PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 178, 240–245 (1985)

Reduced Sympathetic Neuronal Uptake ($Uptake_1$) in a Genetic Model of Desoxycorticosterone–NaCl Hypertension[1] (42005)

LAWRENCE R. KRAKOFF, DRORI BEN-ISHAY, AND JUDITH MEKLER

Hypertension Division of the Department of Medicine, Mount Sinai School of Medicine, CUNY, New York, New York, and Department of Medicine, Hadassah Hospital, Mount Scopus, Jerusalem, Israel

Abstract. The effect of desoxycorticosterone (DOC)–NaCl treatment upon sympathetic neuronal uptake of norepinephrine ($uptake_1$) was evaluated in strains of hypertension-resistant (SBN) and -prone (SBH) rats. Uninephrectomized animals were given either a placebo pellet sc and tapwater (untreated) or a 25-mg DOC pellet and 1% NaCl. Four weeks later, tail systolic pressure was significantly higher in DOC–NaCl SBH, 183 ± 3 mm Hg, than SBN, 141 ± 2 ($P < 0.01$). [^{3}H]Norepinephrine (NE) uptake was determined in heart slices of all four groups by incubation in Krebs buffer at 37°C for 20 min at several concentrations. Preliminary studies confirmed that this is a measure of $uptake_1$. Heart slices of DOC–NaCl-treated SBN and SBH rats had significantly reduced NE uptake at concentrations of 10–80 nM ($P < 0.01$); there was no significant difference between SBN and SBH in this regard. Untreated SBH rats have been shown to have a defect in baroreflex regulation when normotensive. The results raise the possibility that the greater increase in arterial pressure caused by DOC–NaCl in SBH compared to SBN may be related to both the inborn difference in reflex function and an acquired reduction in inactivation of norepinephrine by sympathetic neuronal uptake.

Sympathetic neuronal uptake ($uptake_1$) contributes to physiological inactivation of norepinephrine which has been released from adrenergic neurons or has been secreted (or infused) into the circulation (1, 2). This process may be the major route of inactivation of transmitter in the heart and in small muscular arteries and arterioles, i.e., resistance vessels (3). $Uptake_1$ is a sodium-dependent transport process linked to the activity of Na/K-ATPase (1, 4–6).

Recent reports indicate that Na/K-ATPase activity is reduced in several models of experimental hypertension, possibly due to a circulating, ouabain-like inhibitor of the enzyme (7). Although there is no evidence that neuronal Na/K-ATPase is altered in experimental hypertension, these observations prompted us to evaluate norepinephrine $uptake_1$ *in vitro,* in a model which is characterized by inherited resistance or sensitivity to the hypertensive action of treatment with desoxycorticosterone and high salt intake (8).

Methods. *Norepinephrine uptake.* Tissue uptake of [^{3}H]norepinephrine was determined according to methods similar to those of Sharma and Banerjee (4). Rats were rapidly anesthetized with ether; the hearts were removed and placed in cold Krebs buffer until beating had ceased. The atria were then removed and 0.5-mm ventricular slices weighing 20–40 mg were prepared using a Stadie-Riggs microtome. Individual slices were placed in separate counting vials containing 10 ml Krebs buffer at 4°C. The buffer solution had the following composition (in mM): NaCl, 122; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 5.5 (100 mg/dl); Na_2EDTA, 1 mg/dl; and ascorbic acid, 2 mg/dl. Inhibitors were added before incubation. The vials were placed in a water bath at 37°C and O_2/CO_2, 95/5%, bubbled through each vial through a polyethylene tube connected to a manifold for maintenance of pH 7.4. Control vials were kept in crushed ice at 4°C.

After a 20-min preincubation period, [^{3}H]norepinephrine with varying amounts of unlabeled norepinephrine as needed to achieve given concentrations was added and incubation continued for a period of 20 min. At the end of the incubation period, slices

[1] Presented to the Fifth Scientific Meeting of the Inter-American Society of Hypertension, March 18, 1983.

0037-9727/85 $1.50

were removed and washed in 10 ml of cold buffer, blotted on filter paper, weighed, and then placed in 2 ml 0.4 *N* $HClO_4$ at 4°C. Tissue slices were extracted for 21 hr at 4°C, centrifuged at 3000 rpm for 10 min, then duplicates of 0.5 ml were removed for counting. Duplicating 0.1-ml samples of the incubation medium were also placed in perchloric acid and aliquots were taken for isotope counting. Tritium concentration was determined in 5 ml PicoFlour (Packard) in a Packard Model 3255 liquid scintillation spectrometer. Correction for quenching was made for each sample. Counting efficiency was in the range of 28–32%.

