Biochemical Pharmacology, Vol. 22. pp. 549–560. Pergamon Press, 1973. Printed in Great Britain.

EFFECTS OF RESERPINE ON THE CONTENT AND PROPERTIES OF RAT ADRENAL MEDULLARY STORAGE VESICLES*

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(*Received* 10 *August* 1972; *accepted* 15 *September* 1972)

Abstract—Male rats were given reserpine (2·5 or 10 mg/kg, i.p.) (preceded by chlorisondamine, 10 mg/kg, i.p., to prevent reflex neural stimulation), and the subcellular distributions of adrenal storage vesicle components (catecholamines, ATP and dopamine β-hydroxylase) were determined along with the ability of the vesicles to incorporate a catecholamine (^{14}C-epinephrine) and a non-catecholamine (^{3}H-metaraminol). One and 4 hr after the low dose of reserpine, epinephrine uptake was inhibited 60 per cent, while there was little change in metaraminol uptake; epinephrine uptake returned to normal in 24 hr, at which time there was a small decrease in catecohamines and ATP and a slight shift of dopamine β-hydroxylase toward lower density particles. One hr after the high dose of reserpine, inhibition of epinephrine uptake was nearly complete, and metaraminol uptake decreased by 35 per cent. There was little or no change in catecholamines, ATP and dopamine β-hydroxylase 1 and 4 hr after reserpine, but there was a marked shift at 24 hr, at which time catecholamines and ATP had decreased. Total dopamine β-hydroxylase activity was unchanged at 24 hr, but the distribution was markedly shifted toward lighter particles. Subsequently, the uptake of epinephrine recovered gradually to 80 per cent of controls at 4 days, while metaraminol uptake returned to normal almost immediately (4–24 hr). By 3 days after reserpine, dopamine β-hydroxylase exceeded control levels in all subcellular fractions, but was highest in the fraction containing storage vesicles with below normal catecholamine contents. Catecholamine and ATP levels decreased to 34 per cent of controls between 4 hr and 2 days and then increased to 80 per cent of controls at 4 days. These data and previous studies provide evidence for different degrees of reserpine-sensitivity in different species and indicate that the blockade of ATP–Mg^{2+}-stimulated uptake may be partly reversible, since recovery of the uptake mechanism commences at a time when new vesicle synthesis is not occurring. The rapid recovery of metaraminol uptake also suggests that vesicles partly or completely emptied of their ATP and catecholamine content can still incorporate metaraminol, although their ability to incorporate epinephrine is impaired.

THE MASSIVE loss of catecholamines from the adrenal medulla produced by neural stimulation or pharmacological intervention results in the initiation of a sequence of events designed to accelerate the recovery of those stores. Thus, after depletion following hypoglycemic shock from insulin administration, there is an increase in rate of synthesis of new storage vesicles,[1,2]§ as well as increases in catecholamine-synthesizing enzymes.[3,4] The former effect is reflected in the appearance of vesicles

* This work was supported by grants from the Duke Endowment Fund, United Health Services of N.C., Inc., and USPHS MH 11947 and RR 05405.

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with higher than normal ratios of dopamine β-hydroxylase (DBO) to catecholamines (CA), which result from the fact that catecholamine re-synthesis probably does not keep pace with the more rapid re-synthesis of storage vesicles.[1,2,*] Thus, at intermediate stages during recovery after insulin, the newly formed vesicles have properties which are markedly different from the normal vesicle population: they have lower buoyant densities, lower catecholamine contents and altered specificities for uptake of non-catecholamines vs. catecholamines.[1,2*] However, the properties of the vesicles, the sequence of events during recovery and the magnitude of the changes appear to be species dependent; for instance, the rate of new vesicle synthesis is higher in the rat than in the rabbit, and the recovery of the ability of the vesicles to take up and store amines recovers at different relative rates in rats, rabbits and guinea pigs.[1,2,5*] Consequently, the apparent rate-limiting step in recovery of adrenal amine stores varies from species to species: in the rat, amine resynthesis is probably rate limiting;* in the guinea pig, vesicular uptake or storage properties may play a determining factor;[5] in the rabbit, either of these processes may be implicated in limiting the rate of recovery.[2]

