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Potassium Turnover and Norepinephrine Sensitivity in the Thoracic Aorta of the Dahl Rat (41739)

JACQUELYN M. SMITH AND ALLAN W. JONES*

**Department of Physiology, University of Missouri, Columbia, Missouri 65212*

Abstract. This *in vitro* study evaluated the basal ^{42}K turnover and response to norepinephrine (NE) in the thoracic aorta removed from Dahl salt-sensitive (S) and salt-resistant (R) rats. Five-week-old S and R rats were placed on either a high-salt (HS) or low-salt (LS) diet. After 5 weeks of the diet, systolic blood pressure, aortic weight/length ratio, and the cellular pool of K^+ were elevated in the S-HS group only. In contrast, the steady state turnover of ^{42}K, the NE ED_{50}, and the response to a supramaximal dose of NE were the same in both groups of salt-sensitive and salt-resistant rats. These results suggest that, despite the presence of a greatly elevated systolic blood pressure and evidence of aortic hypertrophy, the intrinsic electrolyte metabolism of the vascular smooth muscle in the Dahl hypertensive rat is the same as that of the Dahl normotensive rat.

Two strains of rats generated by Dahl in 1962 develop hypertension (salt-sensitive, S rat) or remain normotensive (salt-resistant, R rat) during excess salt intake (1, 2). Increased blood and plasma volume (3), elevated levels of an adrenal mineralocorticoid, 18OH-DOC (4), and hyperresponsiveness to intravenous injection of various pressor agents (5–7) have been reported in the hypertensive S rat when compared to normotensive controls. In models of hypertension induced by mineralocorticoid plus salt (8–10) as well as the SHR (spontaneously hypertensive rat) form of genetic hypertension (11), norepinephrine supersensitivity has been associated with altered ionic transport in vascular smooth muscle. It is feasible, then, that the vascular smooth muscle of the genetically salt-sensitive Dahl S rat may exhibit similar ionic transport alterations.

In this laboratory, isotope flux methodology has been used to assess the membrane transport properties and drug supersensitivity in hypertensive models. Using ^{42}K efflux as a measure of potassium turnover, an increase in the basal ^{42}K turnover and response to norepinephrine have been found in rats made hypertensive by treatment with salt plus deoxycorticosterone acetate (DOCA) or aldosterone (8–10). The purpose of the present study was to assess the basal ^{42}K turnover and its response to norepinephrine in the vascular smooth muscle of the Dahl rat.

Methods. *Animal and tissue preparation.* Salt-sensitive (S) and salt-resistant (R) Dahl male rats were obtained from Dr. Iwai of Brookhaven National Laboratory. At age 5 weeks, half of the S and R rats were placed on an 8% NaCl diet (HS) while the remaining S and R rats were placed on a 0.4% NaCl diet (LS) (Agway, Inc., Syracuse, N.Y.). The rats received the experimental diets for a minimum of 5 weeks with free access to water.

Systolic blood pressure was determined the day before the experiment by the tail cuff technique. The animals were decapitated and the heart and thoracic aorta were quickly removed. The heart was weighed after removal of atria and right ventricle. The thoracic aorta was placed in K-free dissection solution containing 0.25 m*M* Ca^{2+} to reduce endogenous K contents in preparation for ^{42}K equilibration. Loose connective tissue was removed from the vessel which was then slit lengthwise. The length was measured before mounting on a stainless-steel holder. The wet weight of the tissue was determined at the end of the experiment and used to calculate a weight (milligrams) to length (centimeters) ratio.

Solutions. The normal physiological solution used had the following millimolar composition: Na, 146.2; K^+, 5.0; Mg^{2+}, 1.2; Ca^{2+}, 2.5; Cl^-, 143.9; HCO_3^-, 13.5; H_2PO_4, 1.2; and dextrose, 11.2. Solutions were gassed with a mixture of 97% O_2–3% CO_2 to obtain pH 7.4.