Preliminary experiments established that [^{3}H]norepinephrine tissue uptake was linear with time over 30 min. Nonspecific tissue accumulation of [^{3}H]norepinephrine was assessed by incubation of tissue at 4°C (4). Active tissue uptake was calculated as the difference between tissue accumulation at 37°C and that at 4°C and calculated as picomoles of norepinephrine per gram per minute. DL-[^{3}H]Norepinephrine with a specific activity of 12–15 Ci/mmole was routinely used at a concentration of 4–6 n*M* for these studies. Unlabeled L-norepinephrine in the range 10–80 n*M* final concentration was added as indicated. Two slices from each heart were incubated at each concentration of norepinephrine.

Recovery of [^{3}H]norepinephrine from both incubation media and tissue was 70–75% when assessed by adsorption and elution from acid-washed alumina at pH 8.4 according to the method of Snyder (9). In preliminary studies, the formation of *O*-methylated metabolites of norepinephrine was found to be less than 15% in both media and tissue samples. The formation of total deaminated metabolites was assessed by extraction with ethyl acetate at pH 2.0 and found to be less than 10%.

Animals. Preliminary studies for characterization of the effect of inhibitors on [^{3}H]norepinephrine uptake were performed in heart slices from normotensive Sprague–Dawley rats. Hypertension-prone (SBH) and -resistant (SBN) rats derived from the Sabra strain at Hebrew University (8) were kept on regular chow until 8 weeks of age, and then underwent unilateral nephrectomy. Each substrain was divided into two groups. One received a subcutaneous placebo implant and was kept on the same food and tap water. The other had a 25 mg desoxycorticosterone pellet implanted and was placed on 1% saline as drinking fluid. Norepinephrine uptake studies were performed 4 weeks later.

Systolic arterial pressure was determined in the tail arteries of prewarmed animals (8) on the day before [^{3}H]norepinephrine uptake experiments. Body weight was measured on the day of the experiments. Heart weight was based upon the combined weight of all slices plus remaining ventricular tissue for each heart and thus includes both right and left ventricle; heart weight is expressed as milligrams per 100 g body wt.

Data analysis. Statistical analysis was performed by analysis of variance and conventional linear regression. Calculations were performed by Techtronix 31 system and the SAS system of the CUNY computer center. Individual means were compared by the Duncan's multiple range test. Comparisons between means were made only if the *F* statistic for overall analysis of variance indicated that significance ($\alpha = 0.05$) was reached.

Because of possible regional differences in innervation between heart slices, [^{3}H]norepinephrine uptake analyses were not averaged for the duplicates of each heart at each concentration. Instead all slices were considered individually for the analysis of variance.

Results. Preliminary experiments were performed in order to assure that our uptake method gave results comparable to a number of previously published studies. In heart slices of normotensive rats, imipramine (10^{-4} *M*) *in vitro* reduced [^{3}H]norepinephrine uptake by 90% ($P < 0.001$). [^{3}H]norepinephrine uptake was reduced 86% ($P < 0.001$) in heart slices of rats given 6-OH dopamine, 100 mg/kg intravenously, 24 hr before the experiment.

Systolic arterial pressure in the group of untreated SBN rats used for [^{3}H]norepinephrine uptake studies was 127 ± 2 mm Hg ($n = 6$). In the untreated SBH rats, systolic arterial pressure was 148 ± 2 mm Hg ($n = 6$). The difference between the two was significant, $P < 0.05$. DOC–NaCl-treated SBN rats had an average systolic pressure of 141 ± 2 mm Hg ($n = 5$) while that of the SBH rats was 183 ± 3 mm Hg ($n = 6$). The

difference was highly significant, $P < 0.01$. Furthermore, the absolute difference in arterial pressure between untreated and treated SBN rats (+14 ± 3 mm Hg) was significantly lower than that of the SBH rats (+35 ± 4 mm Hg, $P < 0.01$). The relationship between arterial pressure and ventricular weight is shown in Fig. 1; a highly significant direct correlation is evident.

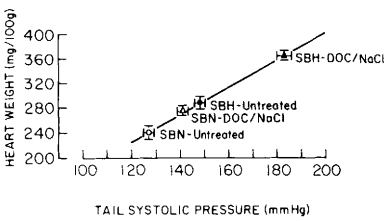

FIG. 1. Relationship between systolic arterial pressure and ventricular weight in untreated and desoxycorticosterone-NaCl-treated SBN and SBH rats. Heart weight = (2.2 ± 0.2) × systolic pressure −38. $r = 0.90$, $P < 0.001$. Results are expressed as means ± SE.