In addition to species differences, the agent used to evoke the depletion of amine stores plays an important role in determining the pattern of loss and recovery. While insulin administration causes neurogenic, all-or-none secretion of vesicle contents, depletion by reserpine proceeds by at least two processes: (1) reflex stimulation of the splanchnic nerve to produce neurogenic secretion similar to that produced by insulin,[6] and (2) depletion due to blockade of ATP–Mg^{2+}-stimulated catecholamine uptake into storage vesicles.[7] By prior administration of a ganglion blocker (chlorisondamine), the former mechanism can be prevented, and the effects of non-neurogenic depletion can be selectively examined.[6]

Previous reports have described the events following reserpine-induced depletion in rabbits,[3,6] as well as insulin-induced depletion in rabbits and rats.[1,2,*] In the present study, the effects of two different doses of reserpine have been examined in chlorisondamine-pretreated rats, by measuring the amounts and subcellular distributions of adrenal storage vesicle components and the ability of the vesicles to incorporate both catechol- and non-catecholamines. These studies further elucidate the nature of the depletion–recovery process in the absence of neural input, as well as establishing whether the species differences are related only to neurogenic (insulin-induced) depletion or whether they can also be observed in non-neurogenic depletion.

METHODS

Treatment of animals. Male Sprague–Dawley rats weighing 200–260 g were given chlorisondamine chloride (10 mg/kg, i.p.), a long-acting ganglionic blocking agent, to prevent neurogenic stimulation of the adrenal gland.[6] Reserpine (2·5 or 10 mg/kg, i.p.) was injected 1 hr after chlorisondamine, and the rats were sacrificed by decapitation at 1, 4 or 24 hr and 2, 3, 4 or 12 days after reserpine administration.

Subcellular distributions of DBO, ATP and CA. The adrenal glands from each animal were excised, cleaned of fat and connective tissue and homogenized (glass-to-glass) in 2·5 ml of ice-cold sucrose–Tris (300 mM sucrose + 25 mM Tris + 0·01 mM iproniazid; adjusted to pH 7 with H_2SO_4). The suspension was centrifuged at 800 *g*

* T. A. Slotkin and N. Kirshner, *Molec. Pharmac.*, in press.

for 10 min and the supernatant was decanted. The pellet was resuspended by glass-to-glass homogenization in 5 ml of distilled water and analyzed for CA and DBO (fraction A; see Fig. 1). One ml of supernatant was diluted with 1 ml of water, homogenized and assayed (fraction B). Another ml of the 800 *g* supernatant was layered over 2·5 ml of 1·6 M sucrose (pH 7) containing 500 U/ml of beef catalase (Sigma) (required for the maintenance of DBO activity) and centrifuged for 2 hr at 140,000 *g*

