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Plasma Pseudorenin in Rats after Alteration in the Renin-Angiotensin System[1] (39590)

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An apparently new enzyme exhibiting renin-like activity but having a lower pH optimum has been described and partially characterized by Skeggs *et al.* (1), who conveniently refer to this enzyme as pseudorenin. Pseudorenin has been reported to occur in various tissues in the rat, but unlike renin it is not concentrated in the kidney (1). Pseudorenin activity is also present in the plasma of rats and humans. Preliminary studies showed no correlation between plasma pseudorenin and blood pressure in normal controls and in hypertensive patients (1). Since nothing is known concerning the factors which regulate plasma pseudorenin, we have initiated a study to investigate possible regulatory mechanisms. Initially, we utilized surgical and pharmacological interventions known to alter plasma renin activity. Both renin and pseudorenin levels in plasma were monitored in rats after nephrectomy or β-adrenergic blockade, which lower plasma renin (2-4), and after inhibition of converting enzyme, which has been shown to increase plasma renin (5).

Methods. Male Wistar rats (300-500 g) were anesthetized with pentobarbital sodium (50 mg/kg ip) and the left carotid artery and right jugular vein were cannulated with PE 50 tubing. In four rats, bilateral nephrectomy was performed and 0.3-ml arterial blood samples were collected immediately after nephrectomy and at 30-min intervals up to 130 min. A final sample was obtained at 180 min after nephrectomy. Samples were collected in small tubes containing 15 μl of 300 m*M* EDTA as an anticoagulant and centrifuged for 15 min to yield plasma. Fluid volume was replaced with 0.9% NaCl after each collection.

In four rats, propranolol (Inderal HCl, injectable), 1 mg/kg, was injected iv at zero time and a supplemental dose of 1 mg/kg was given at 30 min after the first injection. Blood samples were obtained as described above at 0, 30, and 60 min, and blood volume was replaced with 0.9% saline. Blood samples were obtained from four control rats following the schedule outlined for the nephrectomized group.

For the experiment utilizing the converting enzyme inhibitor SQ 20881 (CEI), two rats were catheterized and both kidneys of one rat were removed. Two hours after nephrectomy, 400 μg of CEI was injected iv into each rat followed by 400 μg im. Arterial blood samples (0.3 ml) were collected at 0, 5, 10, 20, 40, and 80 min after CEI injection. Volume was replaced with 0.9% NaCl.

Plasma angiotensin generation was measured by the radioimmunoassay technique of Haber *et al.* (6). For renin-dependent angiotensin generation, 30 μl of plasma was incubated for 2 hr at pH 7.0 with bovine serum substrate (500 pmoles/ml) in the presence of angiotensinase inhibitors (BAL, EDTA, and 8-hydroxyquinoline). Bovine substrate was prepared by the method of Lever *et al.* (7). Pseudorenin-dependent angiotensin generation was obtained by incubating 10-μl plasma samples with tetradecapeptide substrate (500 pmoles/ml) at pH 4.5 for 1 hr. The tetradecapeptide substrate was obtained from Schwarz/Mann. Angiotensin was assayed by radioimmunoassay using components from New England Nuclear. Enzyme assays were linear within the range utilized. Results were calculated as nanograms per milliliter per hour of angiotensin generated. For comparison, results are graphically expressed as percentage of the zero-time sample activity.

Results. The effect of bilateral nephrectomy on both pseudorenin and renin levels in the rat is shown in Fig. 1. In the nonoper-

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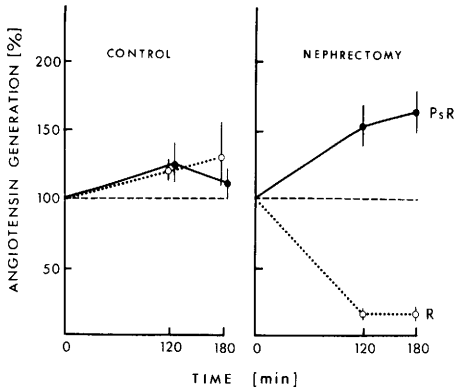

FIG. 1. Effect of bilateral nephrectomy on plasma renin and pseudorenin. (●——●) PsR, pseudorenin; (O-----O) R, renin; both are expressed as percentage of zero-time value. Each point represents mean ± SE of four rats. Zero-time renin and pseudorenin levels were 17.3 ± 3.3 ng/ml/hr of angiotensin and 393 ± 79 ng/ml/hr of angiotensin, respectively.

ated controls, plasma pseudorenin and renin both appear to increase slightly with time, although the only significant increase compared with zero time occurs in the renin levels at 120 min ($P < 0.05$). This apparent increase in plasma renin is probably associated with an increased renin release in response to hemorrhage, as has been demonstrated previously (8, 9). After nephrectomy, renin levels fell to 20 ± 5% of the zero time and remained at this level at 3 hr, whereas pseudorenin significantly increased above the zero time to 165 ± 17% at 180 min ($P < 0.05$) (Fig. 1). These data clearly indicate a nonrenal source for plasma pseudorenin.

