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ADRENAL MEDULLARY STORAGE VESICLES OF THE SPONTANEOUSLY HYPERTENSIVE RAT*

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Abstract—The adrenal medullary vesicles of normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR) were examined in order to determine whether increased sympatho-adrenal activity plays a role in the elevation of blood pressure in the SHR. The uptake of ^{14}C-epinephrine/100 μg of endogenous catecholamines in isolated SHR vesicles was higher than in NWR, while the uptake of ^{3}H-metaraminol was the same as in NWR; thus, SHR vesicles exhibited a higher preference for epinephrine vs metaraminol compared to NWR. The difference in uptake was due to a lower K_m for epinephrine in SHR. The storage of amines was the same in SHR and NWR, as demonstrated by measurements of catecholamine to ATP ratios in purified vesicles, and effluxes from the vesicles of endogenous and newly incorporated amines. The ratio of catecholamines to dopamine β-hydroxylase (DBH, a marker for storage vesicles) was higher in SHR for three reasons: (1) there were fewer vesicles per gland; (2) there was less DBH per vesicle, indicated by an increased precursor/product ratio; and (3) there was a higher catecholamine content per vesicle, as shown by an increase in the ratio of heavy to light vesicles on discontinuous sucrose density gradients. SHR adrenals were depleted of catecholamines after insulin administration to a greater extent than were NWR adrenals, and both SHR and NWR exhibited induction of tyrosine hydroxylase and dopamine β-hydroxylase after insulin. None of these findings is consistent with the view that sympatho-adrenal hyperactivity occurs in the SHR; the data suggest that hypoactivity occurs, perhaps secondarily to the hypertension.

Over the past decade, several useful animal models have been developed to aid in the study of essential hypertension. To date one of the best models is the Wistar-derived spontaneously hypertensive rat (SHR) developed in Japan by Okamoto [1]. These animals typically develop mean arterial blood pressures greater than 180 mm Hg within 10 weeks of birth compared to 120 mm Hg in normotensive Wistar rats (NWR). The incidence of hypertension is 100 per cent and requires no surgical, pharmacological or dietary manipulation.

As in human essential hypertension, considerable attention in the SHR has been devoted to a possible etiological role for the sympathetic nervous system and its endocrine counterpart, the adrenal medulla. Studies from several laboratories have yielded conflicting results. Initially, it was reported that the levels of catecholamines and the catecholamine-synthesizing enzymes, tyrosine hydroxylase and dopamine β-hydroxylase, were elevated in SHR adrenals [2, 3]. Since the activities of these enzymes are usually indicative of the degree of neural input to the tissue [4], it could be hypothesized that the sympatho-adrenal axis was hyperactive and that this was the cause of the hypertension. However, subsequent studies in other laboratories indicated lower catecholamine levels and enzyme activities in the SHR [5, 6]. In fact, recent work by Lovenberg *et al.* [6] has shown that the absolute enzyme activities reflect strain differences and that the comparative levels in SHR could be higher or lower than "normal" depending upon which strain is used as a normotensive control. Catecholamine turnover studies have suggested that a decreased level of synthesis and secretion exists in the sympatho-adrenal axis of the SHR [7–9]; however, this type of study depends upon the administration of a radioactive precursor or a synthesis inhibitor which may not be identically distributed or pooled in SHR and NWR.

Obviously, additional studies are needed to determine whether the sympatho-adrenal axis is hyper- or hypoactive in the SHR. Recent work from our laboratory has detailed stimulation-dependent changes in the properties of the catecholamine storage vesicles of the rat adrenal medulla [10, 11]; these included alterations in the soluble contents of the vesicles, vesicle density and uptake and storage of amines. In the current study, those techniques are used to evaluate differences in adrenomedullary function between SHR and NWR.

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METHODS

Male spontaneously hypertensive rats were obtained from Carworth Farms and used between 10 and 12 weeks of age. Male normotensive Wistar rats were obtained from the same source and were age-matched to the hypertensive rats for each experiment.

Catecholamine content and enzyme activities. Animals were sacrificed by decapitation and the adrenals were removed, cleaned of fat and connective tissue, weighed and homogenized (glass-to-glass) in isotonic KCl (1·25 ml/gland). Aliquots were removed for determinations of catecholamines, dopamine β-hydroxylase and monoamine oxidase activity; the remainder was centrifuged at 26,000 *g* for 10 min and the supernatant analyzed for tyrosine hydroxylase activity. Data are reported in terms of content or activity per gland rather than per mg of tissue or per mg of protein because of the sizable contribution of the cortex to organ weight and protein content.

