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PHARMACOLOGICAL EVIDENCE FOR INVOLVEMENT OF THE SYMPATHETIC NERVOUS SYSTEM IN THE INCREASE IN RENIN SECRETION PRODUCED BY A LOW SODIUM DIET IN RATS

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Summary

To determine the degree to which increased sympathetic activity contributes to the increase in renin secretion produced by a low sodium diet, the β-adrenergic blocking drug propranolol or saline vehicle was injected through indwelling jugular cannulas in rats fed a normal diet and rats fed a low sodium diet for 9 days. Plasma renin activity (PRA) and plasma renin concentration (PRC) were elevated by the low sodium diet, and these values were reduced 42-45% by propranolol, although they were still higher than in the normal diet controls Plasma corticosterone was moderately elevated in cannulated rats on regular diet, compared to decapitated controls, but corticosterone did not differ between cannulated and decapitated rats on low salt diet, propranolol reduced plasma corticosterone. However, PRA and PRC were comparable in cannulated rats and decapitated controls on both the normal and the low sodium diets, and propranolol did not produce a significant reduction in PRA and PRC in rats fed the normal diet This indicates that the effects of propranolol on PRA and PRC in the low sodium rats were not simply due to reduction of a stress-induced increase in renin secretion The results indicate that increased sympathetic activity makes a substantial contribution to the increase in renin secretion produced by 9 days of dietary sodium restriction

When the sympathetic nervous system is stimulated, catecholamines released from renal sympathetic nerve terminals and circulating catecholamines bind to β_1 adrenergic receptors on juxtaglomerular cells in the kidney and increase renin secretion (1). These effects can be blocked by the β-adrenergic blocking drug propranolol A low sodium diet increases renin secretion (2), and administration of propranolol has been reported to reduce plasma renin activity (PRA) in rats fed such a diet (3). However, propranolol was administered intraperitoneally in these experiments, and the stress of intraperitoneal injection itself can increase renin secretion (1; Hargrave and Ganong, unpublished data). Therefore, propranolol could be simply reducing the stress-induced increase in renin secretion In an effort to eliminate this possibility, we injected propranolol in normal and salt-depleted rats with chronically implanted catheters that permitted us to make injections with minimal handling of the animals We also studied decapitated control groups, and we measured plasma ACTH and corticosterone in addition to PRA, plasma renin concentration (PRC), and plasma angiotensinogen

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Materials and Methods

Adult male Long-Evans rats (Simonsen, Gilroy, CA) weighing 240-280g were used in the study The rats were housed in individual metabolic cages in a temperature-controlled animal facility with a 12/12 hour light/dark cycle All were given free access to water

In one experiment, 10 rats were fed low sodium rat chow containing 0 96% K^+, 0 5% Cl^-, and 0.01% Na^+ (Teklab, Madison, WI) Sodium intake in rats on this regimen is usually <1 mEq per week (4) Another 10 were fed Purina rat chow containing 1 1% K^+, 0 48% Cl^-, and 0 28% Na^+ On days 5 and 6 of the low sodium diet, 24 hour urines were collected, and urine volume and sodium and potassium concentrations were measured. On day 7, the rats were anesthetized with pentobarbital, 40 mg/kg intraperitoneally and jugular venous catheters made of PE50 polyethylene tubing were inserted The catheters were filled with heparinized saline, capped and exteriorized On day 8, the jugular catheters were flushed with heparinized saline to maintain their patency and to adapt the animals to the injection procedure On the 9th day of the low sodium diet, 5 of the rats received d,l-propranolol, 1 mg/kg in 1 ml/kg saline intravenously. The other 5 rats received the same volume of isotonic saline Thirty minutes after injection, the animals were removed from their cages and rapidly decapitated Trunk blood was collected in tubes containing EDTA 0 3M (0 1 ml/1 0 ml blood), placed on ice, and promptly centrifuged Plasma was separated and frozen for later analysis of PRA, PRC, angiotensinogen, corticosterone, and ACTH