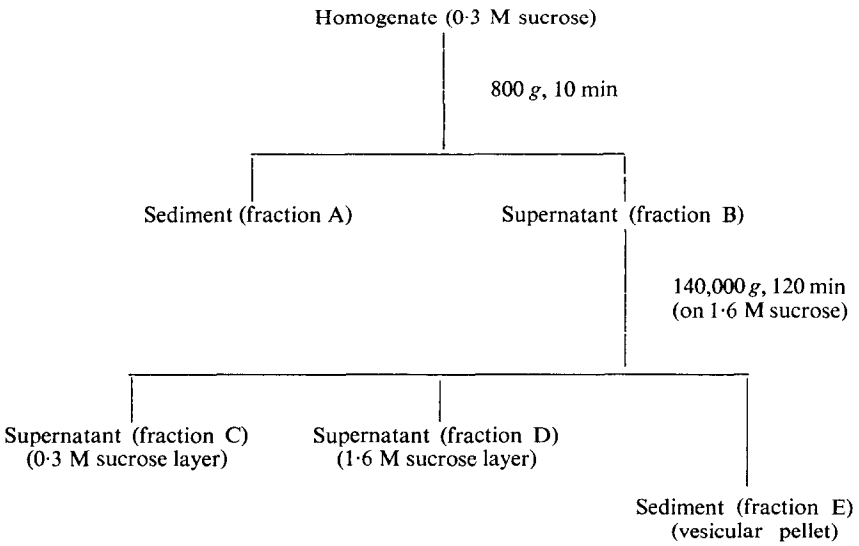

FIG. 1. Subcellular fractionation of adrenal homogenates.

in the number 40 rotor of the Beckman model L2 Ultracentrifuge. This separates intact vesicles from broken vesicle membranes,[1] and from most mitochondrial and lysosomal contaminants,[8] and to some extent from vesicles with lower catecholamine and ATP contents.* The 300 mM sucrose layer (fraction C) and the 1·6 M sucrose layer (fraction D) were diluted with water to a final volume of 2 ml, homogenized and assayed for CA and DBO. Fraction C also contained the C–D interface. The vesicular pellet (fraction E) was resuspended in 2 ml of water and homogenized to lyse the vesicles. One ml was removed for the determination of CA and DBO and the remainder was centrifuged at 26,000 *g* for 10 min to remove the vesicle membranes; the supernatant of this latter centrifugation was analyzed for ATP.

Incorporation of radioactive amines. Adrenal glands were homogenized in 2·2 ml of ice-cold sucrose–Tris as described above, and centrifuged at 800 *g* for 10 min. The supernatant from each sample was divided into four parts, and the ability to incorporate ^{14}C-epinephrine or ^{3}H-metaraminol was measured in sucrose medium containing ATP and Mg^{2+} by the method of Slotkin and Kirshner.[1] The extravesicular CA concentration in each tube was brought to 10^{-4} M by the addition of epinephrine; in tubes in which ^{3}H-metaraminol incorporation was determined, in addition to epinephrine, metaraminol was added to the final concentration of 10^{-4} M for each amine. The additions eliminated and differences in extravesicular amine concentrations among samples. In all cases, results are reported as the temperature-dependent

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component of incorporation (incorporation at 30° minus incorporation at 0°). Incorporation of ^{14}C-epinephrine at 0° was generally about one-fifth of the incorporation at 30° in control samples.

Assays. Catecholamines were assayed fluorometrically and reported as microgram equivalent of epinephrine. Radioactive amines were measured by liquid scintillation spectrometry. ATP was analyzed enzymatically by the firefly method, and DBO was assayed using ^{3}H-tyramine as a substrate. These methods have been previously described in detail.[1]

Statistical analyses. Data are reported in terms of control values, percentages of controls and standard errors of the mean. Levels of significance are calculated by Student's *t*-test.[9]

Materials. Epinephrine-7-^{14}C, metaraminol-7-^{3}H and tyramine-G-^{3}H were obtained from New England Nuclear Corp. Buffered firefly extract was obtained from Worthington Biochemicals. Reserpine phosphate was obtained from Ciba Pharmaceutical Company, epinephrine bitartrate from Winthrop Laboratories and metaraminol bitartrate from Merck, Sharp & Dohme.

RESULTS

Effects of chlorisondamine (10 mg/kg, i.p.) and chlorisondamine plus low dose of reserpine (2·5 mg/kg, i.p.) on incorporation of amines. These data are reported as incorporation per gland and incorporation per 100 μg of CA in the vesicles. The former parameter is a measure of the number of functional vesicles in the gland, while the latter describes the ability of each vesicle to incorporate amines relative to the amount of CA already present. Rats given chlorisondamine alone showed no significant differences ($P > 0{\cdot}15$) from controls in CA levels or incorporation of ^{14}C-epinephrine or ^{3}H-metaraminol at 5 and 25 hr after injection (Table 1). In the reserpine-treated group there was no change in total adrenal CA levels at 1 and 4 hr, showing that there was no neurogenic release of CA; in contrast, epinephrine incorporation at 1 and 4 hr decreased to about 35–45 per cent of controls. At 24 hr after reserpine, adrenal CA had fallen only slightly and epinephrine incorporation had recovered nearly to control levels.