In rats pretreated with propranolol, results similar to those after nephrectomy were obtained. Sixty minutes after initial propranolol treatment, renin plasma levels fell to 33 ± 9% of the zero-time value (Fig. 2). Pseudorenin levels increased significantly to 127 ± 11% of zero time at 30 min ($P < 0.05$), but at 60 min there was no significant increase. These data indicate that pseudorenin release is not under tonic β-adrenergic influence unlike that demonstrated for renin release (8, 9). Preliminary work in our laboratory has also suggested that pseudorenin is not released in response to α-adrenergic or cholinergic stimulation, although more experiments are needed to verify this.

Since plasma pseudorenin levels tended to increase with a decrease in plasma renin, we decided to see if pseudorenin release is under feedback control of angiotensin II as has been demonstrated for renin release (3, 10–12). In the rat with functional kidneys, CEI treatment caused plasma renin levels to increase to 350% of the zero-time level 20 min after treatment, whereas pseudorenin levels failed to change significantly from the zero-time level (Fig. 3). In the nephrectomized rat, renin levels were at less than 10% of the zero-time level of the nonnephrectomized rat when CEI was injected. As expected, renin levels did not increase at any time after CEI treatment. Pseudorenin again did not change significantly from the zero time (Fig. 3).

Discussion. Evidence presented here demonstrates further the presence of an enzyme in rat plasma which exhibits renin-like activity *in vitro* first described by Skeggs *et al.* (1).

Although both renin and pseudorenin will generate angiotensin *in vitro,* this probably does not occur in plasma with pseudorenin *in vivo* since it is inactive on plasma substrate at physiological pH (1). Lack of response of plasma pseudorenin levels to alterations in plasma renin produced by nephrectomy, β-blockade, or converting enzyme inhibition, also suggests that pseudorenin is not involved in the renin–angiotensin system. The physiological role of circu-

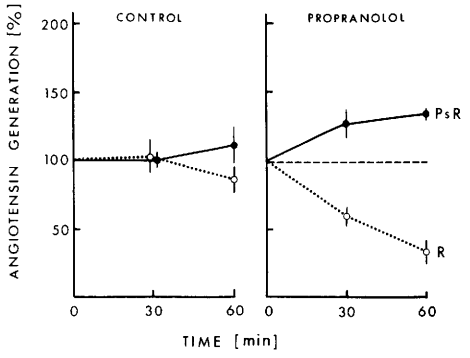

FIG. 2. Effect of propranolol pretreatment on plasma renin and pseudorenin. (●——●) PsR, pseudorenin; (O-----O) R, renin; both are expressed as percentage of zero-time activity. Each point represents mean ± SE of four rats. Zero-time renin and pseudorenin levels were 28.7 ± 6.0 ng/ml/hr of angiotensin and 470 ± 71 ng/ml/hr of angiotensin, respectively.

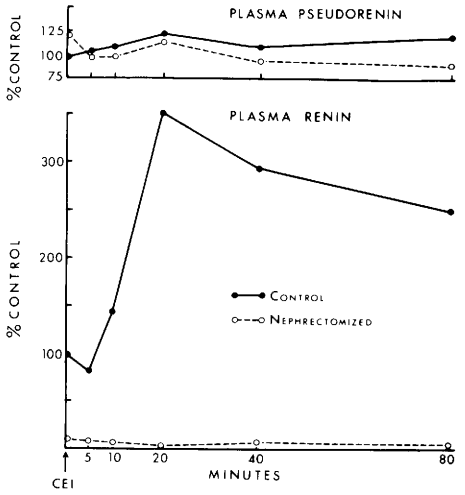

FIG. 3. Effect of treatment with SQ 20881 (400 μg, iv) on plasma pseudorenin and renin levels in a normal and a nephrectomized rat. (●——●) Control; (○-----○) nephrectomized. Top portion represents pseudorenin values expressed as percentage of the zero-time activity of the nonnephrectomized rat. Lower portion shows renin levels expressed as percentage of the zero-time activity in the nonnephrectomized rat.

lating pseudorenin, if one exists, is not known. More investigation into the site of production and physiological mechanisms which govern formation and release of pseudorenin is needed before any conclusions concerning its physiological role can be drawn.

Summary. Plasma levels of renin and pseudorenin were measured at various times in control rats, and after bilateral nephrectomy, treatment with propranolol, or the converting enzyme inhibitor (CEI) SQ 20881. After nephrectomy or propranolol, renin levels decreased but pseudorenin levels increased. Following treatment with CEI, renin levels rose in a nonoperated animal and remained very low in a nephrectomized animal. Pseudorenin levels did not change appreciably after CEI in either animal.

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