EFFECTS OF RESERPINE ON THE ADRENAL MEDULLA OF THE SPONTANEOUSLY HYPERTENSIVE RAT

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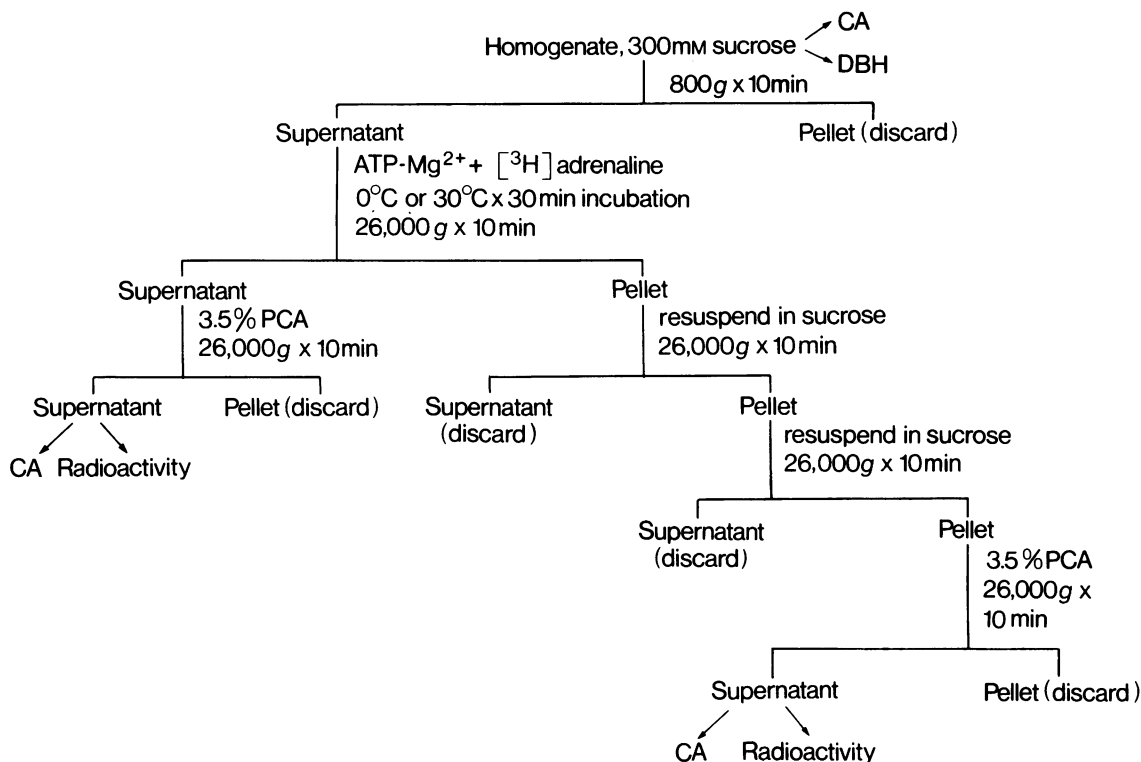
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1   Reserpine administration resulted in a larger initial decline in adrenal catecholamines in spontaneously hypertensive rats (SHR) than in normotensive Wistar rats (NWR); the difference was eliminated by pretreatment with chlorisondamine.

2   Reserpine also produced a larger increase in SHR catecholamines and dopamine β-hydroxylase several days later; chlorisondamine pretreatment did not prevent the increases, although it did slightly slow the increases.

3   Vesicles from SHR or NWR incubated with reserpine *in vitro* demonstrated equivalent inhibition of adenosine $5'$-triphosphate (ATP)-Mg^{2+} -stimulated adrenaline uptake.

4   Recovery of uptake was more rapid in SHR than in NWR after reserpine inhibition, and this was associated with a burst of new vesicle synthesis in the SHR; chlorisondamine pretreatment reduced the number of new, immature vesicles in reserpine-treated SHR.

5   Both SHR and NWR secreted equal proportions of their adrenal catecholamine contents after nicotine administration.

6   These data suggest that the sympatho-adrenal system of the SHR exhibits an enhanced reflex response to reserpine but that reserpine is equally effective in SHR and NWR in producing blockade of vesicular catecholamine transport; these alterations can affect markedly the actions of autonomic drugs in the SHR.