The effect of a low dose of reserpine on ^{3}H-metaraminol incorporation was considerably different (Table 1). There was a small initial decrease (1 hr) followed by a return to normal by 4 hr after reserpine administration.

Effects of chlorisondamine plus high dose of reserpine (10 mg/kg, i.p.) on incorporation of amines. The patterns of decrease and recovery of epinephrine uptake and CA levels differed markedly after high doses of reserpine (Table 2). There was an immediate, complete blockade of epinephrine uptake which was not associated with a decrease in catecholamines (1–4 hr). Epinephrine uptake per gland then increased steadily and was normal at 4 days, while CA dropped markedly to one-third of control between 4 hr to 2 days, and then recovered to 80 per cent by 4 days. Because recovery of epinephrine uptake preceded catecholamine recovery, the uptake per 100 μg of CA was elevated above control levels after 2 days.

The changes in metaraminol incorporation after high doses of reserpine were considerably different from those of epinephrine uptake (Table 2). At 1 hr, when epinephrine incorporation had fallen to nearly zero, metaraminol incorporation per gland

TABLE 1. INCORPORATION OF AMINES BY ISOLATED ADRENAL STORAGE VESICLES*

Treatment and time of sacrifice	Catecholamines	Epinephrine incorporation (per gland)	Epinephrine incorporation (per 100 μg CA)	Metaraminol incorporation (per gland)	Metaraminol incorporation (per 100 μg CA)	No. animals
Control	13·6 ± 0·6†	2·44 ± 0·15‡	18·6 ± 1·3§	0·55 ± 0·04‡	4·15 ± 0·39§	68
			Control (%)			
Chlorisondamine alone (10 mg/kg)						
5 hr	102 ± 5	97 ± 7	94 ± 6	97 ± 6	105 ± 9	18
25 hr	94 ± 7	105 ± 6	108 ± 3	93 ± 5	97 ± 5	6
Chlorisondamine plus reserpine (2·5 mg/kg)						
1 hr	101 ± 4	36 ± 5	36 ± 4	80 ± 5	79 ± 3	6
4 hr	101 ± 5	41 ± 6	45 ± 7	100 ± 7	97 ± 8	5
24 hr	80 ± 6	86 ± 9	104 ± 6	88 ± 6	111 ± 5	6

* Values are expressed as mean ±S.E.
† Micrograms per gland.
‡ Nanomoles per gland/30-min incubation.
§ Nanomoles per 30-min incubation/100 μg of catecholamines in vesicles.

TABLE 2. INCORPORATION OF AMINES BY ISOLATED ADRENAL STORAGE VESICLES*

Treatment and time of sacrifice	Catecholamines	Epinephrine incorporation (per gland)	Epinephrine incorporation (per 100 μg CA)	Metaraminol incorporation (per gland)	Metaraminol incorporation (per 100 μg CA)	No. animals
Control	13·6 ± 0·6†	2·44 ± 0·15‡	18·6 ± 1·3§	0·55 ± 0·04‡	4·15 ± 0·39§	68
			Control (%)			
Chlorisondamine plus reserpine (10 mg/kg)						
1 hr	96 ± 1	7 ± 1	7 ± 1	64 ± 4	67 ± 5	6
4 hr	93 ± 4	13 ± 3	13 ± 3	86 ± 6	92 ± 4	5
24 hr	56 ± 6	39 ± 6	76 ± 10	85 ± 5	166 ± 15	12
2 days	34 ± 7	54 ± 8	191 ± 27	84 ± 7	321 ± 38	11
3 days	50 ± 7	70 ± 6	151 ± 21	97 ± 3	217 ± 37	6
4 days	81 ± 6	97 ± 5	120 ± 8	103 ± 6	130 ± 15	5
12 days	105 ± 7	108 ± 9	99 ± 3	113 ± 7	110 ± 4	6

* Values are expressed as mean ±S.E.
† Micrograms per gland.
‡ Nanomoles per gland/30-min incubation.
§ Nanomoles per 30-min incubation/100 μg of catecholamines in vesicles.

