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Role of Prostaglandins, β-Adrenoceptors, and the Central Nervous System in the Control of Renin Release in Conscious Sodium-Depleted Rats (40951)

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Abstract. The interrelationship of prostaglandins, β-adrenoceptors, and the central nervous system in the control of renin release in conscious sodium-depleted rats was investigated by acute pharmacologic blockade with systemic administration of indomethacin and propranolol and intracerebroventricular injection of clonidine. Indomethacin and propranolol lowered plasma renin activity significantly with respect to control. When both were administered to the same experimental animals, their renin-lowering effect was additive. Intracerebroventricular clonidine and intraperitoneal propranolol or the injection of both produced a similar renin-lowering effect on sodium-depleted rats. It is concluded that the central nervous system, through an α-adrenergic pathway peripherally mediated by β-adrenergic receptors, plays an important role in the renin release in the conscious sodium-depleted rat. A prostaglandin-mediated pathway is also involved in the renin response to the sodium-depleted state, acting in parallel to the sympathetic nervous system. Since renin does not return to normal after blockade of both pathways, other mechanisms may also be operative.

The role of different factors in the regulation of renin release has received considerable attention (1). Mechanisms implicated in the striking increase in renin secretion observed during low sodium intake or diuretic therapy include a change in the delivery of sodium or chloride to the macula densa (2, 3), the activation of a renal vascular baroreceptor (4), and an increase in sympathetic nervous activity (5). Central nervous system stimulation has been shown to increase renin release (5, 6). The dominant role of the sympathetic nervous system in renin release induced by hypotensive drugs in conscious rats has recently been demonstrated (7). The influence of the central and peripheral nervous system on renin release during chronic sodium depletion has remained controversial. Some investigators have shown that denervation reduced the renin response to sodium depletion (8–10), whereas others have failed to see any effect (11, 12). Modulation of diuretic-induced renin secretion by the renal nerves has been demonstrated (13). In chronic situations with renal denervation, other release mechanisms may take over, giving rise to the conflicting evidence in the literature.

More recently, prostaglandins have been shown to stimulate renin release (14–16). We have investigated the role of humoral and nervous influences (i.e., prostaglandins, β-adrenoceptors, and the central nervous system) on renin secretion in conscious sodium-depleted rats by acute pharmacologic blockade of the different mechanisms possibly involved. Indomethacin, an inhibitor of prostaglandin cyclo-oxygenase (17), was used to block prostaglandin synthesis. Propranolol was employed to block β-adrenergic receptors mediating renin release (18). Clonidine, a centrally acting presynaptic α-adrenergic drug which produces a decrease in sympathetic nervous activity (19, 20), was administered intracerebroventricularly (icv) to evaluate the role of the central nervous system in renin release after sodium depletion. Clonidine has previously been shown to reduce renin secretion by acting on the central nervous system (21).

Materials and methods. Male Sprague–Dawley rats weighing 200 to 250 g were kept in individual cages and exposed to light by an automated system from 6 AM

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to 6 PM. Rats were fed a sodium-deficient diet containing less than 5 mmole/kg of sodium (Hartroft Test diet, United States Biochemical Corp., Cleveland, Ohio) for 2 weeks. They drank demineralized water *ad libitum*. Furosemide (10 mg/kg/day) was administered intraperitoneally on the first 2 days.

Prostaglandin synthesis and β-adrenoceptor blockade. On the day of an experiment, sodium-depleted rats were injected subcutaneously (sc) with 0.2 ml of olive oil or indomethacin (5 mg/kg) in 0.2 ml of olive oil. One hour later 0.3 ml of 5% dextrose in water or propranolol (10 mg/kg) in 0.3 ml of 5% dextrose in water was injected intraperitoneally (ip). Rats were decapitated 45 min later and blood from the trunk was collected on ice in glass tubes containing EDTA during the first 5 sec after decapitation. Blood was immediately centrifuged at 4°, and plasma was separated and stored at −20° until assayed for plasma renin activity (PRA).

Intracerebroventricular clonidine and β-adrenoceptor blockade. Rats were sodium-depleted as described above and 48 hr before the experimental day an icv cannula was implanted as described by Donaldson *et al.* (22). On the day of an experiment, rats received ip 0.3 ml of 5% dextrose in water or propranolol (10 mg/kg) in 0.3 ml of 5% dextrose in water. After 15 min, 20 μl of 0.9% NaCl or 0.9% NaCl containing 3 μg of clonidine was injected icv. Thirty minutes later rats were decapitated and blood was collected as described for PRA determination.

Clonidine−HCl was kindly provided by Dr. P. G. Fontana of Boehringer Ingelheim (Canada) Ltd. Propranolol−HCl and indomethacin were purchased from Sigma Chemical Company.

PRA was measured by the radioimmunoassay of angiotensin I generated during a 2-hr incubation of plasma at 37° and pH 6.5 (optimum pH for PRA in rats), as previously described (23). Normal values for conscious rats after subcutaneous injection of olive oil and intraperitoneal injection of 5% dextrose in water as for the first group of sodium-depleted rats were 3.22 ± 0.40 ng of angiotensin I generated per milliliter per hour (ng AI/ml/hr) (n = 6). Conscious rats implanted with an intraventricular cannula and injected intraperitoneally with 5% dextrose and with 20 μl of 0.9% NaCl icv as for the second group of sodium-depleted rats had a PRA of 1.80 ± 0.23 ng AI/ml/hr (n = 9).

Results are expressed as means ± SE. Statistical evaluation was done by one-way analysis of variance and the Newman−Keuls a posteriori test. When Bartlett's test showed that variances were not homogeneous, an analysis of variance was performed on the logarithm transform of the results (24). The null hypothesis was rejected when $P < 0.05$.