The effect of DOC-NaCl treatment on [^{3}H]norepinephrine uptake in heart slices of the four groups of rats is given in Table I. Statistical analysis by ANOVA indicated a significant effect ($P < 0.01$) at each concentration of norepinephrine employed for the comparison of combined groups (untreated SBH + SBN versus treated SBN + SBH). By comparing the means of the four groups individually at each concentration, it is apparent that DOC-NaCl treatment significantly reduced [^{3}H]norepinephrine uptake in both SBN and SBH rat heart slices compared those of both untreated groups at the two lower concentrations (10 and 20 nM). At the two higher concentrations, the significant differences among means were the reductions of DOC-NaCl-treated SBH slices compared to those of the two untreated groups (40 nM) or only the untreated SBN (80 nM).

Discussion. Abnormal metabolism of norepinephrine has previously been documented in experimental hypertension due to desoxycorticosterone-NaCl. Reduced concentration of unlabeled norepinephrine, reduced tissue accumulation of [^{3}H]norepinephrine, and increased turnover of the catecholamine have been described in several tissues outside the central nervous system (10–16). Increased plasma norepinephrine concentration has also been found in desoxycorticosterone-NaCl hypertension (15). Such studies suggest that increased sympathetic tone contributes to elevation of arterial pressure. A pattern of increased sympathetic nerve activity with enhancement by hypothalamic stimulation has been described in this model (17).

Enhanced vascular responsiveness to norepinephrine has uniformly been observed in experimental desoxycorticosterone-NaCl hypertension (17, 18) and in human subjects given α-fluorhydrocortisone (19). Dahl salt-sensitive (S) rats fed a high salt diet have greater vascular responses to sympathetic

TABLE I. NOREPINEPHRINE UPTAKE IN HEART SLICES

	Norepinephrine (nM)			
	10	20	40	80
SBN untreated (12)	4.3 ± 0.3	6.5 ± 0.3	11.2 ± 0.9	18.7 ± 2.7
SBH untreated (12)	3.9 ± 0.2	7.0 ± 0.8	9.8 ± 0.7	16.5 ± 1.2
SBN DOC-NaCl (10)	2.7 ± 0.3[a,b]	3.8 ± 0.5[a,b]	9.0 ± 1.5	12.3 ± 1.3
SBH DOC-NaCl (12)	1.9 ± 0.3[a,b]	2.4 ± 0.4[a,b]	6.2 ± 0.8[a,b]	11.3 ± 1.5[a]
F	13.67	13.28	4.55	3.81
P	<0.0001	<0.0001	<0.01	0.02

Note. Norepinephrine uptake rates are given as pmole/g/min. Means ± SE are shown. Comparisons by one-way ANOVA were made at each concentration of norepinephrine. $\alpha = 0.05$, by Duncan's multiple range test. Numbers of slices incubated at each concentration are given in parentheses.

[a] Significantly different ($\alpha = 0.05$) from SBN untreated.

[b] Significantly different ($\alpha = 0.05$) from SBH untreated.

nerve stimulation compared to R rats suggesting greater sensitivity to released neurotransmitter (20). These observations suggest that reduced inactivation of norepinephrine released from or available to nerve endings may occur as a result of sodium retention.

Evaluation of [^{3}H]norepinephrine uptake in desoxycorticosterone–NaCl hypertension was first studied in isolated perfused hearts. No differences were found between hypertensive and control hearts in [^{3}H]norepinephrine accumulation (10). It is not certain that the observed [^{3}H]norepinephrine accumulation was entirely due to neuronal uptake as compared to extra-neuronal uptake (uptake$_2$) or distribution by diffusion into extracellular space (i.e., not temperature dependent). More recently, [^{3}H]norepinephrine uptake was evaluated in heart slices of desoxycorticosterone–NaCl hypertensive and control rats of the Holtzman strain. Some reduction in [^{3}H]norepinephrine uptake was found in the hearts of the hypertensive rats. However, variation was large and the results were not statistically significant (16).

