

# Altered Norepinephrine Turnover in the Brown Fat of Rats With Chronic Renal Failure<sup>1</sup>

Roberto Bigazzi, Ella Kogosov and Vito M. Campese<sup>2</sup>

R. Bigazzi, E. Kogosov, V.M. Campese, Division of Nephrology, Department of Medicine, University of Southern California, Los Angeles, CA

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## ABSTRACT

Disturbances of the sympathetic nervous system (SNS) have been described in chronic renal failure, but their role in the metabolic derangements of uremia has not been well established. In these studies, SNS activity has been measured in the ventromedial hypothalamic (VMH) nuclei and in the intercostal brown adipose tissue (IBAT) of Sprague Dawley 5% nephrectomized or sham-operated rats. SNS activity was determined by calculating the norepinephrine (NE) turnover rate (in picograms per milligram per hour) 3, 6, and 12 h after the inhibition of NE synthesis with L-methyltyrosine. The endogenous NE concentration was significantly ( $P < 0.01$ ) higher in the VMH ( $14,567 \pm 1,130$  pg/mg wet wt) and IBAT ( $17,902 \pm 2,308$  pg/mg wet wt) of uremic than control rats ( $9,600 \pm 1,110$  and  $5,752 \pm 320$  pg/mg wet wt, respectively). The turnover rates of NE in the VMH ( $582 \pm 146$  pg/mg per hour) and in the IBAT ( $1,432 \pm 179$  pg/mg/hr) of uremic rats were significantly faster ( $P < 0.01$ ) than in control rats ( $192 \pm 96$  and  $173 \pm 58$  pg/mg per hour, respectively). These studies demonstrate a significant increase in NE turnover in the VMH nuclei and IBAT of uremic rats. It is suggested that increased efferent sympathetic nerve discharge from the VMH to the IBAT may play a role in the pathogenesis of malnutrition in uremia.

**Key Words:** Chronic renal failure, norepinephrine, turnover, brown fat

Alterations of the nutritional status are frequently present in uremia. The pathophysiology of these alterations is complex and probably multifactorial (1).

The activity of the sympathetic nervous system (SNS) may influence the nutritional status. A reduction in the activity of the SNS appears to be associated with obesity (2–4). Animals with hypothalamic obesity manifest impaired fat (4) and reduced free fatty acid mobilization (2). More recently, direct connections have been established between ventromedial hypothalamic (VMH) nuclei and brown adipose tissue in the rat (5,6). After lesions of the VMH nuclei, the electrical firing rate of these sympathetic filaments to the interscapular brown fat tissue (IBAT) was reduced (5). The administration of glucose in the VMH produced a dose-dependent increase in the firing rate of the sympathetic nerves (6). The turnover of norepinephrine (NE) in postganglionic fibers in the IBAT or white fat of genetically obese rodents was reduced compared with that of controls (7,8). Thus, it seems plausible that enhanced sympathetic activity from the VMH may reduce body fat stores and body weight.

A large body of evidence indicates that functional abnormalities of the SNS are present in uremia (9,10). Increased blood levels of NE, a marker of SNS activity, were observed in patients with chronic renal failure (11–14). More recently, Converse *et al.* (15) performed direct microelectrode recordings of postganglionic sympathetic action potentials in the peroneal 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.

The content of NE in the cerebral cortex, brain synaptosomes, or peripheral tissues of uremic rats was found to be reduced (16–18). On the other hand, the endogenous concentration of NE was found to be increased in the posterior hypothalamus and in the locus coeruleus of uremic rats (19). These results, however, are difficult to interpret because alterations in monoamine levels in the plasma or brain are the net result not only of synthesis, but also of catabolism, uptake, vesicular storage capacity, and density of sympathetic innervation. Because alterations of NE metabolism and excretion have been consistently observed in chronic renal failure (20–23), an increase in the concentration of NE may not necessarily reflect an increase in SNS activity. Determination of

<sup>1</sup> Received April 28, 1993. Accepted September 6, 1993.

<sup>2</sup> Correspondence to Dr. V.M. Campese, Division of Nephrology, LAC/USC Medical Center, 2025 Zonal Avenue, Los Angeles, CA 90033.

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the turnover rate of NE, by measuring the fall in NE content after the inhibition of synthesis, provides a better index of neuronal activity (24). To determine the role of the VMH nuclei in the genesis of metabolic abnormalities in uremia, we have measured NE turnover in the VMH and in the IBAT of  $\frac{5}{6}$  nephrectomized rats.