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TABLE I. AORTIC DIMENSIONS AND ^{42}K CONTENTS AND TURNOVER IN DAHL SALT-SENSITIVE (S) AND SALT-RESISTANT (R) RATS ON EITHER HIGH- (HS) OR LOW- (LS) SALT DIET

Group	N	Body weight (g)	Blood pressure (mm Hg)	Aortic weight / length ($mg \cdot cm^{-1}$)	Heart weight / body weight ($mg \cdot g^{-1}$)	^{42}K slow ($mmole \cdot kg^{-1}$)	k (min^{-1})	NE Δk_{max} (min^{-1})	NE ED_{50} (M)	Steady state ^{42}K efflux ($mmole \cdot kg^{-1} \cdot min^{-1}$)
S-LS	4	$305^{a,b}$ ± 15	138^{a} ± 2	$10.8^{a,b}$ ± 0.5	2.55^{a} ± .09	$34.0^{a,b}$ ± 2.3	0.0087^{a} ± 0.0002	0.0174^{a} ± 0.0002	$0.9 \times 10^{-9\,a}$ ± 0.1	$0.295^{a,b}$ ± 0.015
S-HS	6	287^{b} ± 7	190^{b} ± 5	11.7^{b} ± 0.3	3.48^{c} ± 0.15	38.4^{b} ± 1.8	0.0097^{a} ± 0.0007	0.0162^{a} ± 0.0008	$1.0 \times 10^{-9\,a}$ ± 0.7	0.379^{b} ± 0.044
R-LS	5	341^{a} ± 13	120^{c} ± 3	$10.7^{a,b}$ ± 0.5	2.06^{b} ± 0.07	30.4^{a} ± 1.4	0.0088^{a} ± 0.0006	0.0176^{a} ± 0.0014	$1.7 \times 10^{-9\,a}$ ± 0.6	0.266^{a} ± 0.010
R-HS	4	299^{b} ± 16	122^{c} ± 4	10.1^{a} ± 0.5	$2.25^{a,b}$ ± 0.03	30.1^{a} ± 2.7	0.0086^{a} ± 0.0003	0.0180^{a} ± 0.0013	$1.4 \times 10^{-9\,a}$ ± 0.6	0.257^{a} ± 0.025

Note. Within each column, values sharing superscript letters are *not* significantly different ($P < 0.05$) as evaluated by 2×2 analysis of variance.

Solutions containing norepinephrine (NE, Winthrop) were made by serial dilution with medium containing 3.4×10^{-6} propranolol and 0.1 mM EDTA.

Isotope techniques. The procedures have been previously used (8, 11) and evaluated (12). Briefly, the aorta was incubated for 3 hr at 37°C in a physiological solution containing ^{42}K (University of Missouri Nuclear Reactor). After a 2-sec rinse, the aorta was moved through a series of tubes containing nonradioactive solution to determine steady state turnover. The tissue then was passed through three tubes containing NE for a 10-min exposure. This was followed by two 10-min washes before exposure to the next dose. A gamma well was used to count ^{42}K. The fraction exchanged per minute for each washout period was computed which, under steady state conditions, represents the rate constant, k (min^{-1}). Under steady state conditions, it is assumed that the efflux of ^{42}K equals the influx. Values for the 30- to 40-min period (just before the first NE exposure) were used for statistical comparisons. A digital computer was used to process the data (12). Dose–response relations were derived from standard normalizing procedures used for the study of drug supersensitivity (13). The response to a given dose of NE, Δk, was taken as the difference between the highest rate constant in the presence of the agonist and the rate constant for the wash period just before exposure to NE. The maximal response, Δk_{max}, was taken as the response to a supramaximal dose of NE, 6×10^{-6} M (9). The individual responses, Δk, were normalized in terms of Δk_{max} for each aorta and represented as percentage. The median effective dose, ED_{50}, was determined for each aorta by linear interpolation between the log dose just below and just above the 50% response. The logs of ED_{50} values were used to make statistical comparisons of ED_{50} (14). The geometric means of the ED_{50} values are presented in Table I.

The cellular pool of K^+ was estimated from the counts remaining after 1-min washout and the specific activity of the ^{42}K solution. This yielded results equivalent to the extrapolation of the slowly exchanging component to zero time because the extracellular constituents were cleared of ^{42}K much faster than the cells (8, 11). The specific activity was derived from

the ratio of micromoles K^+ (determined by flame photometry) to counts per second (gamma well counter) in a weighed sample of ^{42}K solution. The slow ^{42}K pool (^{42}K slow) representing intracellular K^+ was calculated as millimoles per kilogram, wet weight, of aorta.

Statistics. The mean logs of ED_{50} values were used to make statistical comparisons of the ED_{50}. The means ± standard error of the mean (SEM) are presented. Significance at the level of $P < 0.05$ was determined using 2×2 factorial analysis of variance.

Results. A 2×2 factorial analysis of variance indicated a difference, primarily due to diet, between the body weight of the S-HS and R-LS groups only. Aortic weight/length ratio of the S-HS group was greater than that of the R-HS group but this difference was related to genotype rather than diet. An interaction between genotype and salt contributed to the greatly elevated systolic blood pressure and heart/body weight ratio found in the S-HS group.