The *in vitro* measurements of [^{3}H]norepinephrine accumulation by heart slices in this presentation were designed to assess uptake$_1$, a function of sympathetic neurons. Preliminary experiments in tissues of normotensive animals were consistent with previous studies of uptake$_1$ in that there was highly effective inhibition of [^{3}H]norepinephrine uptake by imipramine (1) and by chemical sympathectomy, which is consistent with the disappearance of sympathetic nerve terminals (21).

It is unlikely that our measurements of [^{3}H]norepinephrine uptake included uptake$_2$. This process occurs only at relatively high concentrations of the amine (greater than 10^{-7} M). Uptake$_2$ is reduced by several steroids including desoxycorticosterone (1). However, this steroid has been shown to have no effect upon uptake$_1$ in isolated rat hearts (22).

The effect of desoxycorticosterone–NaCl per se upon [^{3}H]norepinephrine uptake was highly significant at all concentrations of norepinephrine. This effect did not differ between the two strains as [^{3}H]norepinephrine uptake was not significantly different between untreated SBN and SBH strains or between treated strains. Thus, we cannot document that desoxycorticosterone–NaCl treatment has a preferential effect upon norepinephrine uptake of the hypertension-prone strain compared to the resistant one, despite the much greater increase in arterial pressure seen in the former.

SBH rats have reduced atrial norepinephrine concentration when compared to SBN rats (23). It has recently been found that SBH rats also have impaired baroreflex activity in response to a pressor stimulus (phenylephrine) compared to SBN rats without desoxycorticosterone–NaCl treatment. This difference between the two strains is apparent by 7 weeks of age (24). A similar difference in baroreflex pattern has been described in Dahl S compared to R rats on low salt intake (25). In this study DOC–NaCl treatment did cause a small but significant increase in pressure of SBN rats, but a much larger elevation in the SBH group. Taken together, these results suggest that experimental genetic salt-induced hypertension may depend, for its neurogenic component, upon the combination of impaired baroreflex buffering of raised pressure with a defect in inactivation of norepinephrine by sympathetic neuronal uptake.

Our studies do not identify the cause of reduced [^{3}H]norepinephrine uptake as a result of desoxycorticosterone–NaCl treatment. As ventricular enlargement accompanied the changes in arterial pressure, cardiac hypertrophy could be playing some role. Although tissue slices were washed in buffer before incubation, traces of bloodborne factors might have remained. It has recently been reported that high concentrations of plasma from anesthetized normal dogs reduce norepinephrine uptake in isolated canine saphenous vein strips (26). Reduction of tissue Na/K-ATPase activity has been observed in models of salt-dependent experimental hypertension and attributed to circulating factors with ouabain-like activity (7). Such effects could explain the reduced norepinephrine uptake we observed in the desoxycorticosterone–NaCl treated SBH and SBN heart slices. However, there is no direct evidence that sympathetic neuronal Na/K-ATPase is af-

fected in experimental hypertension either at a tissue level or by circulating inhibitors of the enzyme.

In summary, norepinephrine uptake is significantly reduced in heart slices of desoxycorticosterone–NaCL-treated hypertension-prone and -resistant rats, despite differences in the arterial blood pressure of these animals. Perhaps the induced changes in uptake have no relationship to pathogenesis of this form of experimental hypertension. Alternatively, the results suggest that the neurogenic component of salt-dependent hypertension may require more than a single abnormality; predisposition to defective baroreflex function, when combined with reduction in sympathetic neuronal uptake of norepinephrine and increased sympathetic tone, might account for the quantitative increase in arterial pressure that defines genetic hypertension due to excess salt accumulation.

This work was supported by the Lady Davis Foundation of the Hebrew University, Jerusalem, Israel, the Elefant Research Fund, and USPHS Grant HL 13479. The authors would like to express their appreciation to Ruth Gonsky and Elaine Grohman for the preparation of the manuscript.

