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PERMEABILITY CHANGES INDUCED IN THE ADRENERGIC NEURONE BY RESERPINE*

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Abstract—Rabbit heart slices preloaded with *l*- or *d*-metaraminol (*l*- or *d*-MA) showed relatively little washout of either amine, although only *l*-MA is stored in the adrenergic intraneuronal storage granules. Washout of either amine was enhanced by desipramine or ouabain, while tetrabenazine had no effect. Reserpine pretreatment caused a marked increase in the efflux rate of both *l*- and *d*-MA in control slices, as well as those treated with desipramine or ouabain. The peak effect of reserpine occurred 18 hr after injection and after a dose of 0·5 mg/kg or higher. The dose and time required for effect on amine efflux differed from those required for maximal effects on the granular amine storage mechanism. Lowering the re-incubation temperature greatly inhibited the rate of amine efflux from control as well as drug-treated slices. It is concluded that high doses of reserpine greatly enhance, and cold effectively inhibits, the permeability of the adrenergic neurone membrane to the outward movement of amines.

WHILE the action of reserpine on the adrenergic neurone is generally thought to be directed intraneuronally at the level of the amine storage granules,[1–3] certain lines of evidence have suggested that reserpine may also influence the passage of amines through the adrenergic neuronal membrane. Thus, this laboratory has reported that while reserpine does not affect amine uptake by the neurone membrane transport system under control conditions, the drug does potentiate the inhibitory effect of ouabain and lowered sodium concentration on this system.[4, 5]

To further examine possible effects of reserpine on the adrenergic neuronal membrane, we have in the present study measured the effect of reserpine on the efflux of the nonmetabolizable amines, *l*- and *d*-metaraminol (*l*-MA, *d*-MA), *l*-MA being highly bound to intraneuronal granules where it displaces norepinephrine (NE) essentially on a mole-for-mole basis;[6, 7] whereas *d*-MA, although transported efficiently into the neurone by the membrane amine transport system, is not bound to granules and does not displace NE, and in contrast to *l*-MA, rapidly leaves tissues after its administration *in vivo*.[6] We have also compared reserpine effects on the rate of efflux of *l*- and *d*-MA *in vitro* with the drug's ability to block the intraneuronal amine storage mechanism. The results demonstrate that reserpine markedly enhances the efflux rate of both *l*- and *d*-MA, but only in doses considerably higher than those required to block the intracellular amine concentrating mechanism. Furthermore, incubation in the cold effectively inhibits the efflux rate of both of these amines in control as well as in drug-treated preparations. It is concluded that reserpine in higher doses enhances, while cold

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inhibits, the permeability of the neurone membrane to the outward movement of amines, and that this effect of reserpine is separate from its actions on the amine-concentrating mechanism located in the intraneuronal storage granules.

METHODS AND MATERIALS

Rabbit heart slices were prepared and incubated with amines as described previously.[3] For the purpose of loading slices with amines, the slices were incubated with the amines for 60 min in Krebs phosphate buffer, the medium concentration being 0·2 μg/ml in the case of *l*-MA, and 0·3 μg/ml in the case of *d*-MA. After incubation, the slices were removed, rinsed briefly in cold isotonic saline, and transferred to 20-ml beakers containing 3 ml Krebs phosphate buffer, which in some cases contained ouabain, desipramine or tetrabenazine. The beakers were shaken slowly in an atmosphere of air at 37° for 15 or 30 min unless otherwise specified. Slices and re-incubation medium were then analyzed separately for amine content.[8] The amounts of amine present in the slice and medium were added, and the per cent washout calculated from the sum. Accumulation (the sum) averaged about 1·3 μg/g heart for *l*-MA and 1·2 μg/g for *d*-MA. In experiments on accumulation of *l-m*-octopamine, the medium concentration of *l-m*-octopamine was 0·2 μg/ml. These accumulation studies, which give an estimate of the integrity of the granular storage mechanism, were performed as described previously.[3] Reserpine was injected intravenously via the ear vein into New Zealand albino rabbits.