The control rats fed normal rat chow were treated in the same fashion as the rats fed the low sodium diet, 2 days of metabolic measurements were followed by cannulation under pentobarbital anesthesia, flushing the catheter on the first postoperative day, and sacrifice 30 minutes after propranolol or saline injection on the second postoperative day

Two control experiments were done to determine whether the cannulation and injection procedures caused stress-induced increases in renin secretion In one control experiment, 10 rats fed normal chow were divided into 2 groups Six of these rats were cannulated in the same fashion and sacrificed on the second postoperative day, whereas 4 were simply decapitated. In the second control experiment, 12 rats were fed the low sodium diet On the 7th day, 6 of them were cannulated, and all were sacrificed on the 9th day

Assays

ACTH and corticosterone were measured using commercial radioimmunoassay kits (Incstar, Stillwater, MN for ACTH, ICN Biomedical, Costa Mesa, CA for corticosterone) PRA, PRC, and angiotensinogen were measured by the method of Menard and Catt (5) Urinary sodium and potassium were determined by flame photometry

Statistical Analysis

Values are reported as means ± standard error Differences in PRA and PRC between the groups of rats were analyzed by two-way analysis of variance with post hoc comparisons using Newman-Keuls' test (6) Differences in which $p<0.05$ were considered to be statistically significant

Results

The results in the rats treated with propranolol are summarized in Table 1, and cannulated rats are compared to decapitated controls in Table 2 In rats fed the normal diet, sodium excretion was 2100-2400 μEq/day, whereas in

TABLE 1

Effect of Propranolol (P) or Saline Vehicle (S) in Rats Fed a Normal and a Low Sodium Diet

Diet	Treatment	Sodium Excretion (uEq/day)	PRA (ng AI/ml/2h)	PRC (ng AI/ml/2h)	Angiotensinogen (ng/ml)	ACTH (pg/ml)	Corticosterone (ng/ml)
Normal	S	2140±293	6.6±1.6	16.5±1.6	1019±47	90.7±11.0	230.8±95.5
Normal	P	2375±89	3.8±0.4	16.1±1.8	1022±93	85.8± 8.4	194.0±18.9
Low Sodium	S	18±5	21.2±2.2	49.3±4.2	984±93	94.9±19.7	286.2±55.2
Low Sodium	P	13±2	11.6±1.4	28.8±5.0	1077±66	69.3±14.4	145.2±49.9

TABLE 2

Effect of Jugular Cannulation on Renin, ACTH, and Corticosterone in Rats fed a Normal and a Low Sodium Diet

Diet		PRA (ng AI/ml/2h)	PRC (ng AI/ml/2h)	Angiotensinogen (ng/ml)	ACTH (pg/ml)	Corticosterone (ng/ml)
Normal	Cannula	6.5±1.9	23.5±4.1	1721±305	110.6±19.6	148.9±49.5
Normal	No Cannula	4.1±0.5	23.0±3.3	863±49	66.1±3.7	2.6±0.9
Low Sodium	Cannula	15.6±2.5	74.6±11.1	855±37	114.4±10.3	316.9±37.3
Low Sodium	No Cannula	13.0±1.9	67.6±7.5	983±59	270.8±48.1	367.4±43.4

rats fed the low sodium diet, it was 13-18 μEq/day In the low salt rats injected with saline and sacrificed on the 9th day of the diet, there was a statistically significant elevation in PRA and PRC. Propranolol produced a highly significant 42-45% decrease in PRA and PRC However, PRA and PRC were still significantly higher than in either group of rats fed the normal diet Propranolol did not produce a significant decrease in PRA and PRC in the rats fed the normal diet (Table 1) and PRA and PRC values were comparable in cannulated rats and decapitated controls fed the normal diet and the low sodium diet (Table 2) The high mean value for plasma angiotensinogen in the cannulated rats in Table 2 is due primarily to one very high value, and there were no statistically significant differences in plasma angiotensinogen

