some question concerning the role of renin in the development of the hypertension. Sen et al. (5) claim that renin increases during the early phase of the hypertension, but de Jong et al. (2) claim that plasma renin activity increases after the establishment of the

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**Plasma renin activity in spontaneously hypertensive rats (SHR), normotensive Wistar rats, and Sprague-Dawley rats of various ages. All rats showed a decrease in the response to sodium depletion as they grew older, but the spontaneously hypertensive and Wistar rats had a markedly suppressed response at 6-8 months of age. Asterisks indicate measurement of plasma renin activity by bioassay.**

*Circulation Research, Vol. 35, August 1974*
hypertension. Our basal values showed no difference throughout the age spans we studied. These discrepancies could be related to differences in methodology or collection of blood. Collection of blood by decapitation as performed by us and de Jong gives lower basal levels than does collection under anesthesia (24), which is a stimulus to renin. The plasma renin activity is renal in origin, since bilateral nephrectomy markedly lowered plasma renin activity measured by radioimmunoassay (0.35 ± 0.2 ng/ml hour⁻¹). This finding contrasts with that of de Jong et al. (2) who reported persistence of normal plasma renin activity measured by bioassay after nephrectomy. Renin levels measured by bioassay are usually higher than those measured by radioimmunoassay (25), although a recent study by Kotchen et al. (26) in humans, using methods similar to ours, has shown an excellent correlation between bioassay and radioimmunoassay.

Even though we did not lower renin levels chronically, most of our blood pressure and heart rate measurements were done 1 hour after intramuscular propranolol administration at a time when plasma propranolol levels were highest and were in the pharmacologic range. This fact suggests that at least acute propranolol administration does not lower blood pressure or acutely lower plasma renin activity.

Inability to lower basal plasma renin activity with propranolol (experiment 1) suggests that basal plasma renin activity is not regulated by the beta-adrenergic system in the spontaneously hypertensive rat or that much larger doses of propranolol are needed. We were unable to show an inhibition by propranolol of the renin response to two potent stimuli in the older spontaneously hypertensive rats, since neither ether anesthesia and laparotomy nor sodium restriction stimulated plasma renin activity. This hyporesponsive renin system is not a feature of the rat strain, since young spontaneously hypertensive rats respond appropriately. Age, however, appears to be a factor; normotensive Wistar rats, the strain from which the spontaneously hypertensive rats were developed, also have a diminished response of plasma renin activity to sodium restriction as they age. This phenomenon could be analogous to the increased incidence of "low-renin" hypertension in older patients (27). Hypertension could accelerate the development of a hyporesponsive renin system possibly by degenerative changes in the juxtaglomerular apparatus (28). Although the effect of hypertension on the acceleration of the hyporesponsive renin state was not studied in detail in our experiments, the failure of the 6–8-month-old spontaneously hypertensive rats to respond to sodium depletion even though the 6–8-month-old normotensive Wistar rats had a modest response does suggest that hypertension can hasten the hyporesponsive state. This possibility is supported by the report of Sen et al. (5) who have found elevated plasma renin activity and renal renin content in young hypertensive rats but decreased levels as the rats age. The blood for determination of plasma renin activity was collected during ether anesthesia in their experiment, however, and thus their values represent stimulated rather than basal levels.

The possibility that the hypertension in spontaneously hypertensive rats is caused by oversecretion of adrenal mineralocorticoids such as 18-hydroxy-deoxycorticosterone, as suggested by Melby et al. (29) in humans, is unlikely, since the hypertension persists in the rats after bilateral adrenalectomy if they are given 1% saline to drink (30).

Normotensive Wistar rats and spontaneously hypertensive rats, therefore, may serve as useful models for studying the factors leading to a hyporesponsive renin system and the etiology of human essential hypertension.

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Circ Res. 1974;35:215-221
doi: 10.1161/01.RES.35.2.215

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