Incorporation of amines. Each pair of adrenal glands from individual animals was homogenized (glass-to-glass) in 2·2 ml of 0·3 M sucrose, buffered at pH 7·4 with 0·025 M Tris–sulfate, containing 10^{-5} M iproniazid (irreversible monoamine oxidase inhibitor), and a 0·1-ml aliquot was withdrawn for catecholamine analysis. The homogenate was centrifuged at 800 *g* for 10 min, and the supernatant was utilized for determinations in the presence of 5 mM ATP and Mg^{2+} of the temperature-dependent uptakes of ^{14}C-epinephrine (0·1 mM), or in separate tubes, ^{3}H-metaraminol (0·1 mM with 0·1 mM epinephrine added) as described previously [12]. Incubations lasted 30 min at 30°, and the labeled vesicles were washed twice before analysis of radioactivity and catecholamines. The results were expressed as uptake/gland and as uptake/100 μg of endogenous catecholamines; the former parameter reflects the number of functional vesicles in the adrenal, while the latter indicates the ability of individual vesicles to take up amines relative to their catecholamine contents [10].

Incorporation kinetics were determined in a similar fashion except that glands from 6 to 10 animals were pooled, and the external amine concentration was varied between 5 and 80 μM for epinephrine and between 0·1 and 1 mM for metaraminol. External catecholamine concentrations averaged 5 μM for samples in which metaraminol uptake kinetics were measured.

Activity of dopamine β-hydroxylase in intact vesicles. Vesicles were prepared and analyzed as described for uptake studies except that the incubations contained 5 mM ATP–Mg^{2+}, 1 mM fumarate, 1 mM ascorbate and 5 μCi ^{3}H-tyramine (50 μM). After 30 min at 30°, the vesicles were sedimented, washed twice and lysed with 3·5% perchloric acid. The octopamine formed was assayed as ^{3}H-parahydroxybenzaldehyde formed by periodate oxidation [13] as described previously [14].

Efflux of amines. Pooled storage vesicles were labeled with ^{14}C-epinephrine as described for uptake measurements, washed twice, and resuspended (Teflon-to-glass) in fresh sucrose–Tris. The efflux of endogenous and newly incorporated epinephrine at 30° was followed at various times over a 1-hr period using the technique of Slotkin *et al.* [14].

Subcellular catecholamine distribution and ATP determinations. Adrenals from individual animals were homogenized (glass-to-glass) in 2·5 ml sucrose–Tris–iproniazid and centrifuged at 800 *g* for 10 min to remove debris. The pellet (fraction P_1) was resuspended in 5 ml of 3·5% perchloric acid, centrifuged and the supernatant analyzed for catecholamines. An aliquot of the supernatant (S_1) was also deproteinized and analyzed for catecholamines. One ml of S_1 was centrifuged at 26,000 *g* for 10 min, and the pellet (P_2) was resuspended in 2 ml of 3·5% perchloric acid, centrifuged and analyzed, as was an aliquot of the 26,000 *g* supernatant (S_2). Fraction P_2 contains storage vesicles of all densities, vesicle membranes, mitochondria and other contaminating particles, while S_2 contains the soluble fraction.

Another ml of S_1 was layered on 2·5 ml of 1·6 M sucrose (buffered at pH 7·4 with 25 mM Tris) and centrifuged at 140,000 *g* for 2 hr in the No. 40 rotor of the Beckman model L5-50 ultracentrifuge. This procedure separates intact storage vesicles of high density from most contaminating particles [15], from membranes of lysed vesicles [10], from vesicles of lower density with lower catecholamine concentrations [16], and from the soluble fraction. The 0·3 M and 1·6 M sucrose layers were combined (fraction S_3), deproteinized and analyzed for catecholamines. The heavy vesicle pellet (P_3) was lysed by resuspension (glass-to-glass homogenization) in 2 ml of ice-cold H_2O, and an aliquot was withdrawn for catecholamine analysis. The remainder was centrifuged at 26,000 *g* to remove the vesicle membranes, and the supernatant was analyzed for ATP.

A flow sheet of the fractionation procedure appears in Fig. 1.