Introduction

The sympatho-adrenal axis of the Wistar-derived spontaneously hypertensive rat (SHR) shows marked differences in catecholamine disposition when compared to normotensive Wistar rats (NWR). These include alterations in the levels of catecholamines and the catecholamine-synthesizing enzymes, tyrosine hydroxylase, dopa decarboxylase and dopamine β-hydroxylase (Ozaki, Hotta & Aoki, 1972; Nagatsu, Mizutani, Nagatsu, Umezawa, Matsuzaki & Takeuchi, 1972; Ueba, Mori & Tomomatsu, 1972; Spector, Tarver & Berkowitz, 1972; Lovenberg, Yamabe, deJong & Hansen, 1974), differences in catecholamine turnover (Louis, Spector, Tabei & Sjoerdsma, 1969; Louis, Tabei, Spector & Sjoerdsma, 1969; Nakamura, Gerold & Thoenen, 1972; Spector *et al.*, 1972) and probably a lowering of neural input to the adrenal (Slotkin & Green, 1975). Several of the properties of the catecholamine storage vesicle membrane also appear to be altered: there is less dopamine β-hydroxylase per vesicle and a lowered *Km* for inward transport of adrenaline (Slotkin & Green, 1974). In addition, the SHR exhibits an

enhanced catecholamine secretion upon administration of insulin (Slotkin & Green, 1975). It is not clear whether these differences are a causative factor in the development of hypertension, whether they result from the hypertension or whether they represent ancillary strain-dependent factors unrelated to hypertension (Lovenberg *et al.*, 1974). Regardless of the origin of the altered catecholamine disposition, several of the differences could affect the response of the SHR to autonomic agents; for instance, the lower level of dopamine β-hydroxylase (Lovenberg *et al.*, 1974; Slotkin & Green, 1975) renders the SHR more sensitive to inhibitors of that enzyme, and consequently a prolonged decrease in blood pressure (Nagatsu *et al.*, 1972). In the present study, we have examined the effects of reserpine, which evokes a reflex sympatho-adrenal discharge but also causes catecholamine depletion by inhibiting the adenosine $5'$-triphosphate (ATP)-Mg^{2+} -dependent transport system in the storage vesicle membrane. Since both neural input to the adrenal and catecholamine uptake into the vesicles

Homogenate, 300mM sucrose → CA
                            ↘ DBH
800 g x 10min

Supernatant
ATP-Mg^{2+} + [^{3}H]adrenaline
0°C or 30°C x 30min incubation
26,000 g x 10min

Pellet (discard)

Supernatant
3.5% PCA
26,000 g x 10min

Pellet
resuspend in sucrose
26,000 g x 10min

Supernatant   Pellet (discard)
CA Radioactivity

Supernatant
(discard)

Pellet
resuspend in sucrose
26,000 g x 10min

Supernatant
(discard)

Pellet
3.5% PCA
26,000 g x
10 min

Supernatant   Pellet (discard)
CA   Radioactivity

Figure 1   Flow sheet for treatment of rat adrenal homogenates. CA = catecholamine assay, DBH = dopamine β-hydroxylase assay, PCA = perchloric acid.

appear to be altered in the SHR, it is reasonable to expect that the actions of antihypertensive drugs will also be different in the SHR. The present study demonstrates that differences do occur in SHR in the effects of reserpine and the ganglionic blocking agent, chlorisondamine.

Methods

Female normotensive Wistar rats (NWR) and Wistar-derived spontaneously hypertensive rats (SHR) were obtained from Carworth Farms and used at 10-12 weeks of age. Groups of animals were given one of the following treatments: (1) reserpine, 2.5 mg/kg, (2) chlorisondamine, 20 mg/kg, (3) chlorisondamine, 20 mg/kg, followed 1 h later by reserpine, 2.5 mg/kg, or (4) chlorisondamine, 20 mg/kg, followed 1 h later by reserpine, 10 mg/kg; all drugs were injected subcutaneously. The rats were killed 4, 24, 72, 144 or 240 h after reserpine treatment; the adrenal glands from each animal were removed, cleaned of fat and connective tissue, and homogenized in 2.5 ml of sucrose-Tris (300 mM sucrose buffered