RESULTS

As shown in Tables 1 and 2, re-incubation of slices preloaded with either *l*- or *d*-MA resulted in the efflux of a small fraction of *l*-MA (10–13 per cent), and a somewhat higher fraction of *d*-MA (19–28 per cent) after 15 or 30 min re-incubations. Desipramine and ouabain, in concentrations which have been found to inhibit the membrane amine transport system by about 85 per cent,[3] enhanced the efflux of both *l*- and *d*-MA, but tetrabenazine at 10^{-5} M, a concentration which effectively blocks the intraneuronal (granular) amine concentrating mechanism,[3] had no significant effect on efflux of either amine. In contrast with tetrabenazine, 18-hr pretreatment with reserpine (5 mg/kg) greatly enhanced the efflux of both *l*- and *d*-MA in control as well as desipramine or ouabain-treated slices. Cold (4°) markedly decreased the efflux of both *l*- and *d*-MA from untreated and treated slices.

TABLE 1. EFFLUX OF *l*-MA FROM HEART SLICES OF CONTROL AND RESERPINE-TREATED RABBITS UNDER VARIOUS CONDITIONS*

Treatment	Control		Reserpine	
	15 min	30 min	15 min	30 min
None	10·4 ± 0·5	12·9 ± 0·6	39·8 ± 2·4	44·3 ± 2·9
Desipramine, 5×10^{-7} M	26·1 ± 1·3	32·2 ± 0·9	46·2 ± 2·3	55·8 ± 2·8
Ouabain, 10^{-5} M		36·5 ± 2·0		70·3 ± 2·0
Tetrabenazine, 10^{-5} M	13·5 ± 0·9	16·0 ± 0·6		
Cold (4°)		3·0 ± 0·2		11·3 ± 1·0

* Heart slices were prepared from normal or reserpine-pretreated (5 mg/kg, 18 hr) rabbits, and slices were treated with *l*-MA, rinsed, and incubated in fresh medium for 15 or 30 min as described in the Methods section. Figures denote per cent *l*-MA washed out of slice in the time indicated. Drugs other than reserpine were added to the re-incubation medium. Numbers denote means ± S. E. M. The number of experiments was 6–21.

TABLE 2. EFFLUX OF *d*-MA FROM HEART SLICES OF CONTROL AND RESERPINE-TREATED RABBITS UNDER VARIOUS CONDITIONS*

Treatment	Control		Reserpine	
	15 min	30 min	15 min	30 min
None	19·1 ± 0·9	27·7 ± 0·8	36·6 ± 3·5	44·7 ± 2·1
Desipramine, 5×10^{-7} M	37·2 ± 1·5	55·4 ± 0·7	48·2 ± 2·0	63·6 ± 1·2
Ouabain, 10^{-5} M		48·3 ± 2·0		71·3 ± 2·2
Tetrabenazine, 10^{-5} M		22·8 ± 0·8		
Cold (4°)		9·5 ± 0·6		16·8 ± 2·3

* Heart slices were prepared from normal or reserpine-pretreated (5 mg/kg, 18 hr) rabbits, and slices were treated with *d*-MA, rinsed, and incubated in fresh medium for 15 or 30 min as described in the Methods section. Figures denote per cent *d*-MA washed out of slice in the time indicated. Numbers denote means ± S. E. M.

In other experiments, the time and dose–response relationships of the reserpine effects were examined. As shown in Fig. 1, no enhanced efflux of *l*- or *d*-MA was seen 1 hr after a reserpine dose of 5 mg/kg, even though at this time granular storage function was greatly inhibited as evidenced by the markedly lowered accumulation of *l*-*m*-octopamine. Of those times studied, the reserpine-induced efflux enhancement reached a peak at about 18 hr and had disappeared 44 hr after injection, at which time *l*-*m*-octopamine accumulation was still considerably impaired.