## 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  $\frac{2}{3}$  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 electrospygmanometer 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  $\alpha$ -methyl-DL-*p*-tyrosine methyl ester (DL-2methyl-3-[4-hydroxyphenyl]-alanine methyl ester) hydrochloride (Sigma Chemical Co., St. Louis, MO) diluted in saline (25,26). The endogenous tissue levels then decline at a rate proportional to the initial NE concentrations (27). Rats that were decapitated 12 h after the initial administration of L-methyltyrosine, received an additional dose of L-methyltyrosine (80 mg/kg) 3 h after the initial dose to ensure that the formation of catecholamines in the brain and IBAT 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, IBAT, heart, and kidneys were immediately removed, frozen under powdered dry ice, and stored at  $-80^{\circ}\text{C}$  for no longer than 2 to 3 weeks. The brains were then placed on chucks and cut into consecutive 300- $\mu\text{m}$  sections in a  $-10^{\circ}\text{C}$  cryostat. In a  $4^{\circ}\text{C}$  cold room, 0.5-mm-diameter micropunches were obtained bilaterally from the VMH (28,29). Samples were sonicated in 0.03 N perchloric acid and centrifuged, and the supernatant was assayed for catecholamines. Catecholamines in the VMH and IBAT were measured by the radioenzymatic method of Peuler and Johnson (30). This assay is based on the use of the enzyme catechol-*O*-methyltransferase, which transfers a radioactive

methyl group from S-[methyl- $^3\text{H}$ ]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. Catecholamines in the heart and kidneys were measured by high-pressure liquid chromatography with an electrochemical detector.

The turnover rate of NE was calculated by the method of Brodie *et al.* (24). The log of NE was plotted versus time, and the least-square straight line provided the fractional turnover rate, *k*. The NE turnover (in picograms per milligram per hour) was calculated as the product of *k* times the endogenous concentration of NE. The half-life for the VMH was calculated from the equation  $t_{1/2} = 0.434/k$ . The half-life for the IBAT, heart, and kidney was calculated from the equation  $t_{1/2} = 0.693/k$ . The 95% confidence intervals were determined for the turnover rates by the method of Taubin *et al.* (31). The data were evaluated statistically by analysis of variance. Values are given as means  $\pm$  SE.

## RESULTS

The data on body weight, blood pressure, and serum creatinine are summarized in Table 1. Systolic blood pressure in uremic rats ( $153 \pm 3.6$  mm Hg) was significantly greater ( $P < 0.01$ ) than in control animals ( $123 \pm 1.5$  mm Hg). Serum creatinine in uremic rats ( $1.15 \pm 0.09$  mg/dL) was also significantly higher ( $P < 0.01$ ) than in control animals ( $0.34 \pm 0.02$  mg/dL). The endogenous NE concentration was significantly greater ( $P < 0.01$ ) in the VMH ( $14,567 \pm 1,130$  pg/mg wet wt) and IBAT ( $17,902 \pm 2,308$  pg/mg wet wt) of uremic than control rats ( $9,600 \pm 1,110$  and  $5,752 \pm 320$  pg/mg wet wt, respectively).

The turnover rate of NE in the VMH of uremic rats ( $582 \pm 146$  pg/mg per hour) was significantly faster ( $P < 0.01$ ) than in control rats ( $192 \pm 96$  pg/mg per hour) (Table 2). The NE turnover rate in the IBAT of uremic rats ( $1,432 \pm 179$  pg/mg per hour) was also significantly faster ( $P < 0.01$ ) than in control rats ( $173 \pm 58$  pg/mg per hour) (Figure 1).

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)	$123 \pm 1.5$	$153 \pm 3.6^{\circ}$
Body Wt (g)	$310 \pm 10.6$	$270 \pm 8.5$
Serum Creatinine (mg/dL)	$0.34 \pm 0.02$	$1.15 \pm 0.09^{\circ}$

$^{\circ} P < 0.01$ .

To determine whether the increase in NE turnover was specific for the IBAT and VMH, we also measured NE turnover in the heart and kidney. The NE turnover in the heart of uremic rats was not different that in control animals. On the other hand, the turnover rate in the kidney of uremic rats ( $20.0 \pm 2.9$  pg/mg per hour) was significantly lower than in control animals ( $36.6 \pm 2.2$  pg/mg per hour;  $P < 0.01$ ).

## DISCUSSION

This study has shown that the turnover rate of NE, a marker of SNS activity, in the VMH and IBAT of rats with chronic renal failure is increased. This increase is not a generalized phenomenon due to the uremic state, because the turnover rate in the heart of uremic rats was unchanged and the turnover rate in the kidney of uremic rats was actually decreased. Previous studies from our laboratory also indicate that the increase in the turnover rate of NE in the VMH is not an expression of the generalized activation of the turnover rate of NE in the brain due to the uremic state, because the turnover rate of NE was increased in the posterior but not in the anterior hypothalamic nuclei or in the nucleus tractus solitarius (19). In addition, we have previously shown that the content, as well as the release and reuptake of NE, in synaptosomes isolated from the cerebral cortex was significantly decreased in uremic rats compared with control animals (17).