Secretion evoked by insulin. Rats were starved overnight and administered insulin (5 i.u./kg) via a tail vein. Three and 6 hr later, 20% glucose (3 ml, i.p.) was administered to terminate the hypoglycemic shock. Animals were sacrificed 1, 4 and 7 days later, and adrenal homogenates were analyzed for catecholamines, tyrosine hydroxylase and dopamine β-hydroxylase as described above.

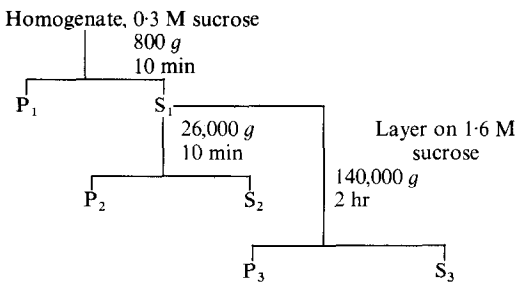

Fig. 1. Flow diagram for subcellular fractionation of adrenal homogenates.

Table 1. Catecholamine content and enzyme activities of adrenals from normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR)*

	Body wt (g)	Adrenal wt (mg/gland)	Catechol-amines (μg/gland)	Enzyme activities (nmoles/gland/hr)		
				Tyrosine hydroxylase	Dopamine β-hydroxylase	Monoamine oxidase
NWR	267 ± 5 (47)	18·5 ± 0·5 (28)	15·3 ± 0·9 (41)	4·6 ± 0·5 (19)	1·53 ± 0·16 (23)	0·97 ± 0·04 (10)
SHR	169 ± 4† (47)	16·5 ± 0·5‡ (28)	11·0 ± 0·7† (41)	3·3 ± 0·3§ (20)	0·69 ± 0·07† (23)	0·83 ± 0·04‖ (10)
SHR as % of NWR	63 ± 2	89 ± 3	72 ± 5	72 ± 7	45 ± 5	85 ± 5

* Data are presented as means ± standard errors of the number of determinations in parentheses.
† $P < 0·001$ vs NWR. ‡ $P < 0·01$. § $P < 0·05$. ‖ $P < 0·02$.

Table 2. Activity of dopamine β-hydroxylase in intact storage vesicles of normotensive (NWR) and hypertensive (SHR) rats*

	Tyramine/octopamine ratio	Per cent conversion
NWR	1·08 ± 0·01 (12)	48·2 ± 0·6
SHR	1·42 ± 0·02† (11)	41·4 ± 0·6†

* Data represent means ± standard errors of the number of determinations in parentheses.
† $P < 0·001$ vs NWR.

Assays. All samples for catecholamine analysis were first deproteinized by addition of perchloric acid to a final concentration of 3·5 per cent, followed by centrifugation at 26,000 *g* for 10 min. Supernatants were then analyzed by the trihydroxyindole method using an autoanalyzer [17]. Radioactive amines were measured by liquid scintillation spectrometry [18].

Tyrosine hydroxylase was measured by the method of Waymire *et al.* [19], using ^{14}C-tyrosine (10^{-5} M) as substrate, dopamine β-hydroxylase by a modification [14] of the method of Friedman and Kaufman [13] using ^{3}H-tyramine (10^{-5} M) as a substrate and para-hydroxymercuribenzoate (1 mM) to inactivate endogenous inhibitors, and monoamine oxidase by a modification [20] of the method of Laduron and Belpaire [21] also using ^{3}H-tyramine (10^{-5} M) as a substrate.

ATP was analyzed by the luciferin-luciferase method of Strehler and Totter [22] using a Farrand ratio fluorometer with filters removed; measurements were taken 20 sec after the addition of enzyme.

Statistical methods. Data are reported as means ± standard errors, and levels of significance as calculated by Student's *t*-test [23]. Uptake kinetics are presented as double-reciprocal plots as described by Lineweaver and Burk [24]; the slopes, intercepts and standard errors are calculated by the method of least-squares [23] using 19 individual uptake determinations for each curve. Efflux curves are fitted by the method of least-squares using a two-compartment model [14].