at pH 7.4 with 25 mM Tris) containing 0.01 mM iproniazid (irreversible monoamine oxidase inhibitor). Aliquots were withdrawn for assay of catecholamines and dopamine β-hydroxylase and the remainder was centrifuged at 800 g for 10 minutes. Aliquots of the supernatant were used for the determination of adrenaline uptake as described previously (Slotkin, 1973); the sucrose incubation medium contained 5 mM ATP, 5 mM Mg^{2+}, 0.1 mM adrenaline and 5 μCi [^{3}H]-adrenaline and samples were incubated for 30 min at 30°C while duplicate tubes were kept on ice. Following two washes and centrifugations at 26,000 g of the vesicular pellet, the labelled vesicles were lysed with 3 ml of 3.5% perchloric acid and analyzed for catecholamines and radioactivity. The incubation medium was also analyzed to determine the specific activity. A flow sheet of the procedure appears in Figure 1. The uptake was calculated as described in an earlier communication (Slotkin & Kirshner, 1973a) and the results expressed as uptake per gland and as uptake per 100 μg endogenous catecholamines. The first parameter measures the net uptake capacity of the entire tissue while the latter

represents the abilities. of individual vesicles to take up amines relative to their catecholamine contents. Under these conditions, the uptake of adrenaline occurs solely into storage vesicles, despite the presence of contaminating particles in the incubation medium (Slotkin & Kirshner, 1971; Slotkin & Kirshner, 1973b).

Studies of the *in vitro* effects of reserpine and harmine on uptake in NWR and SHR adrenal vesicles were conducted in a similar fashion except that animals received no drug treatment; instead the agents were added directly to the incubation medium in concentrations ranging from 2×10^{-8} M to 2×10^{-6} M for reserpine and from 10^{-6} M to 10^{-4} M for harmine.

Nicotine-induced secretion was determined by the administration of 10 mg/kg subcutaneously; the rats were killed 4 h later. Adrenals were homogenized in 5 ml of 3.5% perchloric acid, centrifuged at $26,000\,g$ to remove precipitated protein, and the supernatant analyzed for catecholamines.

Assays

Catecholamines were analyzed by the trihydroxy-indole method (without prior adsorption on alumina) using an autoanalyzer and [^{3}H]-adrenaline was determined by liquid scintillation spectrometry (Merrills, 1963; Slotkin, Ferris & Kirshner, 1971). Dopamine β-hydroxylase activity (DBH, E.C. 1.14.2.1) was determined by a modification (Slotkin *et al.*, 1971) of the method of Friedman & Kaufman (1965) using 1 mM parahydroxymercuribenzoate to inactivate endogenous inhibitors (Duch, Viveros & Kirshner, 1968). Incubations were carried out for 1 h at 37°C using 1 μCi [^{3}H]-tyramine (10 μM) as substrate.

Statistical analysis

Data are reported as means ± standard errors and levels of significance determined by Student's *t*-test.

Drugs

Adrenaline-[7-^{3}H] and tyramine-[G-^{3}H] were obtained from New England Nuclear Corporation, chlorisondamine chloride and reserpine phosphate from Ciba Pharmaceuticals, tyramine hydrochloride, parahydroxymercuribenzoate and nicotine from Sigma Chemical Company, and adrenaline bitartrate from Winthrop Laboratories.

Results

Effects of chlorisondamine (Table 1)