TABLE 3. SUBCELLULAR DISTRIBUTION OF CATECHOLAMINES, DOPAMINE β-HYDROXYLASE AND ATP*

Treatment and time of sacrifice	Catecholamines and ATP† A	B	C	D	E	Total (A + B)	ATP (Fraction E)	No. animals
Control‡	1·81 ± 0·25	10·1 ± 0·6	1·38 ± 0·10	0·65 ± 0·03	5·80 ± 0·34	11·9 ± 0·5	100 ± 3§	48
				Control (%)				
Chlorisondamine alone (10 mg/kg)								
5 hr	100 ± 2	99 ± 1	80 ± 6	94 ± 6	102 ± 2	99 ± 1	106 ± 1	6
25 hr	99 ± 2	101 ± 3	84 ± 8	94 ± 6	109 ± 9	99 ± 4	112 ± 3	12
Chlorisondamine plus reserpine (2·5 mg/kg)								
4 hr	67 ± 1	88 ± 4	88 ± 4	95 ± 3	88 ± 3	81 ± 2	89 ± 3	6
24 hr	65 ± 4	75 ± 5	103 ± 10	78 ± 3	66 ± 4	73 ± 5	67 ± 4	11

Treatment and time of sacrifice	Dopamine β-hydroxylase† A	B	C	D	E	Total (A + B)	No. animals
Control‖	0·17 ± 0·02	0·77 ± 0·02	0·15 ± 0·02	0·02 ± 0·01	0·43 ± 0·04	0·94 ± 0·09	48
				Control (%)			
Chlorisondamine alone (10 mg/kg)							
5 hr	117 ± 6	115 ± 6	89 ± 9	179 ± 42	111 ± 3	115 ± 5	6
25 hr	106 ± 3	110 ± 4	120 ± 6	100 ± 11	100 ± 3	110 ± 4	12
Chlorisondamine plus reserpine (2·5 mg/kg)							
4 hr	97 ± 3	120 ± 5	133 ± 10		104 ± 4	114 ± 4	6
24 hr	93 ± 6	110 ± 5	190 ± 10	61 ± 16	92 ± 5	106 ± 4	11

* Values are expressed as mean ±S.E.
† A = 800 *g* pellet; B = 800 *g* supernatant; C = 0·3 M sucrose layer + C–D interface; D = 1·6 M sucrose layer; and E = 140,000 *g* pellet.
‡ Micrograms per gland.
§ Per cent of control.
‖ Nanomoles per gland/hr.

was about 65 per cent of controls. At 4 hr, when epinephrine incorporation was still very low, metaraminol incorporation per gland had recovered to 80 per cent and returned to normal at 24 hr. Because the return of metaraminol incorporation exceeded that of CA during the recovery phase, the incorporation per 100 μg of CA was elevated at all times after 24 hr.

Isolation of storage vesicles. The subcellular distributions of CA, dopamine β-hydroxylase and ATP from adrenal glands of untreated rats are shown in Table 3. Approximately 15 per cent of the total CA and 20 per cent of total DBO were found in the 800 *g* pellet; this probably represents the fraction of cells not disrupted during homogenization and was fairly consistent from preparation to preparation. Of the amount placed on the discontinuous gradient, about 15 and 20 per cent of the CA and DBO, respectively, were found associated with fraction C (0·3 M sucrose) indicating that about one-sixth of the vesicles were lysed during the preparation. Only 6 per cent of the CA and less than 3 per cent of the DBO were found in the 1·6 M sucrose layer (fraction D). The intact vesicular pellet (fraction E) contained about 60 per cent of the CA and DBO placed on the gradient or about 50 per cent of the CA and DBO in the whole gland. Total recoveries of CA and DBO from the discontinuous gradient averaged about 80 per cent of the amount in fraction B. The values for the subcellular distribution of CA and DBO agree closely with those of Smith and Winkler,[8] Viveros *et al.*[2,6] and Slotkin and Kirshner.[1,*]