Materials. DL-Epinephrine-7-^{14}C, DL-metaraminol-7-^{3}H, L-tyrosine-carboxyl-^{14}C and tyramine-G-^{3}H were obtained from New England Nuclear Corp. Epinephrine bitartrate was obtained from Winthrop Laboratories, metaraminol bitartrate from Merck, Sharp & Dohme, iproniazid phosphate from Sigma Chemical Corp. and regular insulin (80 i.u./ml) from Squibb Pharmaceuticals. Buffered firefly extract used

Table 3. Incorporation of ^{14}C-epinephrine (0·1 mM) and ^{3}H-metaraminol (0·1 mM) by isolated adrenal storage vesicles of normotensive (NWR) and hypertensive (SHR) rats*

	Epinephrine		Metaraminol	
	(nmoles/gland)	(nmoles/100 μg endogenous catecholamines)	(nmoles/gland)	(nmoles/100 μg endogenous catecholamines)
NWR	3·46 ± 0·19 (24)	25·3 ± 1·1 (24)	0·81 ± 0·09 (24)	5·62 ± 0·54 (24)
SHR	3·11 ± 0·15 (24)	30·3 ± 1·3† (24)	0·65 ± 0·08 (23)	5·94 ± 0·39 (23)

* Data represent means ± standard errors of the number of determinations in parentheses.
† $P < 0·01$.

in the ATP determinations was purchased from Worthington Biochemicals.

RESULTS

Spontaneously hypertensive rats (SHR) were considerably smaller in size than normotensive Wistar rats (NWR) of the same age, but the differences in adrenal weight were much less marked (Table 1). The catecholamine content and tyrosine hydroxylase activity of SHR adrenals were about one-fourth lower than in NWR, but the dopamine β-hydroxylase activity was less than half that of NWR (Table 1); monoamine oxidase activity was only slightly lower in the SHR.

To measure the dopamine β-hydroxylase activity in intact vesicles, the vesicles were made to incorporate ^{3}H-tyramine and were then washed extensively. Upon subsequent lysis of the vesicles, the intravesicular ratio of tyramine to its β-hydroxylated product, octopamine, was measured (Table 2). The precursor/product ratio was close to unity for NWR, but was approximately 1·4 for SHR. The calculated percentage conversion was significantly lower in SHR (Table 2) but the difference was not as large as when dopamine β-hydroxylase activities were measured with lysed preparations (Table 1).

Despite the large difference between SHR and NWR in two vesicular components (catecholamines and dopamine β-hydroxylase), there were no significant differences in the uptakes per gland of 0·1 mM epinephrine or metaraminol in isolated vesicles (Table 3). When uptake was expressed in units of nmoles/100 μg of endogenous catecholamines (a measure of the abilities of individual vesicles to take up amines relative to endogenous stores), a small but significant increase in epinephrine uptake was observed in SHR, but metaraminol uptake was the same in SHR and NWR (Table 3).

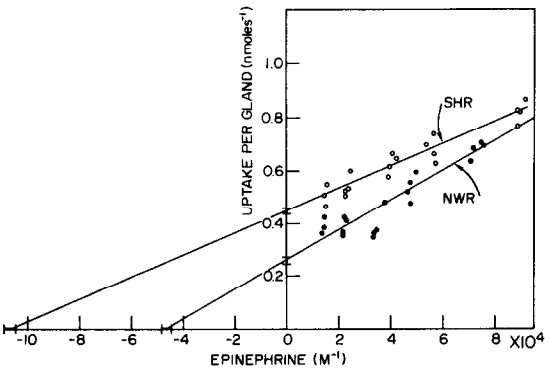

Fig. 2. Lineweaver–Burk plot for uptake of epinephrine/gland in isolated adrenal storage vesicles of spontaneously hypertensive rats (SHR) (○) and normotensive Wistar rats (NWR) (●). Bars denote standard errors of intercepts. Intercepts on both ordinate and abscissa are significantly different for SHR compared to NWR ($P < 0{\cdot}001$). For NWR, $K_m = 21{\cdot}8 \pm 0{\cdot}9$ μM, $U_{max} = 3{\cdot}88 \pm 0{\cdot}15$ nmoles/gland; for SHR, $K_m = 9{\cdot}35 \pm 0{\cdot}17$ μM, $U_{max} = 2{\cdot}24 \pm 0{\cdot}04$ nmoles/gland.