Twenty-five hours after the administration of chlorisondamine there were no significant changes in adrenal catecholamines, dopamine β-hydroxylase or in adrenaline uptake in isolated vesicles in either normotensive Wistar rats (NWR) or spontaneously hypertensive rats (SHR).
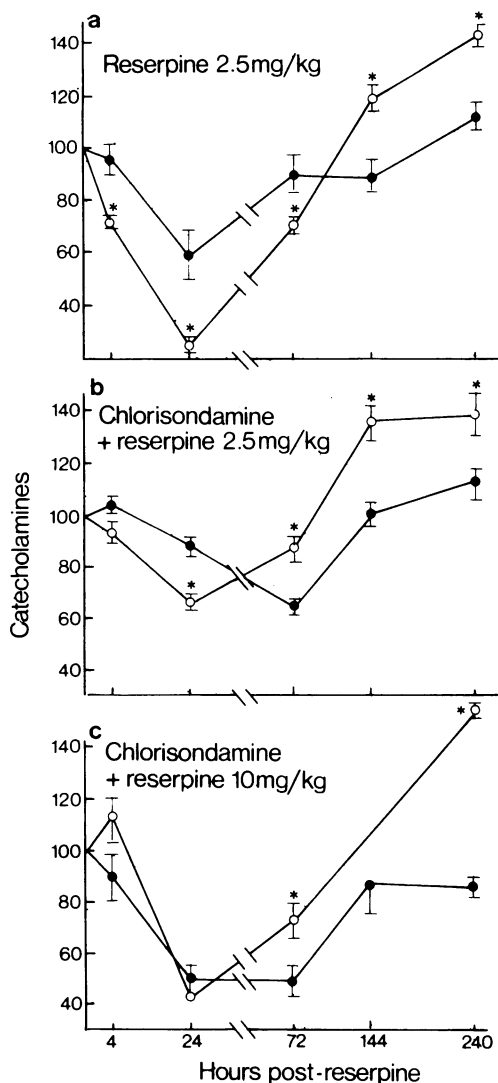
Effects of reserpine on catecholamines

Four hours after administration of 2.5 mg/kg of reserpine to NWR, there was no change in catecholamines, but levels were decreased to 60% of control by 24 h (Figure 2a). On the other hand, catecholamines in the SHR were markedly lower 4 h after 2.5 mg/kg and were 25% of control by 24 h (Figure 2a). By 3 days post-reserpine, catecholamines had increased nearly to control levels in NWR, but only to 70% in SHR. However

Table 1   Effects of chlorisondamine (20 mg/kg s.c.) on adrenal medullae of normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR).

Rat strain	Treatment	Catecholamines (µg/gland)	Dopamine β-hydroxylase (nmol/h per gland)	Adrenaline uptake		Number of animals
				nmol per gland	nmol per 100 µg catecholamines	
NWR	None	15.0 ± 0.5*	1.57 ± 0.05	3.04 ± 0.13	20.5 ± 1.0	37
	25 h after chlorisondamine	14.5 ± 0.2	1.70 ± 0.08	3.07 ± 0.12	21.3 ± 0.8	5
SHR	None	11.2 ± 0.4	0.69 ± 0.02	2.74 ± 0.08	24.6 ± 1.8	39
	25 h after chlorisondamine	10.4 ± 0.3	0.73 ± 0.05	2.74 ± 0.14	26.1 ± 0.5	7

*Mean ± s.e.

depletion after 24 h was less in both NWR and SHR than after reserpine alone, although SHR were still depleted to a greater extent than NWR at this time (Figure 2b). Three days after reserpine and chlorisondamine, SHR catecholamines were 90% of controls but NWR were only 65%; at subsequent times, the levels in SHR were substantially supranormal while NWR were at or slightly above control (Figure 2b).

Chlorisondamine pretreatment also prevented any initial (4 h) catecholamine-depleting effect of high doses of reserpine (Figure 2c). Twenty-four hours later, both NWR and SHR were depleted to 40-50% of control catecholamine levels. NWR catecholamines remained depleted between 1 and 3 days post-reserpine and increased toward control levels by 6-10 days, while in the SHR some recovery was evident by 3 days and catecholamines were 150% of controls by 10 days (Figure 2c).

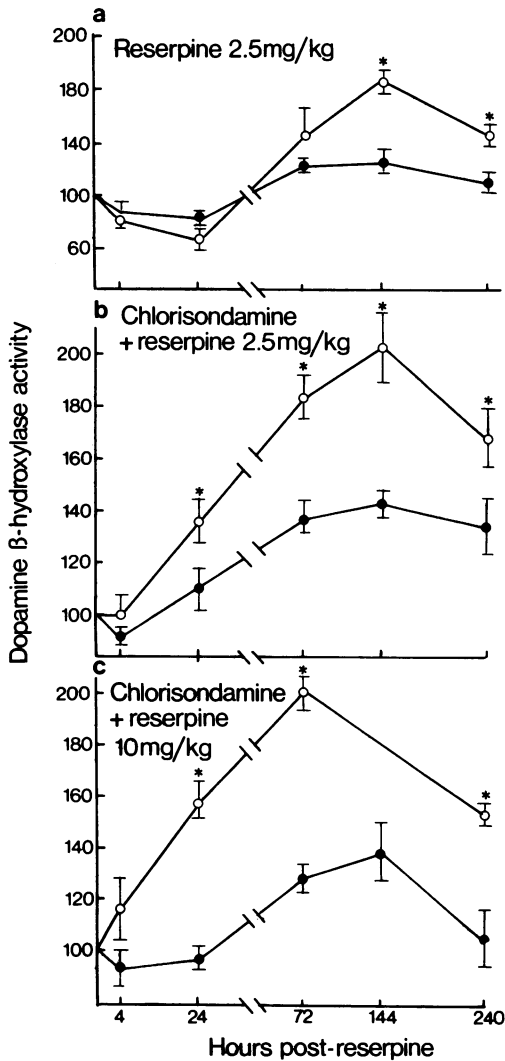### Effects of reserpine on dopamine β-hydroxylase (DBH) activity