On the other hand, plasma corticosterone was significantly elevated in the cannulated rats fed the normal diet, while ACTH was elevated in the non-cannulated group on low salt diet (Table 2) A small but statistically significant reduction in plasma corticosterone was produced by propranolol in the rats fed the normal diet and the rats fed the low sodium diet (Table 1) Plasma ACTH values showed the same trend, but not to a statistically significant degree

Discussion

There are two main ways to approach the question of whether the moderate increase in renin secretion produced by simply restricting dietary sodium is mediated by the sympathetic nervous system one is renal denervation and the other is β-adrenergic blockade with propranolol or a related β-adrenergic blocking drug The effects of renal denervation on the PRA response are controversial (1) In dogs, renal denervation has been reported to delay (7) or abolish (8) the renin response to a low sodium diet However, Gotschall et al (9) found no difference in the PRA response to sodium depletion in denervated dogs One difficulty with studies of this type is that if the sodium deprivation is prolonged or a diuretic is also administered, enough sodium can be lost to activate intrarenal nonneural renin-stimulating mechanisms (2) in addition to any sympathetic component that may be present In addition, denervation studies do not rule out the possibility of circulating catecholamines affecting renin secretion

Some years ago, Ganong and associates (10) reported that propranolol lowered PRA in pentobarbital-anesthetized dogs fed a low sodium diet without lowering PRA in dogs fed a normal sodium diet However, pentobarbital can modify autonomic responses In normal human volunteers, Omvik, Enger, and Eide reported (11) that chronic treatment with propranolol did not produce a significant decrease in the PRC response to 5 days on a low sodium diet, but they did not demonstrate that β-adrenergic receptors were blocked at the time of blood sampling In salt-depleted rats, Pettinger and associates (12) did not observe a decrease in PRA when propranolol was administered, but their rats had received three days of treatment with the potent diuretic furosemide in addition to dietary salt restriction In another study (13) propranolol administered in the food and water failed to block the increase in PRA produced by a low sodium diet, but the actual intake of propranolol was not measured In a third study (3), propranolol was reported to decrease PRA in rats fed a low salt diet However, stressful stimuli including intraperitoneal injections can produce sympathetically mediated increases in PRA (1, Hargrave and Ganong, unpublished data), and since the propranolol was injected intraperitoneally in these experiments, the decrease in PRA could have been due simply to inhibition of the stress response For this reason, we studied unanesthetized, unrestrained rats with chronically implanted cannulas which permitted us to inject propranolol or saline vehicle with

minimal handling of the rats. The dose of propranolol that we employed abolishes the increase in PRA produced by the psychological stress of immobilization (14).

Were the cannulated rats acutely stressed to a sufficient degree to increase renin secretion? Their plasma corticosterones were increased to a significant degree compared to decapitated controls in rats on the regular diet, and so were their plasma ACTHs, although the ACTH increases did not reach statistical significance. In contrast, in the low salt group, plasma corticosterones were the same in both groups, and ACTHs were higher in the decapitated rats than in the cannulated rats It is interesting in this regard that propranolol seemed to reduce the pituitary-adrenal activation, possibly because of its calming, anti-anxiety effect (15) However, propranolol did not produce a statistically significant decrease in PRA or PRC in the rats fed the normal diet, and PRA and PRC values in cannulated rats were comparable to those in decapitated controls Consequently stress-induced increases in renin secretion were not observed in our experiments

It should be noted that despite the marked reduction in PRA and PRC produced by propranolol in the rats fed the low sodium diet, these values remained significantly greater than in the saline-injected rats fed the normal diet This indicates that after 9 days of dietary salt restriction, nonsympathetic factors also contribute to the increase in renin secretion. However, the fact that β-adrenergic blockade reduced the PRA and PRC response by 42-45% indicates that the sympathetic nervous system plays a major role in the response

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