Altered Norepinephrine Turnover in the Brain of Rats With Chronic Renal Failure

Roberto Bigazzi, Ella Kogosov, and Vito M. Campese

R. Bigazzi, E. Kogosov, V.M. Campese, Division of Nephrology, Department of Medicine, University of Southern California, Los Angeles, CA (J. Am. Soc. Nephrol. 1994; 4:1901–1907)

ABSTRACT
Disturbances of the sympathetic nervous system have been described in chronic renal failure, but their role in the genesis and maintenance of hypertension frequently associated with this condition has not been established. The neuroadrenergic activity in brain nuclei involved in the regulation of blood pressure in uremic animals has also not been previously evaluated. In these studies, the neuroadrenergic activity was measured in the anterior, lateral, and posterior hypothalamic nuclei, in the locus coeruleus, and in the nucleus tractus solitarius of Sprague Dawley rats % nephrectomized or sham operated 4 wk before the experiments. Neuroadrenergic activity was determined by calculating norepinephrine (NE) turnover rate (in picograms per milligram per hour), 3, 6 and 12 h after inhibition of NE synthesis with i- methylyrosine. The endogenous NE concentration was significantly greater in the posterior hypothalamic nuclei (21,501 ± 1,777 pg/mg wet wt) and in the locus coeruleus (16,152 ± 1,114 pg/mg wet tissue) of uremic compared with control rats (12,213 ± 1,404 and 7,991 ± 622 pg/mg wet wt, respectively).

On the other hand, the endogenous NE content of the nucleus tractus solitarius and the anterior hypothalamic nuclei did not differ between uremic and control rats. The turnover rate of NE in the posterior hypothalamic nuclei of uremic rats (2150 ± 430 pg/mg per hour) was significantly faster (P < 0.05) than in control rats (977 ± 244 pg/mg per hour). The turnover rate of NE in the locus coeruleus of uremic rats (2,584 ± 323 pg/mg per hour) was also significantly faster than in control animals (400 ± 140 pg/mg per hour; P < 0.01). On the other hand, the turnover rate of NE in the anterior and lateral hypothalamus and in the nucleus tractus solitarius did not differ between uremic and control rats. The administration of a neurotoxin, 6-OH-dopamine, in the posterior hypothalamic nuclei caused a significant decrease in blood pressure from 161 ± 8.4 to 139 ± 6.8 mm Hg (P < 0.01), whereas the injection of the vehicle caused no change in blood pressure (166 ± 7.7 and 163 ± 8.3 mm Hg, respectively). These studies demonstrate a significant increase in NE turnover in the posterior hypothalamic nuclei and locus coeruleus of uremic rats, suggesting that increased neuroadrenergic activity in these nuclei may play a role in the pathogenesis of hypertension in uremia.

Key Words: Hypertension, chronic renal failure, norepinephrine, norepinephrine turnover, neuroadrenergic activity

A large body of evidence indicates that functional abnormalities of the sympathetic nervous system (SNS) are present in uremia (1,2). However, to what extent these abnormalities contribute to the genesis and maintenance of hypertension associated with chronic renal failure remains to be established.

Increased blood levels of norepinephrine (NE), an indirect marker of SNS activity, have been shown in patients with chronic renal failure (3–6). Some investigators have shown a direct and significant correlation between plasma concentrations of catecholamines and blood pressure in a small group of patients with hypertension and chronic renal failure and interpreted these data to support a role for the SNS in the maintenance of hypertension in this condition (3). Other investigators, however, failed to find a consistent relationship between blood levels of NE and blood pressure in uremic patients (1,4). Plasma concentrations of NE depend not only on the rate of release from sympathetic nerve terminals, but also on the rate of reuptake, metabolism, and excretion. Because alterations of NE metabolism and excretion have been consistently observed in patients with chronic renal failure (7–10), increased plasma concentrations of NE may not necessarily reflect an increase in SNS activity. More recently, Converse et al. (11) performed direct microelectrode recordings of postganglionic sympathetic action potentials in peripheral nerves of chronic hemodialysis patients with and without bilateral nephrectomy. They found that the rate of sympathetic nerve discharge was much
higher in dialysis patients than in control subjects. In uremic patients, plasma NE levels varied widely and no correlation was found between those levels and sympathetic nerve discharge in the peroneal nerves.