Administration of reserpine (2.5 mg/kg) alone produced a small decline in DBH in NWR and SHR at 4 and 24 h, but an increase at subsequent time periods (Figure 3a). The elevation was much more pronounced in SHR than in NWR.

Chlorisondamine pretreatment prevented the initial reserpine-induced decline in DBH at 4 and 24 h, and in fact resulted in a significant increase in DBH in SHR at 24 h (Figure 3b); subsequent increases in DBH of NWR and SHR equalled or exceeded those seen at 3, 6 and 10 days after reserpine alone (Figure 3b). A similar pattern of change in DBH was seen after chlorisondamine + 10 mg/kg of reserpine (Figure 3c).

Effects of reserpine on adrenaline uptake per gland

Reserpine (2.5 mg/kg) produced nearly total blockade of adrenaline uptake per gland in isolated storage vesicles in both NWR and SHR at 4 h (Figure 4a). There was only slight recovery at 24 h, but by 3 days, uptake per gland was normal in NWR and supranormal in SHR; uptake per gland remained elevated in SHR up to 10 days post-reserpine (Figure 4a).

Chlorisondamine pretreatment did not alter the reserpine-induced decrease in adrenaline uptake per gland at 4 or 24 h but appeared to slow the recovery in both NWR and SHR (Figure 4b). Uptake per gland reached control levels by 6 days post-reserpine and, in SHR, exceeded control levels at 10 days (Figure 4b). Similar results were obtained with chlorisondamine followed by 10 mg/kg of reserpine (Figure 4c).
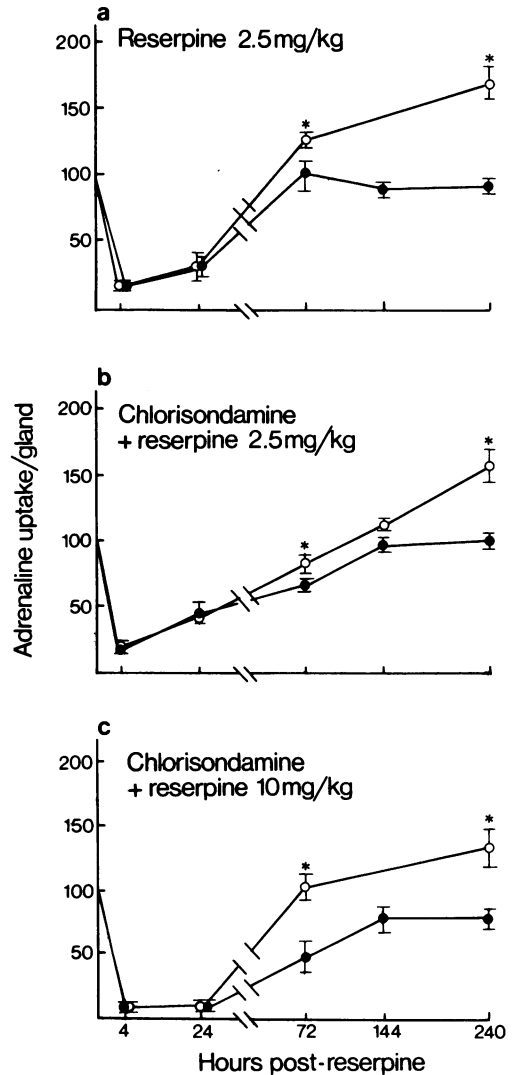
Figure 2 Effects of reserpine on adrenal catecholamines in normotensive Wistar rats (NWR) (●) and spontaneously hypertensive rats (SHR) (○). In (b) and (c), chlorisondamine (20 mg/kg) was administered 1 h before reserpine. Each point represents mean of 5-6 animals in terms of percent of control; vertical bars show s.e. mean. Control values are given in Table 1.
* Significant differences ($P < 0.05$ or better) between SHR and NWR values.