Results. Prostaglandin synthesis and β-adrenoceptor blockade. PRA in sodium-depleted rats injected sc with oil and ip with 5% dextrose was 29.4 ± 4.3 ng AI/ml/hr (Fig. 1). Rats treated with indomethacin sc had a lower PRA of 18.9 ± 2.6 ng AI/ml/hr ($P < 0.05$). Propranolol ip produced markedly lower PRA values of 11.6 ± 2.0 ng AI/ml/hr ($P < 0.01$ vs control; $P < 0.05$ vs indomethacin-treated). When rats were injected with both indomethacin and propranolol, PRA was reduced to a still greater extent to 5.8 ± 0.9 ng AI/ml/hr ($P < 0.05$ vs propranolol-treated).

Intracerebroventricular clonidine and β-adrenoceptor blockade. To study the actual participation of the central nervous system in the β-adrenergically stimulated renin release produced by sodium depletion, the individual and combined effects of icv clonidine and ip propranolol on PRA of

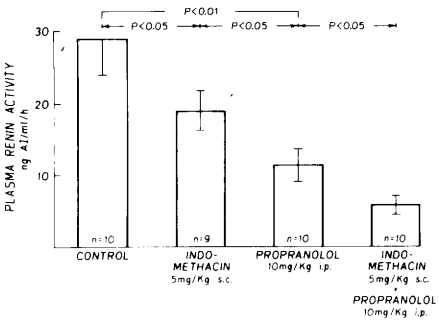

FIG. 1. Plasma renin activity of sodium-depleted rats receiving sc indomethacin, ip propranolol, or both.

sodium-depleted rats were compared. PRA in rats injected with 20 μl of 0.9% NaCl icv was 13.2 ± 1.9 ng AI/ml/hr. Sodium-depleted rats injected icv with clonidine exhibited a lower PRA of 8.5 ± 1.4 ng AI/ml/hr ($P < 0.05$) 30 min later (Fig. 2). Rats receiving propranolol ip and 0.9% NaCl icv showed a similarly decreased PRA of 7.9 ± 1.4 ng AI/ml/hr ($P < 0.05$ vs control, not significantly different from clonidine-treated rats). Rats receiving both clonidine and propranolol revealed no additive effect, as their PRA was 9.6 ± 1.2 ng AI/ml/hr ($P < 0.05$ vs control, not significantly different from clonidine- or propranolol-treated rats).

Discussion. These results suggest that humoral and nervous factors play a role in the renin release observed during a negative sodium balance. The effects of prostaglandin synthesis inhibition by indomethacin and β-adrenoceptor blockade by propranolol are additive. These data may be interpreted to the effect that prostaglandins and β-adrenergic receptors act through different pathways on renin release. Similar evidence has been obtained by Henrich *et al.* (25) for renin secretion during hemorrhage in the dog. Seymour and Zehr (26) have suggested that prostaglandins act directly on juxtaglomerular cells independently of β-mediated renin release. These findings are somewhat at variance with evidence produced by Campbell *et al.* (27), who found that renal prostaglandins are important mediators of sympathetically stimulated renin release. These authors suggested that prostaglandins act at a site distal to the β-adrenergic receptor in the normal rat. After sodium deprivation, prostaglandin synthesis in the kidney is greatly increased (28). Under these conditions prostaglandin-mediated renin release may become independent from adrenergic stimulation.

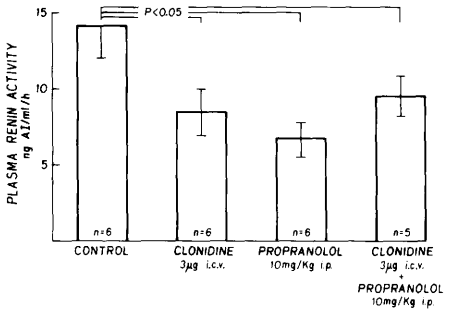

FIG. 2. Plasma renin activity of sodium-depleted rats receiving icv clonidine, ip propranolol, or both.

Circulating catecholamines stimulate renin release (29, 30). β-blockade-induced reduction of renin secretion does not prove a role for the renal nerves (31). However, a centrally acting drug like clonidine decreases renin to the same extent as propranolol. The combination of the two drugs does not decrease PRA further in sodium-depleted rats. The response to icv clonidine, which blocks noradrenergic pathways in the central nervous system through its α-adrenergic presynaptic agonist effect (19, 20), suggests that β-adrenoceptors may mediate the action of the central nervous system by way of the renal nerves. Intracerebroventricular administration of clonidine avoids the α-adrenergic postsynaptic agonist effect observed when the drug is administered peripherally, which might increase renin (21). A spilling of clonidine from the cerebral ventricles to the blood stream cannot be excluded and under the present experimental circumstances may reduce the renin-lowering effect of central noradrenergic blockade. An effect on circulating catecholamines cannot be altogether excluded.

It must be noted that control values for PRA in sodium-depleted rats in the first experiment were significantly higher than control values in sodium-depleted rats in the second experiment. The manipulation of animals in the two experimental situations was different. In the first, rats were injected with olive oil sc; in the second, rats had been implanted with an icv cannula 48 hr before and received 20 μl of 0.9% NaCl icv. However, the explanation for the observed differences in control rats in both experiments remains to be discovered.

In conclusion, these data indicate that the central nervous system acting through an α-adrenergic pathway peripherally mediated by β-adrenergic receptors, plays an important role in renin release in the

conscious sodium-depleted rat. A prostaglandin-mediated pathway also plays a role in renin secretion in the sodium-depleted state and seems to act in parallel to the sympathetic nervous system. Since renin does not return to normal after blockade of either pathway, other mechanisms may also be operative.

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