Studies performed in animals with experimentally induced chronic renal failure have failed to clarify the pathogenetic role of the SNS in the maintenance of hypertension. Most investigators, however, have studied the content of NE in the entire brain or in brain synaptosomes isolated from the cerebral cortex or in peripheral tissues and have usually observed a reduction rather than an increase in the content of this amine in uremic rats (12–14). These results, however, are difficult to interpret because alterations in monoamine content in the brain are the net result not only of synthesis, but also of catabolism, uptake, vesicular storage capacity, and density of sympathetic innervation. Determination of the turnover rate of NE, by measuring the fall in stores after inhibition of synthesis, provides a better index of neuronal activity than measurements of stores alone (15). In addition, measurements of NE stores in the cerebral cortex as performed in previous studies (11,12) may not necessarily be indicative of changes in brain nuclei directly involved in blood pressure regulation: Several nuclei in the hypothalamus and brain stem participate in the control of blood pressure (16–18). Studies of NE turnover in these nuclei may provide more useful information on their participation in blood pressure regulation in uremic animals. In these studies, we have measured NE turnover in the hypothalamus (anterior, lateral, and posterior nuclei), locus coeruleus, and nucleus tractus solitarius of the brain stem in the renal ablation model of renal failure in the rat.

MATERIAL AND METHODS

Male Sprague-Dawley rats weighing 200 to 300 g were used for these studies. The animals were fed normal rat chow (ICN Nutritional Biochemical, Cleveland, OH) throughout the study. The rats were anesthetized with a short-acting barbiturate, Brevital (75 mg/kg, ip). After anesthesia, rats underwent 3/4 nephrectomy of the right kidney and, 1 wk later, total nephrectomy of the left kidney. Age-matched rats underwent sham operations and were used as controls. Blood pressure was measured weekly by the tail-cuff method with an electromyographmomanometer and physiograph recorder MK-III (Narco Bio-Systems, Houston, TX).

NE Turnover Rate

Four weeks after the left total nephrectomy or sham operation, NE turnover rate was calculated by assaying the endogenous NE concentration at time 0 and 3, 6, and 12 h after the ip injection of 80 mg/kg of α-methyl-DL-p-tyroserine methyl ester (DL-2methyl-3-[4-hydroxyphenyl]-alanine methyl ester) hydrochloride (Sigma-REA St. Louis, MO) diluted in saline (19,20). The endogenous tissue levels then decline at a rate proportional to the initial NE concentrations (21). Rats that were decapitated 12 h after the initial administration of L-methylyrosine, received an additional dose of L-methylyrosine (80 mg/kg) 3 h after the initial dose to ensure that the formation of catecholamines in the brain was blocked throughout the study. Each datum point includes a minimum of 6 and a maximum of 10 rats. Rats were killed by decapitation and without anesthesia. After decapitation, the brains were immediately removed and frozen under powdered dry ice and stored at −80°C for no longer than 2 to 3 wk. The brains were then placed on chucks and cut into consecutive 300-μm sections in a −10°C cryostat. In a 4°C cold room, one 0.5-mm-diameter micropunch was obtained bilaterally from the anterior, lateral, and posterior hypothalamic nuclei, starting from 7.6 mm interaural, and from the locus coeruleus. Micropunches of the nucleus tractus solitarius were also obtained, starting from 2.8 mm interaural (22,23). The punch cuts of brain tissue weighed 0.1 mg. Samples were sonicated in 0.03 N perchloric acid and centrifuged, and the supernatant was assayed for catecholamines by the radioenzymatic method of Peuler and Johnson (24). This assay is based on the use of the enzyme catechol-O-methyltransferase, which transfers a radioactive methyl group from S-[methyl-3H]adenosyl-L-methionine to an endogenous catecholamine to form a radioactive O-methyl catecholamine derivative. The sensitivity of this method for NE is 1 to 2 pg.