A similar dissociation of reserpine-induced effects on efflux and *l*-*m*-octopamine accumulation may be seen in the dose response curves depicted in Fig. 2, which show that 18 hr after a dose of 0·1 mg/kg of reserpine, no enhancement of *l*- or *d*-MA efflux could be noted, while *l*-*m*-octopamine accumulation was greatly impaired. Maximal effects of reserpine on efflux rates were seen at 0·5 mg/kg doses or higher.

The results of a more detailed study of the effect of lowered temperature on amine

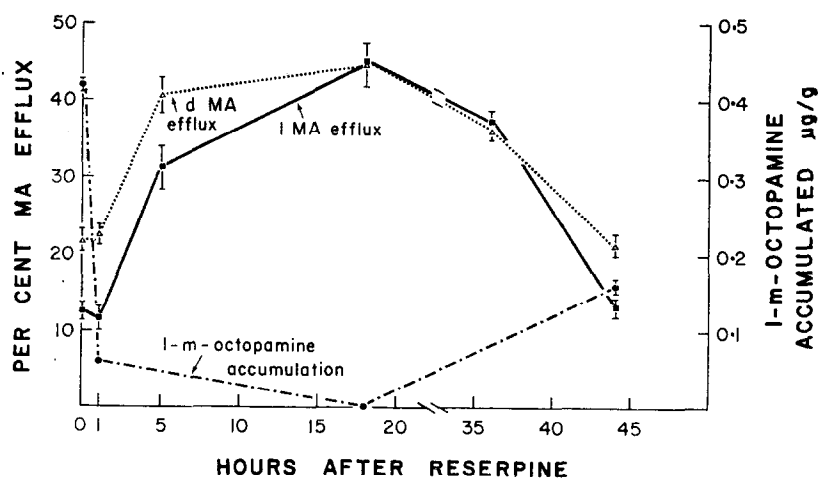

FIG. 1. Efflux of *l*- and *d*-MA and accumulation of *l*-*m*-octopamine at various times after administration of reserpine (5 mg/kg). Rabbits were killed at the indicated times after reserpine. Heart slices were preloaded with *l*- or *d*-MA and re-incubated as described in Methods. Other slices were incubated only with *l*-*m*-octopamine, and the extent of accumulation measured. The latter is an estimate of the state of the granular storage mechanism.[3] Points indicate mean values ± S.E.M.

efflux are described in Table 3. It can be seen that relatively small temperature changes markedly altered the efflux rates. Thus, incubation at 24° caused almost as much inhibition of *d*-MA efflux as incubation at 4°. Lowering the medium temperature from 37° to 24° also greatly inhibited the enhanced efflux caused by a high concentration of ouabain or by a low sodium concentration.

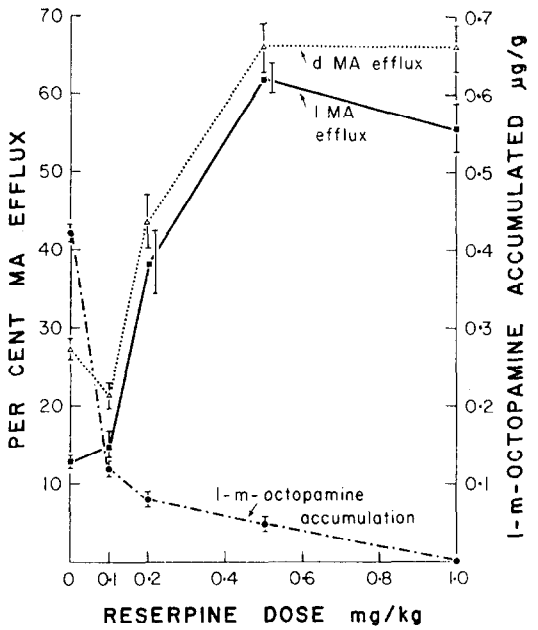

FIG. 2. Effect of various reserpine doses on efflux of *l*- and *d*-MA and on accumulation of *l-m*-octopamine. Rabbits were killed 18 hr after reserpine treatment. Heart slices were preloaded with *l*- or *d*-MA and re-incubated as described in Methods. Other slices were incubated only with *l-m*-octopamine and the extent of accumulation measured. The latter is an estimate of the state of the granular storage mechanism.[3] Points indicate mean values ± S.E.M.