1. Iversen LL. Role of transmitter uptake mechanisms in synaptic transmission. Brit J Pharmacol **41**:571–591, 1971.
2. Paton DM, ed. The Mechanism of Neuronal and Extraneuronal Transport of Catecholamines. New York, Raven Press, 1976.
3. Bevan J. Some bases of differences in vascular response to sympathetic activity. Circ Res **45**:161–171, 1979.
4. Sharma VK, Banerjee SP. Inhibition of ^{3}H-norepinephrine uptake in peripheral organs of some mammalian species by ouabain. Eur J Pharmacol **41**:417–429, 1977.
5. Sharma VK, Banerjee SP. Regeneration of ^{3}(H)-ouabain binding to ($Na^+ + K^+$)-ATPase in chemically sympathectomized cat peripheral organs. Mol Pharmacol **15**:35–42, 1979.
6. Sharma VK, Pottick LA, Banerjee SP. Ouabain stimulation of noradrenaline transport in guinea pig heart. Nature (London) **21**:817–819, 1980.
7. Pamnani M, Huot S, Buggy J, Clough D, Haddy F. Demonstration of a humoral inhibitor of the Na^+–K^+ pump in some models of experimental hypertension. Hypertension **3**:6–101, 1981.
8. Ben-Ishay D, Saliternik R, Welner A. Separation of two strains of rats with inbred dissimilar sensitivity to DOCA-salt hypertension. Experientia **28**:1321–1322, 1972.
9. Snyder SH, Green AJ, Hendley ED. Kinetics of ^{3}H-norepinephrine accumulation into slices from different regions of the rat brain. J Pharmacol Exp Ther **164**:90–102, 1968.
10. deChamplain J, Krakoff LR, Axelrod J. Catecholamine metabolism in experimental hypertension in the rat. Circ Res **20**:136–145, 1967.
11. Krakoff LR, de Champlain J, Axelrod J. Abnormal storage of norepinephrine in experimental hypertension in the rat. Circ Res **21**:583–591, 1967.
12. Kazda S, Pohlova I, Bibr B, Kockova J. Norepinephrine content of tissues on DOCA-hypertensive rats. Amer J Physiol **216**:1472–1475, 1969.
13. de Champlain J, Mueller RA, Axelrod J. Turnover and synthesis of norepinephrine in experimental hypertension in rats. Circ Res **25**:285–291, 1969.
14. Nakamura K, Gerold M, Thoenen H. Experimental hypertension of the rat: Reciprocal changes of norepinephrine turnover in heart and brain stem. Naunyn Schmiedebergs Arch Pharmacol **268**:125–139, 1971.
15. Reid JL, Zivin JA, Kopin IJ. Central and peripheral adrenergic mechanisms in the development of deoxycorticosterone-saline hypertension in rats. Circ Res **37**:569–579, 1975.
16. Giachetti A, Rubinstein R, Clark TL. Noradrenaline storage in desoxycorticosterone-saline hypertensive rats. Eur J Pharmacol **57**:99–106, 1979.
17. Takeda K, Bunag RD. Augmented symathetic nerve activity and pressor responsiveness in DOCA hypertensive rats. Hypertension **2**:97–101, 1980.
18. Beilin LJ, Wade DN, Honour AJ, Cole TJ. Vascular hyperreactivity with sodium loading and with desoxycorticosterone induced hypertension in the rat. Clin Sci **39**:793–810, 1970.
19. Chobanian AV, Volicer L, Tifft CP, Gavras H, Liang C, Faxon D. Mineralocorticoid-induced hypertension in patients with orthostatic hypotension. N Engl J Med **301**:68–73, 1979.
20. Takeshita A, Mark AL. Neurogenic contribution to hindquarters' vasoconstriction during high sodium intake in Dahl strain of genetically hypertensive rat. Circ Res **43**(Suppl 1):86–91, 1978.
21. Thoenen H, Tranzer JP. Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxy dopamine. Naunyn Schmiedebergs Arch Pharmacol in Exp Pathol **261**:271–288, 1968.
22. Salt PJ. Inhibition of noradrenaline uptake$_2$ in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines. Eur J Pharmacol **20**:329–340, 1972.

23. Le Quan-Bui KH, Devynck MA, Pernollet MG, Ben-Ishay D, Meyer P. Variations in cardiac noradrenaline content during sodium loading in hypertension prone and resistant rats. Experientia **37**:169–170, 1981.
24. Shorer D, Weinstock M, Ben-Ishay D. Increased baroreceptor reflex sensitivity in hypertension-resistant Sabra rats. In: Rascher W, Clough D, Ganten D, eds. Hypertensive Mechanisms. Shattauer Verlag, pp371–374, 1982.
25. Gordon FJ, Matsuguchi H, Mark A. Abnormal baroreflex control of heart rate in pre-hypertensive and hypertensive Dahl genetically salt-sensitive rats. Hypertension **3**(Suppl 1):135–141, 1981.
26. Freas W, Muldoon SM, Haddy FJ. Accumulation of (^{3}H)norepinephrine in canine saphenous vein: Influence of plasma. Amer J Physiol **243**:424–433, 1982.

Received February 27, 1984. P.S.E.B.M. 1985, Vol. 178.
Accepted October 15, 1984.