The turnover rate of NE was calculated by the method of Brodie et al. (15). The log of NE was plotted versus time, and the least-square straight line provided the fractional turnover rate, k. The NE turnover (picograms per milligram per hour) was calculated as the product of k times the endogenous concentration of NE. The half-life was calculated from the equation $t_{1/2} = 0.434$ divided by the slope. The 95% confidence intervals were determined for the turnover rates by the method of Taubin et al. (25).

Two subgroups of uremic rats (six each) were anesthetized with pentobarbital (30 mg/kg, ip) and mounted on a stereotaxic apparatus (David Kopf Instruments Co., Tujunga, CA). Burr holes were placed in the cranium at sites that would allow introduction of 30-gauge stainless steel cannulas into the posterior hypothalamic area, using coordinates (anteroposterior, 4.4 mm; from midline, 0.4 mm; and dorsoventral, 8.8 mm). Before the cannula is lowered into position, the needle and attached PE 50 tubing
were filled with 6-OH-dopamine (6-OHDA) or with normal saline by the use of microsyringes. Five micrograms of 6-OHDA was dissolved in 2.5 μL of isotonic sodium chloride solution, containing 1 mg/mL of ascorbic acid, adjusted to pH 5.5 with sodium hydroxide. One microliter of 6-OHDA was infused over 6 min. Rats treated with vehicle received 1 μL of saline over 6 min. Blood pressure was measured 7 days later in both groups of rats. The placement of the catheters into the posterior hypothalamus was confirmed by dissection of the brains after the completion of the experiment. In addition, NE content was measured in these nuclei after decapitation of the animals.

The data were evaluated statistically by unpaired t test and by regression analysis with the computer program Statview and Graphics. Values are given as means ± SE.

RESULTS

The data on body weight, blood pressure, and serum creatinine are summarized in Table 1. In uremic rats, systolic blood pressure and serum creatinine were significantly higher (P < 0.01) than in control animals.

The endogenous concentration of NE in the locus coeruleus and lateral and posterior hypothalamic nuclei was higher in uremic than in control rats. On the other hand, there was no significant difference in the concentration of NE between the two groups in the anterior hypothalamic nuclei.

The turnover rate of NE in the posterior hypothalamic nuclei and in the locus coeruleus of uremic rats was significantly faster (P < 0.05) than in control rats (Table 2; Figures 1 and 2). The turnover rate of NE in the anterior and lateral hypothalamic nuclei and in the nucleus tractus solitarius of uremic rats did not differ from that of control rats.

The administration of 6-OHDA into the posterior hypothalamic nuclei caused a significant decrease in blood pressure from 161 ± 8.4 to 139 ± 6.8 mm Hg (P < 0.01), whereas the injection of vehicle caused no change in blood pressure (166 ± 7.7 and 163 ± 8.3 mm Hg, respectively) in the uremic rats (Figure 3). After the administration of 6-OHDA, the content of NE in the posterior hypothalamic nuclei of uremic rats was 5.139 ± 413 pg/mg of tissue, significantly less (P < 0.01) than in control uremic rats (8.762 ± 7520 pg/mg of tissue). The values of NE in control uremic rats are lower than the baseline values. It is possible that, because of tissue destruction from the placement of the cannulas, the punched tissues did not correspond exactly to the posterior hypothalamic nucleus.