ATP was assayed in the intact vesicular pellet (fraction E). ATP values in other subcellular fractions were small and quite variable.

Effects of chlorisondamine and chlorisondamine plus low dose of reserpine (2·5 *mg/kg, i.p.*) *on the subcellular distributions of CA, DBO and ATP.* Five and 25 hr after chlorisondamine administration, there were small increases in DBO levels in several fractions and in ATP in fraction E, while CA remained unchanged (Table 3). The DBO values in fraction D are unreliable because of the small quantities involved.

In the reserpine-treated group, there was a decrease in total CA to about 75 per cent of controls at 24 hr (Table 3), which was accompanied by a relatively small shift in distribution toward the light fraction (C). DBO also increased in fraction C at 4 hr and a greater increase of DBO in fraction C was seen at 24 hr. However, there was no significant change in total DBO or in DBO in the intact vesicle fraction (E). ATP levels in the intact vesicle fraction decreased parallel to CA.

Effect of chlorisondamine plus high dose of reserpine (10 *mg/kg, i.p.*) *on subcellular distributions of CA, DBO and ATP* (Table 4). Four hr after administration of reserpine, total CA levels (A + B) dropped slightly, while DBO levels were unchanged. There was no significant shift in the subcellular distributions of CA or DBO. Twenty-four hr after reserpine administration, total CA had dropped to about 55 per cent of controls. Although total DBO was unchanged, DBO in fraction C doubled while DBO in fraction E decreased to 70 per cent of controls. Three days after reserpine and administration, CA levels recovered to 75 per cent while DBO exceeded controls in all subcellular fractions, with the largest increase occurring in fraction C. Vesicular ATP levels mirrored CA content.

It was important to establish whether reserpine-induced changes in DBO distribution were attributable to increased vesicle fragility, which could result in increased lysis during homogenization. To test this, adrenals were homogenized in sucrose–Tris,

* T. A. Slotkin and N. Kirshner, *Molec. Pharmac.*, in press.

TABLE 4. SUBCELLULAR DISTRIBUTION OF CATECHOLAMINES, DOPAMINE β-HYDROXYLASE AND ATP*

Treatment and time of sacrifice	Catecholamines and ATP† A	B	C	D	E	Total (A + B)	ATP (Fraction E)	No. animals
Control‡	1·81 ± 0·25	11·1 ± 0·6	1·38 ± 0·10	0·65 ± 0·03	5·80 ± 0·34	11·9 ± 0·5	100 ± 3§	48
	Control (%)							
Chlorisondamine plus reserpine (10 mg/kg)								
4 hr	79 ± 5	82 ± 4	87 ± 4	90 ± 4	87 ± 5	81 ± 4	94 ± 2	6
24 hr	42 ± 5	60 ± 7	71 ± 9	53 ± 11	61 ± 8	57 ± 7	63 ± 5	6
3 days	85 ± 7	74 ± 3	75 ± 2	92 ± 1	73 ± 3	74 ± 3	72 ± 3	6

Treatment and time of sacrifice	Dopamine β-hydroxylase† A	B	C	D	E	Total (A + B)	No. animals
Control‖	0·17 ± 0·02	0·77 ± 0·02	0·15 ± 0·02	0·02 ± 0·01	0·43 ± 0·04	0·94 ± 0·09	48
	Control (%)						
Chlorisondamine plus reserpine (10 mg/kg)							
4 hr	103 ± 7	101 ± 5	108 ± 5	104 ± 10	92 ± 5	101 ± 5	6
24 hr	102 ± 13	111 ± 6	192 ± 20	92 ± 17	70 ± 8	110 ± 7	6
3 days	156 ± 9	141 ± 7	259 ± 27		137 ± 12	143 ± 7	6

