**Adrenergic Component of Renin Release Induced by Vasodilating Antihypertensive Drugs in the Rat**

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**ABSTRACT**

The capacity of the vasodilating drugs, minoxidil and hydralazine, for inducing renin release was characterized in rats according to chronology and dose response. Propranolol inhibition of this renin release was also characterized and related to serum levels of propranolol. Minoxidil and hydralazine (1.0 mg/kg) induced sevenfold elevations of serum renin activity. Treatment with propranolol, resulting in plasma propranolol concentrations as low as 50 ng/ml, impaired vasodilatory drug-induced renin release. The propranolol inhibition suggests a beta-adrenergic component to this type of renin release and the potential for a clinically efficacious drug interaction.

**KEY WORDS**

hydralazine beta-receptor blockade propranolol minoxidil

The combination of a vasodilating drug and propranolol can control hypertension in man without precipitating many untoward effects (1-5). Vasodilating agents elicit renin release from the kidney which results in elevated serum renin activity and increased angiotensin formation (6, 7). The angiotensin thus formed probably antagonizes the antihypertensive effect of vasodilating drugs (8) such as hydralazine and minoxidil. Since combined therapy with propranolol and vasodilating drugs has been found to be particularly effective (1-5), this study was designed to characterize vasodilatory drug-induced renin release in the rat and to determine the effect of beta-receptor blockade on this release.

**Methods**

Male Wistar rats (180-250 g) were housed in individual cages and exposed to light by an automated system from 6 AM to 6 PM. The rats ingested tap water and Purina rat chow containing 152 mEq sodium/kg ad libitum.

Both hydralazine and minoxidil were administered intraperitoneally, and propranolol was injected subcutaneously. An equal volume (0.5 ml) of saline was administered as a placebo to the control rats. Doses of drugs were calculated for the salt in mg/kg.

Propranolol was solubilized in 0.9% NaCl by adding several drops of glacial acetic acid. Propranolol was administered subcutaneously because of the constant, sustained plasma levels following this route of administration (Fig. 1). Intraperitoneal injection of propranolol resulted in a rapid rise and fall and a high degree of variability in serum levels among the rats. Alternatively, subcutaneous administration resulted in more sustained plasma propranolol levels with minimal variability among the rats. Thus, the sustained plateau (Fig. 1) from the subcutaneous administration permitted the experiments to be done at relatively constant, predictable propranolol serum levels.

In the experiments involving beta-receptor blockade, propranolol was injected at 0 time and the vasodilating drug was injected at 10 minutes; the rats were decapitated at 30 minutes. The rats treated with a vasodilating drug alone or with saline were killed 20 minutes after injection.

At the times indicated in Results, the unanesthetized rats were decapitated, and aortic blood samples were collected in iced, siliconized glass tubes during the first 4 seconds after decapitation. In propranolol-treated rats, the remaining blood collected after the first 4 seconds was used for assay of propranolol. All samples were allowed to clot and were centrifuged at 12,000 g at 4°C for 20 minutes; the serum was frozen (−20°C) in capped plastic tubes until assay.

**RENIN ASSAY**

Serum renin activity was quantified as previously described (9) with several modifications. After slowly thawing the samples on ice, the serum was treated with the angiotensinase inhibitors diisopropylfluorophosphate (DFP) and ethylenediaminetetraacetate (EDTA), and the pH was adjusted with 1.0M citrate buffer, pH 5.35 (v/v:1/10). The resulting pH in the treated samples ranged from 6.4 to 6.55, which is the plateau portion of the pH optimum curve for rat renin determined in our laboratory. This plasma mixture was incubated at 37°C for 3 hours; it was then immersed in an ice bath, and angiotensin was quantified by radioimmunoassay (9). Radioiodinated angiotensin I (Schwarz/Mann) was purified chromatographically (9) and diluted with 0.1M Tris buffer at pH 7.5 (containing 0.2% lysozyme) to...
PROPRANOLOL BLOCK OF RENIN RELEASE

Figure 1

Serum propranolol concentrations in the rat at intervals following administration of 15 mg/kg, sc or ip. Each value is the mean ± se for five rats.

Results

The vasodilating drugs, minoxidil and hydralazine, induced a dose-related increase in serum renin activity (Figs. 2 and 3). The chronology of renin release with these two agents is given in Figures 4 and 5. The peak increase in serum renin activity with hydralazine occurred earlier (20 minutes) than that with minoxidil (45 minutes), but the latter may have a longer duration of action. The serum renin activity remained significantly elevated above control levels for both vasodilating agents 5 hours after administration. Treatment of rats with a 0.3-mg/kg dose of propranolol inhibited (P < 0.01) minoxidil- and hydralazine-induced renin release by 78% and 85%, respectively (Figs. 6 and 7). Comparable decreases for a 1.5-mg/kg dose of propranolol were 89% and 91%, respectively. A 15-mg/kg dose of propranolol had no greater effect than did a 1.5-mg/kg dose. Thus, the 0.3-mg/kg and 1.5-mg/kg doses of propranolol are near the plateau portion of the dose-response curve. Serum propranolol levels for the 0.3-mg/kg and 1.5-mg/kg doses were 49 ± 4 and 220 ± 19 ng/ml, respectively. Thus, the majority of the impairment of renin release was

Propranolol assays

Propranolol concentration in serum was determined according to the procedure of Shand et al. (10) with several modifications. A 1-ml sample of serum was alkalized with 0.25 ml of 1.0N NaOH and extracted with redistilled heptane containing 1.5% isoamyl alcohol. The organic phase was removed after 10 minutes of shaking, and the propranolol was reextracted into 1.5 ml of 0.1N HCl. Fluorescence of the acid layer was quantified in an Aminco-Bowman fluorescent spectrophotometer (maximum excitation 300 nm and maximum emission at 360 nm). The standard curve was constructed by using 0.1N HCl which had been used in the extraction of serum from control (saline-injected) rats, since quenching was consistently present from serum. The percent of recovery using this technique varied from 85% to 95%, and the results were corrected accordingly.