DISCUSSION

The pathogenesis of hypertension associated with chronic renal failure is complex. A variety of factors,

| TABLE 1. Blood pressure, body weight, and serum creatinine in control and uremic rats |
|---------------------------------|-----------------|-----------------|
| Parameter                      | Control         | Uremic          |
| No. of Rats                    | 28              | 34              |
| Systolic Blood Pressure (mmHg) | 123 ± 1.46      | 153 ± 3.6<sup>o</sup> |
| Body Wt (g)                    | 310 ± 10.6      | 270 ± 8.5       |
| Serum Creatinine (mg/dL)       | 0.34 ± 0.02     | 1.15 ± 0.09<sup>o</sup> |

<sup>o</sup> P < 0.01.

| TABLE 2. Endogenous NE concentration and turnover rate in the anterior (AH), lateral (LH), and posterior (PH) hypothalamic nuclei, locus coeruleus, and nucleus tractus solitarius (NTS) of ¾ nephrectomized (U) and control (C) rats |
|-------------------------------------------------|-----------------|-----------------|
| Endogenous NE (pg/mg of tissue)                  | Fractional Turnover (h⁻¹) | Turnover Rate (pg/mg per hour) | t<sub>1/2</sub> (h) |
| C       | U    | C       | U    | C       | U    | C       | U    |
| AH      | 6,282 ± 648 | 6,546 ± 462 | 0.05 ± 0.02 | 0.04 ± 0.01 | 314 ± 125 | 262 ± 65 | 8.68 | 10.8 |
| LH      | 11,621 ± 769 | 13,971 ± 816<sup>o</sup> | 0.09 ± 0.01 | 0.10 ± 0.02 | 1,045 ± 116 | 1,397 ± 279 | 4.8 | 4.34 |
| PH      | 12,213 ± 1,404 | 21,501 ± 1,777<sup>o</sup> | 0.08 ± 0.02 | 0.10 ± 0.02 | 977 ± 244 | 2,150 ± 430<sup>o</sup> | 5.4 | 4.34 |
| NTS     | 5,075 ± 568 | 7,775 ± 1,479 | 0.06 ± 0.02 | 0.06 ± 0.02 | 305 ± 102 | 467 ± 156 | 7.2 | 7.2 |
| Locus Coeruleus | 7,991 ± 622 | 16,152 ± 1,114<sup>o</sup> | 0.05 ± 0.02 | 0.16 ± 0.02 | 400 ± 160 | 2,584 ± 323<sup>o</sup> | 8.7 | 2.7 |

<sup>a</sup> P < 0.05.
<sup>b</sup> P < 0.01 compared with controls.
including an increased activity of the SNS, have been proposed to be responsible for the rise in blood pressure in this condition (26). In this study, to measure the activity of the SNS in brain nuclei directly involved in blood pressure regulation, we have evaluated NE turnover rate.

Our studies have shown that uremic rats manifest substantial abnormalities of noradrenergic activity in the posterior hypothalamic nuclei and the locus coeruleus of rats with chronic renal failure. NE turnover rate has been extensively used to determine the activity of the SNS in the brain. It is important, however, to underscore the fact that this method depends in part on the capacity of the inhibitor to penetrate into various brain nuclei and that uremia may alter the capacity of drugs to diffuse into the brain. This, however, cannot explain our findings, because we observed differences in NE turnover only in certain brain nuclei. Direct measurements of NE release from brain nuclei would be a better methodologic approach to these issues.

The posterior hypothalamus has been identified as a pressor region. Electrical stimulation of this area increased blood pressure in the rat (27–29), whereas destruction of the posterior hypothalamic area decreased blood pressure in spontaneously hypertensive rats (SHR) (30). Local perfusion of hypertonic saline or phenylephrine in the posterior hypothalamus elicited an increase in blood pressure and tachycardia and a rise in NE release (31). Blockade of α-adrenergic receptors with phenoxybenzamine prevented the cardiovascular effects caused by the administration of NaCl or phenylephrine in this area, indicating that these effects depend on the stimulation of α-adrenergic receptors. The increase in NE turnover in the posterior hypothalamic nuclei suggests that increased noradrenergic outputs from this area may play a role in the maintenance of hypertension in uremic rats.