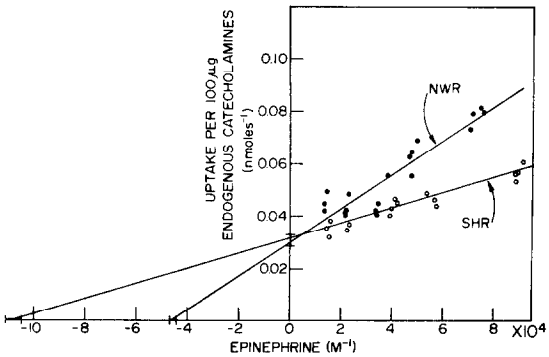

Fig. 3. Lineweaver–Burk plot for uptake of epinephrine/100 μg of endogenous catecholamines in SHR (○) and NWR (●). Bars denote standard errors of intercepts. Intercepts on the abscissa are significantly different for SHR compared to NWR ($P < 0{\cdot}001$), while intercepts on the ordinate are not. For NWR, $K_m = 21{\cdot}8 \pm 0{\cdot}9$ μM, $U_{max} = 33{\cdot}2 \pm 1{\cdot}3$ nmoles/100 μg of catecholamines; for SHR, $K_m = 9{\cdot}35 \pm 0{\cdot}17$ μM, $U_{max} = 31{\cdot}8 \pm 0{\cdot}7$ nmoles/100 μg of catecholamines.

To evaluate the nature of the alteration in amine uptake, the concentration dependencies of incorporation of epinephrine and metaraminol were examined. A Lineweaver–Burk plot of epinephrine uptake per gland indicated a lower K_m for SHR vesicles compared to NWR, but also a lower maximal uptake (U_{max}) (Fig. 2). However, on the basis of uptake per unit of catecholamines, there was no significant difference in U_{max}, but K_m was still lower for SHR (Fig. 3). For metaraminol, there were no differences between SHR and NWR in K_m or in U_{max} either for uptake per gland (Fig. 4) or uptake per unit of catecholamines (Fig. 5).

In order to determine whether altered storage of vesicular amines could account for differences in incorporation, the effluxes of newly incorporated and endogenous catecholamines were measured. The rate of loss of amines from isolated storage vesicles was the same in both SHR and NWR (Fig. 6).

The subcellular distribution of catecholamines in SHR and NWR appears in Table 4. There were no differences in P_1 and S_1 (800 g spin), indicating that the glands from either group were homogenized equally well. Similarly, the distributions in P_2 and S_2 (26,000 g spin) were identical; since this spin separates the catecholamines from broken vesicles (soluble) from catecholamines in intact vesicles (particulate), the data indicate that the fragility of the vesicles is the same in SHR and NWR, as indicated by the ratio of amines in broken/intact vesicles (Table 4). When the S_1 preparation was spun through 1·6 M sucrose, a procedure which separates heavy vesicles from lighter and broken vesicles, there was a significant increase in heavy vesicles (P_3) in SHR vs NWR and a significant decrease in

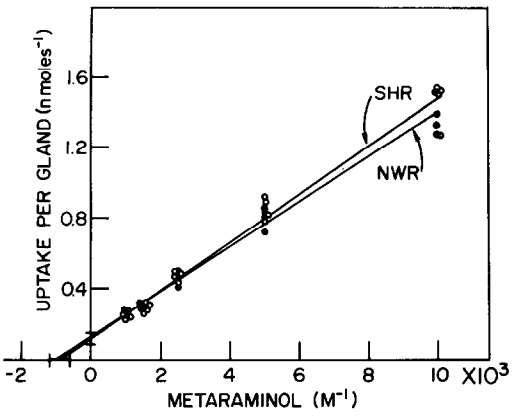

Fig. 4. Lineweaver–Burk plot for uptake of metaraminol/gland in SHR (○) and NWR (●). Bars denote standard errors of intercepts. Intercepts on abscissa and ordinate are not significantly different for SHR compared to NWR. For NWR, $K_m = 1010 \pm 130$ μM, $U_{max} = 7{\cdot}9 \pm 0{\cdot}8$ nmoles/gland; for SHR, $K_m = 1220 \pm 160$ μM, $U_{max} = 8{\cdot}8 \pm 1{\cdot}1$ nmoles/gland.

S_3, which contains catecholamines from light and broken vesicles (Table 4). The fraction of amines in light vesicles was obtained by subtracting the amines from broken vesicles (S_2) from fraction S_3; if the distribution into heavy and light vesicles was then expressed as a ratio, it was evident that there was a shift toward heavier vesicles in the SHR (Table 4). There was no difference between SHR and NWR in the ratio of catecholamines to ATP in the heavy vesicle fraction (Table 4).