Give 8,000–10,000 counts/0.002 ml. The antibody to angiotensin I and other components of the assay have been previously described (9).

Figure 2

Dose response of minoxidil-induced renin release. Blood for assay of serum renin activity (S.R.A.) was collected 40 minutes after administration of minoxidil. Each value is the mean ± se for five rats. A1 = angiotensin I.

Figure 3

Dose response of hydralazine-induced renin release. Blood for assay of serum renin activity (S.R.A.) was collected 20 minutes after administration of hydralazine. Each value is the mean ± se for five rats. A1 = angiotensin I.
accomplished by plasma propranolol levels as low as 50 ng/ml.

Discussion

Various stimuli cause renin release from the juxtaglomerular cells of the kidney (11, 12). In addition to direct neurogenic and hemodynamic stimuli, many drugs induce renin release (6, 13-15). The renin release induced by vasodilating drugs could be mediated by at least four mechanisms: (1) increased sympathetic discharge to the juxtaglomerular cells of the kidney, (2) elevated levels of circulating catecholamines, (3) hemodynamic changes in the kidney due to vasodilation with altered renal perfusion, and (4) a decrease in sodium load reaching the macula densa segment of the distal tubule.

In the present study, propranolol, even at low serum concentrations (50 ng/ml), prevented more than 85% of the renin release induced by the vasodilating drugs, minoxidil and hydralazine. This reduction supports the hypothesis that a significant portion of vasodilatory drug-induced renin release is mediated by the beta-adrenergic component of the sympathetic nervous system. This interpretation is consistent with that of Loeffler and his associates (16) who have shown that direct stimulation of renal nerves in anesthetized dogs causes renin release and that this response can be blocked with propranolol. An increase in circulating catecholamines can also contribute to renin release by a beta-adrenergic mechanism (6), and this effect would likewise be blocked by propranolol.

The possibility that other mechanisms contribute to this type of renin release is suggested by the fact that propranolol did not suppress the increase in serum renin activity induced by vasodilating drugs to, or below, the mean control values in any of our experiments. The chronology and the pattern of renin release (Fig. 5) are similar to those for the lowering of blood pressure by identical doses of hydralazine (8) in DOC-NaCl hypertensive rats. With higher doses of hydralazine (6 mg/kg), which induce greater reductions in blood pressure, renin release was less effectively (50%) blocked by propranolol. Further increments in the dose of propranolol did not overcome the effect of high
doses of vasodilating drugs. Incidentally, other pharmacokinetic studies have shown that plasma propranolol concentrations of 50 ng/ml and 220 ng/ml cause a 3.5-fold and a 20-fold displacement to the right, respectively, of the isoproterenol (tachycardia) dose-response curve in rats. Consequently, these doses are effective beta-blocking doses of propranolol. Thus, hemodynamic effects may contribute in some way to the non-beta-adrenergic component of this renin release, particularly with extremely high doses of vasodilating drugs.

The potential importance of drug-induced alteration of renin release is suggested by the recent correlation between the impairment of renin release and the antihypertensive activity of propranolol alone in hypertensive patients by Buhler et al. (17). If propranolol-induced impairment of endogenous renin release is relevant as an antihypertensive mechanism, then its effect on vasodilatory drug-induced renin release may be extremely important in the hypotensive effects of this drug combination. Propranolol alone induces a modest blood pressure reduction in severely hypertensive patients (17). However, when it is combined with minoxidil during chronic therapy, the blood pressure can be reduced virtually to normal levels (2, 5).

The angiotensin formed from increased renin release would be expected to antagonize the antihypertensive activity of vasodilating drugs because of the dynamic interrelationships of these vasoactive substances at the vascular smooth muscle level (8). The increment in angiotensin formation should cause sodium retention due to excess aldosterone secretion resulting in further impairment of the lowering of blood pressure by vasodilating drugs alone. Also, angiotensin possesses numerous pharmacologic activities, each of which contribute qualitatively to the elevation of blood pressure (18-24) and, under certain circumstances, induce pathologic vascular lesions (25, 26). Because of these multiple activities of angiotensin, the blockade of renin release by propranolol in...
patients treated with vasodilating agents could be an important factor contributing to the efficacy of this drug combination.

Preliminary hemodynamic investigations show synergistic hypotensive effects in rats when pranorolol is combined with hydralazine or minoxidil (27). These observations are consistent with an important hypertensive role of impaired renin release by propranolol in the drug combination. Also, since propranolol adds to the lowering of blood pressure while impairing renin release, the lowering of blood pressure per se is not the sole mechanism of the renin release.

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References

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