One could speculate that the increase in NE turnover in the posterior hypothalamus might be secondary to the rise in blood pressure, rather than being its cause. This, however, is unlikely because several reports have demonstrated that NE turnover in this region decreases rather than increases in response to a rise in blood pressure; NE turnover in this region, on the other hand, increases when arterial pressure falls (32,33).

The locus coeruleus contains almost half of the NE-synthesizing neurons in the brain (34), and it has many connections with the SNS (35). Electrical (36) or l-glutamate-induced (37) stimulation of the locus coeruleus evokes a rise in arterial pressure, which is more pronounced in SHR (38). Lesions of the posterior hypothalamus attenuated the blood pressure response to electrical stimulation or to microinjection of l-glutamate into the locus coeruleus (35,39), suggesting that noradrenergic neurons in the locus coe-
ruleus may project to the posterior hypothalamus and result in the elevation of blood pressure.

In order to confirm whether activation of neuroadrenergic neurons in the posterior hypothalamus contributes to the rise of blood pressure in the uremic rat, we measured blood pressure before and 1 wk after the administration of the neurotoxin 6-OHDA in this area of the brain. The injection of 6-OHDA, but not of vehicle, significantly lowered blood pressure in uremic rats. The injection of 6-OHDA in the posterior hypothalamus of normal rats does not modify blood pressure, suggesting that this nucleus is not involved in the maintenance of baseline blood pressure in the normal rat (35).

The lateral hypothalamus has also been implicated in the control of the autonomic nervous system (40-41). Electrical stimulation of this region decreases the sympathetic nerve firing rate and may increase parasympathetic activity (42,43). On the other hand, lesions of the lateral hypothalamus produce a significant increase in NE turnover in peripheral tissues (44). Our data do not suggest that dysfunctions of the lateral hypothalamic nuclei participate in the maintenance of hypertension in rats with chronic renal failure.

The nucleus tractus solitarius plays an important role in the control of blood pressure via modulation of the baroreflex arc (45). The absence of significant abnormalities of NE turnover in this region of the brain does not support a role for these nuclei in the pathogenesis of hypertension in rats with chronic renal failure.

The posterior hypothalamic area has been identified as a depressor region, and lesions of this area produce fulminating hypertension in the normotensive rat (46-48). This region has been implicated in the pathogenesis of hypertension in salt-sensitive SHR (SHR-S). Chen et al. (49) have shown that dietary salt loading exacerbates the severity of hypertension in SHR-S and decreases endogenous NE content and turnover in the anterior hypothalamic area, thus decreasing the release of NE from noradrenergic nerve terminals in this region. They interpreted these findings to support the hypothesis that reduced noradrenergic input to depressor neurons in the anterior hypothalamus is related to salt sensitivity in these rats. The absence of abnormalities in NE turnover rate in the anterior hypothalamus suggests that alterations of depressor neurons in this region do not play a significant role in the maintenance of hypertension in rats with chronic renal insufficiency.

In conclusion, these studies provide evidence that increased activity of noradrenergic neurons in the locus coeruleus and posterior hypothalamic nuclei may contribute to the pathophysiology of hypertension in rats with chronic renal insufficiency. The data are also in keeping with the notion that projections from the locus coeruleus into the posterior hypothalamus may increase sympathetic nerve activity and blood pressure in uremic rats.

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If, to the time of Bowman, morphology guided the course of functional investigation by hypothetical implication, since the work of Richards and Smith the converse is true, for structural investigation has become largely an effort to rationalize the morphologist's findings with firmly established functional patterns of renal activity. And in these correlations there is again observed the effect of methodology, both in the posing of problems and in suggesting possible means of their resolution. A characteristic of functional data is that they are quantitative, and a correlation, to be meaningful, must therefore be an equation in which both terms, structural and functional, are so expressed. Microdissection and isolation of the essential organs, the nephrons, are the only means which can reveal the entirety of their dimensions in number, length, diameter, and calculable mass.