* Values are expressed as mean ±S.E.
† A = 800 *g* pellet; B = 800 *g* supernatant; C = 0·3 M sucrose layer + C–D interface; D = 1·6 M sucrose layer; and E = 140,000 *g* pellet.
‡ Micrograms per gland.
§ Per cent of control.
‖ Nanomoles per gland/hr.

centrifuged at 800 *g*, and the supernatant was centrifuged at 26,000 *g* for 10 min. The latter centrifugation sediments intact vesicles and particulate DBO from soluble DBO (released on vesicle lysis); a greater amount of soluble DBO in the 26,000 *g* supernatant should appear after reserpine treatment if reserpine increases vesicle fragility. However, 24 hr after reserpine treatment (10 mg/kg), there was no change in the DBO in the 26,000 *g* supernatant (control = 0·05 ± 0·01 nmole/gland/hr; reserpine = 0·04 ± 0·01 nmole/gland/hr), indicating that there was no change in vesicle fragility.

DISCUSSION

In their studies utilizing rabbit adrenals, Viveros *et al.*[3] observed at nearly complete depletion of catecholamines within 24 hr of administration of 1 mg/kg of reserpine, and there was little recovery of either catecholamines or the ability of the vesicles to take up amines until significant new vesicle synthesis had occurred, as indicated by increases in DBO levels. In contrast, administration of 2·5 mg/kg of reserpine in rats produced only a small net loss of catecholamines after 24 hr, and the ability of the vesicles to incorporate exogenous epinephrine returned rapidly to normal. The recovery of uptake occurred at a time when there was little synthesis of new vesicles (no net increase in DBO), and thus the recovery occurred in the vesicles which had been exposed to reserpine. This implies that the blockade by reserpine of ATP-Mg^{2+}-stimulated uptake is reversible to some extent in the rat; the data of Viveros *et al.*[3] imply that reversibility does not occur to a significant extent in rabbits given 1 mg/kg of reserpine. Studies utilizing amine vesicles from various sources exposed to reserpine *in vitro* have also indicated variable degrees of reversibility. Thus, while bovine adrenal medullary vesicles demonstrate irreversible blockade,[7] Klein and Lagercrantz[10] have found that the blockade in isolated nerve trunk vesicles is indeed reversible.

After a high dose (10 mg/kg) of reserpine, there was a prolonged blockade of epinephrine uptake which did require some synthesis of new vesicles to restore the uptake to normal. The finding that the degree of reversibility of reserpine's effect decreased with increased dosage confirms earlier studies *in vitro* that showed that reserpine produced reversible blockade at low concentrations, but irreversible blockade at high concentrations.[10] Even so, the rate of recovery of uptake could not be due solely to new vesicle synthesis, since the increase in DBO accounted for only about 40 per cent of the uptake recovery. It is noteworthy that even at a dose of reserpine ten-times higher than that used in rabbits,[3] there was a more rapid return to normal catecholamines and uptake in rats, thus emphasizing the role that reversibility may play in determining the relative sensitivity of the two species to reserpine.

Metaraminol uptake recovered rapidly after either high or low doses of reserpine. This reflects the fact that metaraminol is taken up by a predominantly ATP-Mg^{2+}-independent (and hence reserpine-resistant) mechanism. The magnitude of the initial inhibition of metaraminol uptake (35 per cent) by high doses of reserpine *in vivo* duplicated the degree of reserpine-sensitivity found upon incubation of vesicles *in vitro* with reserpine.[11,12] Furthermore, the rapid recovery of the partial blockade of metaraminol uptake in the absence of increases in DBO confirms the view that in this system the actions of reserpine *in vivo* must be reversible to some extent.