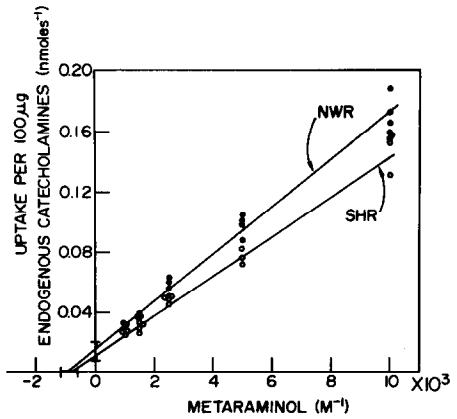

Fig. 5. Lineweaver–Burk plot for uptake of metaraminol/100 μg of endogenous catecholamines in SHR (○) and NWR (●). Bars denote standard errors of intercepts. Intercepts on abscissa and ordinate are not significantly different for SHR compared to NWR. For NWR, $K_m = 1010 \pm 130$ μM, $U_{max} = 65 \pm 9$ nmoles/100 μg of catecholamines; for SHR, $K_m = 1220 \pm 160$ μM, $U_{max} = 86 \pm 10$ nmoles/100 μg of catecholamines.

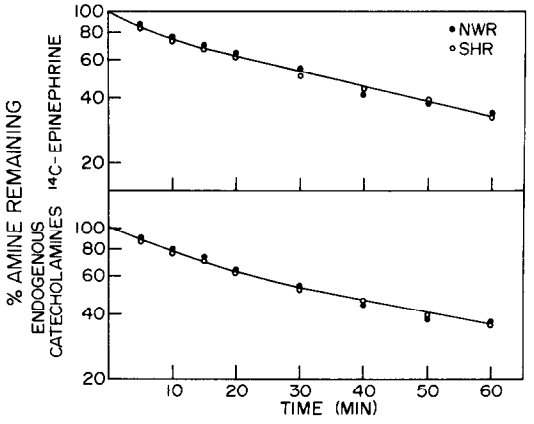

Fig. 6. Efflux of newly incorporated ^{14}C-epinephrine and endogenous catecholamines from adrenal storage vesicles of SHR (○) and NWR (●).

The abilities of SHR and NWR adrenals to secrete and resynthesize catecholamines were tested by administration of insulin, which evokes massive sympathetic discharge. One day after treatment, catecholamine stores had declined by 50 per cent in the NWR, but declined even further in the SHR (Table 5); in both, recovery of amines was nearly complete within 4 days. The activity of tyrosine hydroxylase was elevated equally in both SHR and NWR at 1, 4 and 7 days after insulin, while dopamine β-hydroxylase was decreased in both at 1 day, increased above controls at 4 days, and returned to control levels by 7 days (Table 5).

DISCUSSION

Stimulation of the adrenal medulla is associated with changes in the content and properties of storage vesicles [10, 11, 25–27]. Figure 7 represents in schematic form the sequence of events in secretion and resynthesis of a vesicle and its contents. In the normal state, an intact vesicle contains ATP, catecholamines (CA) and soluble proteins; most of the CA is bound with ATP in a storage complex, while a small amount is bound loosely to other vesicle constituents [10, 11, 14, 18]. The vesicles can incorporate epinephrine into the stable and labile binding pools and metaraminol (MA) primarily into the labile pool [14, 18]. Upon stimulation, all-or-none, exocytotic secretion of the soluble content of the vesicle occurs, leaving the membrane behind either to be reused or destroyed [10, 11, 27]. The first step after secretion is the resynthesis of the vesicle and its protein content [10, 11, 27]. Because these new vesicles are deficient in catecholamines and ATP, they differ from normal vesicles: first, they have a lower density than vesicles with normal soluble constituents [11, 12, 27]. Second, they have a decreased preference for uptake of epinephrine vs metaraminol because the major binding site for epinephrine (ATP) is absent [10–12].