6 and 10 days post-reserpine SHR catecholamines were substantially elevated above control while NWR levels were normal.

When chlorisondamine was administered prior to reserpine (2.5 mg/kg), the initial (4 h) decline in SHR catecholamines seen with reserpine alone did not occur (Figure 2b). Furthermore, the degree of

Figure 3 Effects of reserpine on adrenal dopamine β-hydroxylase activities in normotensive Wistar rats (NWR) (●) and spontaneously hypertensive rats (SHR) (○). In (b) and (c), chlorisondamine (20 mg/kg) was administered 1 h before reserpine. Each point represents mean of 5-6 animals in terms of percent of control; vertical bars show s.e. mean. Control values are given in Table 1.
* Significant differences (*P* < 0.05 or better) between SHR and NWR values. Vertical scale is compressed in (a).
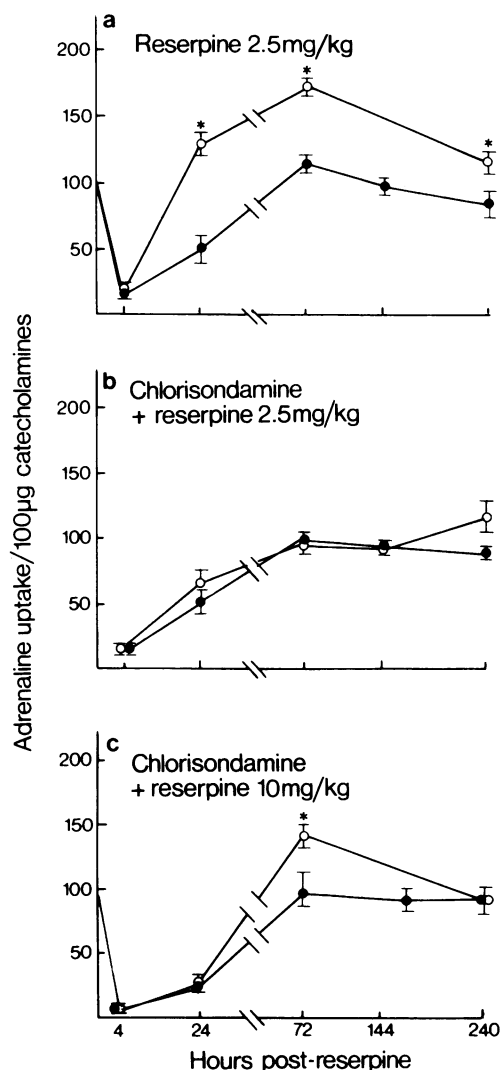
Figure 4 Effects of reserpine on [³H]-adrenaline uptake per gland in isolated adrenal storage vesicles from normotensive Wistar rats (NWR) (●) and spontaneously hypertensive rats (SHR) (○). In (b) and (c), chlorisondamine (20 mg/kg) was administered 1 h before reserpine. Each point represents mean of 5-6 animals in terms of percent of control; vertical bars show s.e. mean. Control values are given in Table 1.
* Significant differences (*P* < 0.05 or better) between SHR and NWR values.

Effects of reserpine on adrenaline uptake per 100 μg endogenous catecholamines

Reserpine (2.5 mg/kg) alone reduced uptake/100 μg catecholamines to about 20% of controls at 4 h in both NWR and SHR (Figure 5a). However, by 24 h NWR values had returned to 50% of control and SHR uptakes were elevated above controls. At subsequent times, uptakes/100 μg catecholamines were at or near control levels for NWR; in SHR, the highest value (>170% of control) was reached at 3 days and declined to 115% by 10 days post-reserpine (Figure 5a).