The steady state turnover of ^{42}K, NE ED_{50}, and the response to a supramaximal dose of NE (6.0×10^{-6} *M*) were the same in both the sensitive (HS and LS) and resistant (HS and LS) groups. Analysis of variance indicated there was no interaction between genotype and diet for these three parameters. In contrast, both the cellular pool of K^+ (slow ^{42}K) and the calculated steady state K^+ efflux (slow ^{42}K times turnover) of the S-HS group were greater than the R-LS and R-HS groups, differences attributed to genotype rather than diet by the factorial analysis.

Discussion. The systolic blood pressure change induced in the Dahl rat was dependent on genotype (S-type) and diet (high salt). The ionic properties of the aorta, however, did not exhibit such dual dependency. There were no alterations in the basal ^{42}K turnover or sensitivity to norepinephrine in the thoracic aorta of the Dahl salt-sensitive rat that can be related to either genotype or to a high-salt diet. This observation differs from previous findings of elevated ^{42}K turnover and sensitivity to norepinephrine in aorta from genetically hypertensive rats of the Okamoto strain (SHR) and from Sprague–Dawley rats made hypertensive with mineralocorticoid–salt treatment (8–11). Thus, the elevated levels of 18OH-DOC in the Dahl S rat (4) apparently do not induce the same changes in the potassium turnover as an excess of exogenous DOCA or aldosterone. There are, however, increases in ^{42}K contents and in weight/length ratio of aorta from Dahl S rats which are primarily related to genotype. Increased ^{42}K contents and weight/length ratio were related to elevated cell water contents in aorta from DOCA–salt hypertensive rats (8). Based on these observations, it appears that the thoracic aorta from Dahl S rats contain a greater proportion of cellular material per unit weight. This finding is in contrast to the SHR in which there were no changes in aortic K or cell water contents during early established hypertension (11). Although similar to the early aortic changes associated with DOCA–salt hypertension (8), the underlying factor differ (genotype versus mineralocorticoid–salt treatment).

Previous reports on the properties of vascular smooth muscle in the Dahl S and R rats are varied. *In vitro,* no changes occurred in the membrane potential, intracellular concentration of Na, K, Cl, or the contractile response to norepinephrine in the caudal artery of the Dahl S rat after 5 weeks of a high-salt diet (15, 16). However, in the latter study, systolic blood pressure only reached a level of 161 mm Hg which may have minimized changes in the vascular smooth muscle. In contrast, both the ouabain-insensitive (17) and ouabain-sensitive (3, 17) uptake of ^{86}rubidium was elevated (nanomoles per milligram dry weight) in the tail artery and thoracic aorta (3) of the Dahl S rat. However, our present findings suggest that this increase in the uptake of rubidium in the aorta may reflect an increased proportion of cellular contents rather than intrinsic changes in the vascular smooth muscle membrane. In the present study, the steady state efflux of ^{42}K ($mmole \cdot kg^{-1} \cdot min^{-1}$) was also significantly increased in the Dahl S rat (Table 1). However, this increased ^{42}K steady state efflux resulted primarily from the enlarged cell pool size (slow ^{42}K) in the aorta rather than from changes in the rate constants for potassium.

Dahl S rats are characterized by an increase in peripheral vascular resistance in response to high salt intake (18) and both humoral (19) and neurogenic (20, 21) mechanisms have been implicated. In addition, renal factors also

appear to play a role in the development of hypertension in the Dahl S rat. An intrinsic defect in the salt excretion from isolated perfused kidneys (22, 23) has been reported. Increased concentration of renal α_2 adrenergic receptors (24) and decreased levels of renal prostaglandins (25) lend support to the suggestion that sodium handling in the kidney of the Dahl S rat is altered. *In vivo*, an enhanced pressor response to intravenous norepinephrine has been reported (5–7). It was suggested that a change in baroreceptor sensitivity rather than intrinsic vascular reactivity (6) was responsible for the pressor supersensitivity. In support of this hypothesis, the vasoconstrictor response to norepinephrine was unaltered in a preparation using the autoperfused hindquarters of the Dahl S rat (20). Thus, it is possible that these humoral, neurogenic, and renal factors may influence the vascular reactivity *in vivo*. However, in their absence under *in vitro* conditions, ^{42}K turnover and catecholamine effects on ^{42}K fluxes appear to be relatively unchanged in the Dahl S rat. Because of the location of baroreceptors in the aortic arch, the findings of increased aortic ^{42}K contents and weight/length ratio may have relevance to altered baroreceptor sensitivity in Dahl S rats.

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