Table 4. Subcellular distribution of catecholamines from adrenals of normotensive (NWR) and hypertensive (SHR) rats*

	Percentage of total catecholamines						Ratio of catecholamines in broken/intact vesicles	Ratio of catecholamines in heavy/light vesicles	Molar ratio of catecholamines to ATP in P_3
	P_1	S_1	P_2	S_2	P_3	S_3			
NWR	17 ± 1 (12)	83 ± 1 (12)	73 ± 2 (8)	11 ± 1 (8)	51 ± 1 (16)	30 ± 1 (16)	0·128 ± 0·015 (8)	2·68 ± 0·12 (16)	3·90 ± 0·15 (12)
SHR	15 ± 1 (12)	85 ± 1 (12)	74 ± 1 (8)	10 ± 1 (8)	57 ± 2† (13)	26 ± 1‡ (13)	0·130 ± 0·010 (8)	3·56 ± 0·29‡ (13)	4·03 ± 0·12 (12)

* Data represent means ± standard errors of the number of determinations in parentheses.
† $P < 0·02$ vs NWR. ‡ $P < 0·01$.

Table 5. Effect of insulin (5 i.u./kg, i.v.) on catecholamines, tyrosine hydroxylase and dopamine β-hydroxylase in adrenals of normotensive (NWR) and hypertensive (SHR) rats*

Time after insulin (days)	Per cent of control					
	Catecholamines (μg/gland)		Tyrosine hydroxylase (nmoles/gland/hr)		Dopamine β-hydroxylase (nmoles/gland/hr)	
	NWR	SHR	NWR	SHR	NWR	SHR
0	100 ± 6 (41)	100 ± 6 (41)	100 ± 8 (19)	100 ± 9 (20)	100 ± 4 (23)	100 ± 5 (23)
1	52 ± 4 (8)	35 ± 3† (11)	155 ± 13 (8)	158 ± 6 (11)	78 ± 5 (8)	67 ± 4 (11)
4	87 ± 5 (10)	86 ± 4 (9)	180 ± 7 (11)	179 ± 13 (8)	126 ± 3 (11)	131 ± 9 (9)
7	99 ± 5 (9)	94 ± 5 (11)	140 ± 11 (9)	160 ± 9 (11)	103 ± 9 (9)	107 ± 6 (11)

* Data represent means ± standard errors of the number of determinations in parentheses. Control values are given in Table 1.
† $P < 0·005$ vs NWR.

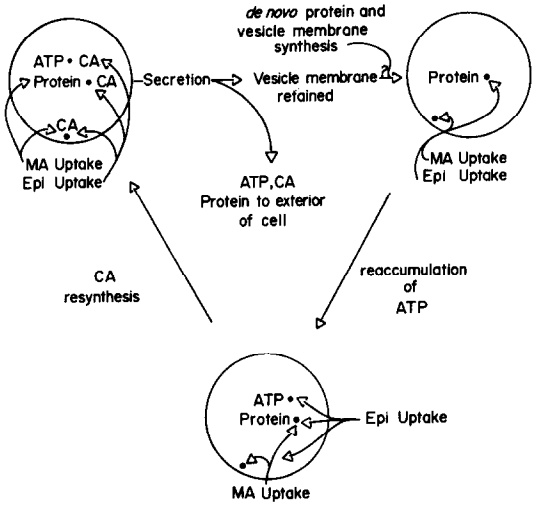

Fig. 7. Schematic representation of secretion from and resynthesis of adrenal catecholamine storage vesicles. CA = catecholamines, MA = metaraminol, Epi = epinephrine.

Within 24 hr after synthesis of a new vesicle, ATP is reaccumulated, and the vesicle re-establishes preference for uptake of epinephrine [11]. The major differences at this time are a lowered ratio of catecholamines to ATP and a lower density. Only after refilling with catecholamines do the ratios and densities approach normal [11]. To summarize, the consequences of increased adrenomedullary stimulation are: (1) decreased preference for epinephrine vs metaraminol; (2) decreased ratio of high density (heavy) vesicles to low density (light) vesicles; (3) decreased ratio of catecholamines to ATP; and (4) decreased catecholamine content per vesicle.

In contrast to these observations for increased activity, the following were observed in spontaneously hypertensive rats: (1) increased preference for epinephrine vs metaraminol (Figs. 3 and 5, Table 3); (2) increased ratio of heavy to light vesicles (Table 4); and (3) no alterations in ratio of catecholamines to ATP (Table 4).

Evaluation of catecholamine content per vesicle in SHR vs NWR is somewhat difficult because of the inability to accurately determine the number of vesicles.