It has been known for many years that lesions in the VMH produce hyperphagia and obesity (32–34). These lesions cause an increased firing rate of the vagus nerve (35) and a reduced firing rate of the sympathetic nerves to the IBAT (36), leading to reduction of the thermogenic properties of the IBAT (37,38). On the contrary, the microinjection of glucose (39) or 3-hydroxybutyrate (40) in the VMH increases the firing rate of the sympathetic nerves to brown adipose tissue, resulting in increased mobili-

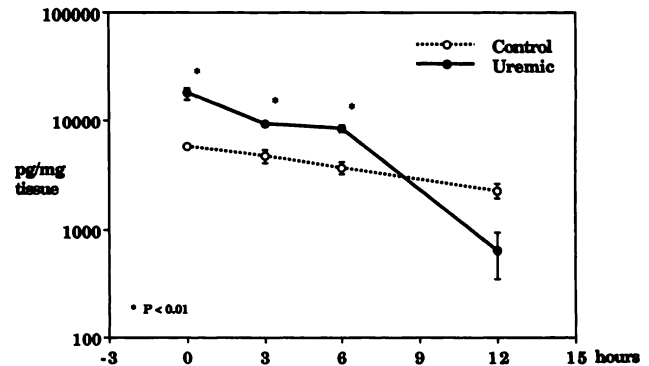


Figure 1. Endogenous NE content before and 3, 6, and 12 h after the inhibition of NE synthesis with L-methyltyrosine (80 mg/kg ip) in the posterior hypothalamic nuclei of rats with chronic renal failure and controls. The data on turnover rate are provided in Table 2.

zation of fat and free fatty acids, increased thermogenesis (41), and reduction in body weight (42).

The presence of increased turnover of NE in the VMH suggests that an increase in sympathetic nerve firing from the VMH may be responsible for the increased NE turnover in the IBAT. The data are also consistent with the hypothesis that uremia may activate the SNS and enhance energy expenditure through brown adipose tissue (43).

We realize that to establish the physiologic significance of these turnover measurements and to more definitely determine a connection between increased NE turnover in the VMH and in the IBAT, we need to measure nerve traffic along the fibers that innervate the IBAT and to measure NE release by push-pull microinfusion or microdialysis. However, these measurements go beyond the scope of this investigation.

This study does not allow us to determine the mechanisms responsible for the increased NE turnover in the VMH and IBAT. The entry of fatty acids or ke-

TABLE 2. Endogenous NE concentration and turnover rate of the IBAT and in the VMH of control (C) and 5/6 nephrectomized (U) rats<sup>a</sup>

	Endogenous NE (pg/mg of tissue)		Fractional Turnover (h <sup>-1</sup> )		Turnover Rate (pg/mg per hour)		t <sub>1/2</sub> (h)	
	C (34)	U (28)	C	U	C	U	C	U
IBAT	5,752 ± 320	17,902 ± 2,308*	0.03 ± 0.01	0.08 ± 0.01	173 ± 58	1,432 ± 179 <sup>b</sup>	23.1	8.6
VMH	9,600 ± 1,110	14,567 ± 1,130 <sup>b</sup>	0.02 ± 0.01	0.04 ± 0.01	192 ± 96	582 ± 146 <sup>b</sup>	21.7	10.8
Heart	1,072 ± 76.8	1,057 ± 64.1	0.06 ± 0.01	0.07 ± 0.01	64.3 ± 10.7	74.0 ± 10.6	11.6	9.9
Kidney	215 ± 14.4	96 ± 9.8 <sup>b</sup>	0.17 ± 0.01	0.21 ± 0.03	36.6 ± 2.2	20.2 ± 12.9 <sup>b</sup>	4.1	3.3

<sup>a</sup> The t<sub>1/2</sub> for the IBAT was calculated by the formula t<sub>1/2</sub> = 0.693/k. The t<sub>1/2</sub> for VMH was calculated by the formula t<sub>1/2</sub> = 0.434/k (24). In brackets are the number of rats studied.

<sup>b</sup> P < 0.01 compared with controls.

tones into the brain could stimulate the sympathetic nerve activity in the VMH (36). The transport of ketones into the brain is regulated by a specific carrier mechanism across the blood-brain barrier (44). One could speculate that either an increase in the blood levels of free fatty acids or ketoacids or an increased activity of this transport system may be responsible for these derangements. However, this hypothesis at this time remains purely speculative. Further studies are needed to ascertain the mechanisms responsible for the increased sympathetic activity in the VMH and IBAT of uremic rats and to determine whether there is a causative relationship between the increase in NE turnover in the VMH and the IBAT.

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