When depletion of rat adrenal catecholamines is evoked by neurogenic secretion (insulin administration), the uptake of epinephrine shows an initial rate of recovery

far higher than after the high dose of reserpine. Furthermore, all of the recovery after insulin can be accounted for by new vesicle synthesis, whereas new vesicle synthesis does not appear to be significant in the initial period after reserpine. This suggests the importance of neural input as a possible regulator of the synthesis of new vesicles. In insulin-treated rats, there is massive neural input to the adrenal glands, and vesicle synthesis is accelerated. In the reserpine-treated rats, neural input was blocked by chlorisondamine (a long-lasting ganglion blocker), and new vesicle synthesis was not apparent until 3 days after reserpine. The hypothesis that neural input alters the rate of vesicle synthesis is supported by the observation in rabbits that doses of reserpine which cause reflex stimulation of the adrenal result in a vesicle synthesis rate in excess of that observed with doses which do not evoke stimulation,[3] and also by the depressed rate of recovery of catecholamines and the smaller increases in DBO after reserpine-induced depletion in adrenal glands which have been denervated.[13]

There were three distinct phases in the loss–recovery cycle for catecholamines and epinephrine uptake with high doses of reserpine. Initially (4 hr), there was a loss of uptake without changes in catecholamine levels, which reflected the lack of neurogenic effect in the presence of chlorisondamine. In the next period (1–2 days), epinephrine uptake recovered to about 50 per cent of control while catecholamines dropped markedly. This indicates the importance of ATP–Mg^{2+}-stimulated uptake—at least half of the activity must be present to prevent loss of the vesicular stores. In the late recovery period (after 2 days), both catecholamines and uptake increased, although the increases in uptake preceded increases in amine content. This latter effect reflects the fact that when new vesicle synthesis is occurring, the replenishment of catecholamines is probably rate limiting in the recovery of depleted stores, as it is after insulin administration.§

After either high or low doses of reserpine, there were shifts in the subcellular distributions of vesicle components which indicated: (1) a parallel loss of ATP and catecholamines in the intact vesicle fraction, with only minor changes in relative distribution of catecholamines; and (2) no initial net loss of DBO, but a shift of activity toward the lighter sucrose layer (C); after the high dose, later increases in total DBO with the largest increases in the light layer.

These data enable the reconstruction of the events probably occurring after reserpine administration: the blockade of stimulated uptake results in the loss of vesicular catecholamines and ATP [14,15] which is not accompanied by the loss of proteins such as soluble DBO. Because ATP and catecholamines represent a significant portion of the vesicle weight, the vesicles become lighter and begin to disappear from fraction E and appear in the light sucrose layer (fraction C). Some of the light vesicles are destroyed, but many recover from the blockade, reaccumulate ATP and catecholamines, become more dense, and once again appear in fraction E. Simulaneously, once neural input is restored (when the effects of chlorisondamine terminate) new vesicles are synthesized and also appear in fraction C until they too are refilled to the point where their density is high enough to appear in fraction E. A scheme of this type is supported by changes in DBO distribution reported after reserpine administration in rabbits;[6] the formation of new, "empty" vesicles has also been reported in rats and rabbits given insulin.[1,6]*

It has been suggested that storage vesicles which lack ATP but have their protein

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complements may be able to incorporate metaraminol,[1,*] or, as a corollary, that metaraminol is loosely bound inside the vesicle rather than being incorporated into the ATP–storage complex. In the present study, metaraminol uptake returned to normal at a time when a large proportion of the vesicular ATP and catecholamines was missing, as reflected both by the ATP levels in fraction E and in the shift of DBO activity toward lighter particles. This tends to support the hypothesis that metaraminol uptake is not readily affected by alterations in the stable storage capacity of the vesicles.

Acknowledgements—The authors thank Mrs. Nannie Jordan for her technical assistance and Dr. Norman Kirshner for his advice and thoughtful comments.

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