Chlorisondamine pretreatment did not alter the

Figure 5 Effects of reserpine on [³H]-adrenaline uptake per 100 μg of endogenous catecholamines in isolated adrenal storage vesicles from normotensive Wistar rats (NWR) (●) and spontaneously hypertensive rats (SHR) (○). In (b) and (c), chlorisondamine (20 mg/kg) was administered 1 h before reserpine. Each point represents mean of 5-6 animals in terms of percent of control; vertical bars show s.e. mean. Control values are given in Table 1.
* Significant differences (*P* < 0.05 or better) between SHR and NWR values.

initial effect of the low dose of reserpine on uptake/100 μg catecholamines in NWR but prevented the later increases which had been seen in SHR after reserpine alone (Figure 5b). There was no difference between NWR and SHR in the inhibition of uptake and subsequent recovery with

chlorisondamine + 2.5 mg/kg of reserpine (Figure 5b). After chlorisondamine + 10 mg/kg of reserpine, the decrease in uptake/100 μg catecholamines in NWR and SHR was more profound at 4 and 24 h than at the lower dose (Figure 5c). NWR recovered to control levels by 3 days, while SHR exceeded control at 3 days and returned to normal by 10 days (Figure 5c).

Effects of reserpine and harmine in vitro *on adrenaline uptake per 100 μg endogenous catecholamines*

Reserpine and harmine in vitro inhibited adrenaline uptake into isolated storage vesicles to approximately the same extent in NWR and SHR. The calculated IC_{50}'s (8 determinations) for reserpine were 0.11 ± 0.02 μM in NWR vesicles and 0.13 ± 0.02 μM in SHR vesicles; for harmine, IC_{50}'s were 20 ± 5 μM in NWR vesicles and 12 ± 2 μM in SHR vesicles.

Nicotine-induced catecholamine secretion

Four hours after the administration of nicotine, adrenal catecholamine levels had decreased to $79 \pm 2\%$ of control in NWR (14 animals); and to $78 \pm 2\%$ of control in SHR (12 animals).

Discussion

The effects of reserpine on the adrenal medulla consist of three distinct actions: an initial reflex stimulation mediated by the splanchnic nerve and resulting in depletion of catecholamine stores *via* exocytotic, all-or-none release of the soluble content of the storage vesicles; a further depletion of catecholamines due to blockade of the $ATP-Mg^{2+}$-dependent uptake system in the storage vesicle membrane; and a later, less intense reflex stimulation in response to the hypotension which results from sympathetic catecholamine depletion. Thus, in comparing the reserpine-induced alterations in catecholamine disposition in normotensive Wistar rats (NWR) and spontaneously hypertensive rats (SHR) it is important to establish which differences are attributable to direct, and which to reflex, effects of the drug.

A recent study (Slotkin & Green, 1975) demonstrated that the vesicular uptake system of the SHR exhibited a lower *Km* for adrenaline than did that of NWR, indicating an enhanced uptake of catecholamines in the SHR. In the current experiments, two competitive inhibitors (reserpine and harmine) of the uptake system (Jonasson, Rosengren & Waldeck, 1964; Green & Slotkin, 1973) were just as effective *in vitro* in SHR

vesicles as in NWR vesicles. These data suggest that the K_i for the inhibitors is reduced in the SHR in a fashion similar to the reduction in the K_m for adrenaline, and that reserpine is therefore equally effective in its direct effect in SHR and NWR. The *in vivo* studies confirm this hypothesis: in all cases, the inhibition of adrenaline uptake per gland or per 100 μg endogenous catecholamines was equivalent in SHR and NWR at 4 h post-reserpine. Consequently, the differences seen between SHR and NWR in the effects of reserpine *in vivo* probably result primarily from differences in reflex stimulation of the splanchnic nerve.