In rats of the same strain, dopamine β-hydroxylase (DBH) activity can be taken as a measure of the number of vesicles, but as is evident in Table 1, there is considerably less DBH in the SHR. In part, this reflects the presence of fewer storage vesicles, as evidenced by the decreased U_{max} for epinephrine uptake per gland. However, it is notable that the ratio of catecholamine content to DBH is high in the SHR compared to NWR, and it is important to establish whether that difference is due to decreased DBH per vesicle or increased catecholamines per vesicle. The measurement of conversion of tyramine to octopamine using intact vesicles provides a relative measure of DBH concentration per vesicle. Although there was a significant lowering of conversion in SHR, the difference (48 vs 41 per cent) could not account for the 50 per cent increase in catecholamine/DBH ratio of SHR compared to NWR. These data suggest that, in addition to lower DBH per vesicle, there is a higher level of catecholamines per vesicle in the SHR, as further confirmed by the greater proportion of heavy vesicles. Once again, this is the opposite of what would be expected if the adrenals were hyperactive in the SHR. There is no evidence, therefore, to support the contention that sympatho-adrenal activity is increased in the SHR; in fact, the properties of the vesicles indicate that activity may be somewhat depressed, confirming earlier studies on catecholamine turnover in peripheral sympathetic tissue [7–9].

The question remains whether the understimulation is a compensatory central reflex in response to the hypertension or whether there is an unrelated genetic defect in adrenomedullary function. To determine which factor operates, SHR and NWR were given insulin, which evokes massive stimulation of the adrenal medulla. One day later, SHR had secreted a greater percentage of catecholamines than NWR, but there was no significant difference in the loss of DBH. Thus, after intense stimulation of both SHR and NWR adrenals, the higher catecholamine to DBH ratio in SHR is no longer evident, but is re-established by 4 days post-insulin. In addition, stimulation-induced increases in tyrosine hydroxylase and DBH were proportionately identical in SHR and NWR. These data all suggest that the hypoactivity of the SHR adrenal is not due entirely to a genetic defect in adrenomedullary function but rather reflects in part a decrease in splanchnic stimulation; if the suppression of neural activity is removed, the catecholamine content per vesicle is no longer elevated. Furthermore, because resynthesis of catecholamines after massive stimulation is not a major factor in the first day after insulin [10, 11, 27], the lower amine level in SHR at 24 hr probably reflects an enhanced secretory response to insulin. This supports the hypothesis that basal adrenal stimulation is low—there is a larger difference between basal and maximal stimulation in SHR than in NWR. On the other hand, the differences in basal activities per gland of tyrosine hydroxylase and DBH may not in themselves constitute a measure of the rate of stimulation, since there are wide variations in activities even among different normotensive strains [6].

Since the effects of stimulation are derived from acute studies using insulin, an additional hypothesis could be advanced to explain the present results: chronic adrenal stimulation may lead to accelerated catecholamine and vesicle turnover, but the properties of the vesicles might return to normal because of the establishment of a new steady state. In order for the vesicles to appear "normal", the rates of synthesis and destruction of vesicle components relative to each other would have to be unchanged despite the fact that the turnovers of each would be increased. However, this alternate hypothesis cannot explain the observed shift in some vesicle parameters in a direction opposite to that seen upon stimulation, nor could it account for the lowered rates of catecholamine turnover previously reported [7–9]. In any case, studies in chronically stimulated adrenals indicate that there is indeed an increase in "immature" vesicles, as typified by lower catecholamine/DBH ratios [28].

It is of additional interest that at least two of the differences between NWR and SHR adrenal vesicles rest in the vesicle membrane, namely the lower DBH per vesicle (DBH in rat adrenal vesicles is primarily membrane-bound [29]) and the increased affinity of the uptake system for epinephrine (decreased K_m). The latter factor cannot represent altered storage, since the efflux of amines and catecholamine to ATP ratio was normal in SHR, and in any case alterations in intravesicular storage should cause changes only in U_{max}, not in K_m [30]. The membrane alterations may represent genetic differences between the two rat strains in a like fashion to those of catecholamine biosynthetic enzymes: whether or not the differences are hypertension related, these alterations may have important consequences in the activity of autonomic agents with actions on the storage vesicle. The lowered DBH activity should render the SHR more sensitive to inhibitors of that enzyme [3]. On the other hand, the lowered K_m for catecholamines vs false transmitters (metaraminol) may decrease the ability of the latter to replace endogenous stores. Since the introduction of an α-methyl group makes the uptake properties of a given amine resemble metaraminol [18], drugs such as α-methyldopa should also be less effective in SHR.

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