Low doses (2.5 mg/kg) of reserpine elicited little or no initial reflex secretion in NWR, as indicated by the absence of a significant decrease in catecholamines at 4 hours. In contrast, there was a prompt decline in SHR catecholamine levels. To test whether this was due to a greater reflex effect in the SHR, another group of rats was pretreated with chlorisondamine, which blocks splanchnic stimulation of the adrenal medulla; under these conditions reserpine failed to produce depletion in SHR at 4 h, indicating that the SHR does exhibit a greater neural reflex effect to reserpine alone than the NWR. Similar results have been obtained with insulin (Slotkin & Green, 1975), which also elicits reflex activity, suggesting that the exaggerated sympatho-adrenal response in the SHR is a general phenomenon and is not specific only to reserpine. To determine whether this property is due to a difference in the adrenal medulla itself or rather to a more proximal alteration in the reflex pathway, NWR and SHR were given nicotine, which stimulates the adrenal medulla directly. Both NWR and SHR secreted equal proportions of their catecholamine stores, indicating that the adrenal glands themselves were equally responsive in the two strains. These data suggest that the site of the alteration may be in central regulation of autonomic reflexes.

If the SHR is in fact abnormally responsive to reserpine-induced stimulation, then other biochemical parameters which are dependent on neural input should also demonstrate differences from NWR. Thus, there was a greater induction of dopamine β-hydroxylase in SHR 6-10 days after reserpine alone than in NWR, indicating a more rapid synthesis of new storage vesicles (Slotkin & Kirshner, 1973b). This explains in part the large increase in adrenaline uptake per gland in SHR 3-10 days after reserpine: despite the greater loss of vesicles from neurogenic secretion, the further increment in neural input from reserpine and the resultant hypotension produces a more rapid vesicle synthesis. The large burst of new synthesis results in the formation of 'immature' vesicles (Slotkin & Kirshner, 1973a,b; Slotkin, 1973) with

low catecholamine to dopamine β-hydroxylase ratios (Figures 2a and 3a) and consequently an increased adrenaline uptake per 100 μg endogenous catecholamines (Figure 5a). Thus, reserpine alone in the SHR causes a greater initial depletion of catecholamines and storage vesicles, and a higher degree of resynthesis at subsequent times.

The data obtained with chlorisondamine-pretreated animals tend to support the hypothesis that stimulation is more intense in the SHR in both initial and late time periods. While ganglionic blockade prevented the initially (4 h) greater depletion of SHR catecholamines, there was still a significant difference between SHR and NWR at 24 h; since chlorisondamine is a competitive antagonist, it is likely that the increased splanchnic stimulation in the SHR results in overcoming of the blockade at an earlier point than in NWR. The prevention by chlorisondamine of the reserpine-induced initial reflex discharge led to a slowing of the depletion phase but also to a slower rate of subsequent increase in catecholamines: reserpine-treated SHR increased by 20% between days 1 and 3 with chlorisondamine pretreatment, but by 45% with reserpine alone; the 25% increase seen in NWR given reserpine alone was reversed (25% decline) with chlorisondamine-pretreatment. Inhibition of stimulation similarly slowed the rate of recovery of adrenaline uptake per gland, suggesting that the large burst of new vesicle synthesis in SHR had been reduced; this was confirmed by the absence of 'immature' vesicles as indicated by a normal adrenaline uptake per 100 μg endogenous catecholamines.

The apparent facilitation by chlorisondamine of the reserpine-induced increases in dopamine β-hydroxylase activity in both SHR and NWR do not indicate a larger degree of induction: the prevention of the initial secretion results in the preservation of a larger number of intact old vesicles, the membranes of which would otherwise have been destroyed or recycled 1 to 2 days after secretion (Slotkin & Kirshner, 1973b). Under these conditions, adrenaline uptake per 100 μg endogenous catecholamines is a better measure of new vesicle formation than is enzyme activity.

The administration of larger doses of reserpine after chlorisondamine resulted in an accentuation of differences between SHR and NWR which could be attributed to a more intense late stimulatory phase in the SHR. The disparities between SHR and NWR catecholamines, dopamine β-hydroxylase and adrenaline uptake per gland were larger and the differences in adrenaline uptake per 100 μg catecholamines suggested that immature vesicles were present at 3 days in the SHR. However, there were fewer immature vesicles present in SHR given chlorisondamine and

10 mg/kg reserpine than after 2.5 mg/kg reserpine alone, indicating the importance of the initial stimulation for the large burst of new vesicle synthesis.

In conclusion, although the basal sympatho-adrenal tone of the SHR appears to be lower than that of the NWR, the SHR exhibits an apparent hyper-reactivity in response to drugs which elicit sympatho-adrenal reflexes. Consequently, con- siderable caution must be exercised in evaluating the activities of autonomic agents in the SHR.

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