TABLE 3. EFFECT OF LOWERED TEMPERATURE ON EFFLUX OF *d*-MA FROM RABBIT HEART SLICES UNDER VARIOUS CONDITIONS*

Treatment	Per cent efflux
Normal (37°)	27·7 ± 0·8
24°	12·6 ± 0·8
4°	9·5 ± 0·6
Low [Na^+], 37°	53·2 ± 1·9
Low [Na^+], 24°	17·0 ± 1·4
Ouabain, $2·2 \times 10^{-5}$ M, 37°	71·5, 65·0
Ouabain, $2·2 \times 10^{-5}$ M, 24°	16·7, 18·0

* Rabbit heart slices were loaded with *d*-MA, rinsed, and incubated in fresh medium at temperatures indicated. Ouabain was added at beginning of re-incubation. Low [Na^+] indicates that washout occurred in Krebs phosphate buffer with choline chloride substituted for NaCl. Na concentration under this condition is 20 mM. Figures denote per cent *d*-MA washed out of slice in 30 min. Numbers denote means + S. E. M. for 6–20 experiments, except in the case of the ouabain experiments.

DISCUSSION

The results described above indicate that reserpine in higher doses enhances the permeability of the neurone membrane to the outward movement of amines. The dissociation of this effect from actions of the drug on the intracellular (granular) storage mechanism is indicated by: (1) the temporal and dosage separation of reserpine effects. Thus, higher doses of reserpine are required, and a longer period of time is required for an effect on efflux to be manifested than for an effect on granular storage, as divulged by the *l-m*-octopamine accumulation study. (2) Essentially identical effects of reserpine are seen on *l*- and *d*-MA efflux, although only the former is granule bound and displaces NE. (3) Tetrabenazine, in a concentration which has been demonstrated to block granular storage function in the adrenergic neurone, does not enhance *l*- or *d*-MA efflux rate. (4) That the effect of reserpine is on membrane permeability is further suggested by the finding that temperature lowering profoundly decreases the efflux rates of both *l*- and *d*-MA in control and reserpine-treated preparations under a variety of conditions.

Still another indication that reserpine, in higher doses, enhances amine efflux in this manner may be seen in the previously described dose–response relation of *l*-MA release by reserpine from rat heart *in vivo*.[9] Thus, considerably higher doses of reserpine were required to effect *l*-MA depletion 3 hr after reserpine injection than were required for NE depletion, and, more importantly, progressive enhancement of *l*-MA depletion was seen up to doses of 0·5 mg/kg, a dose–response relationship similar to that in the present study. If *l*-MA depletion were dependent solely on blockade of intraneuronal storage sites, no such progressive depletion should have been noted, as much smaller doses (e.g. 50 μg/kg) suffice to block the granular storage mechanism.

The dose–response relationship of reserpine on permeability is reminiscent of that required to induce a "stress" response since, for example, it has been shown that considerably higher doses of reserpine are required to cause ACTH release in the rat than are required to release NE and serotonin.[10] It is tempting to speculate that the higher doses of reserpine may induce a permeability change in certain centers—as, for example, the hypothalamus—such that hypothalamic corticotropin-releasing factor as well as other mediators might be freed by nonspecific leakage.

A recent study using labelled reserpine showed that, in the rat, persistently bound reserpine levels associated with NE depletion in the heart were only a little greater 18 hr after a dose of 100 μg/kg than after 25 μg/kg.[11] Other studies have shown that after a large dose of reserpine (5 mg/kg) in the rabbit, the drug was removed rapidly from tissues and only trace amounts remained in the heart and other organs.[12] The effect of large doses of reserpine described in this paper, therefore, suggests the possibility that the permeability changes seen maximally at 18 hr, but not seen at 1 hr, may be an indirect effect of the drug—an effect mediated by an unknown mechanism.

ADDENDUM

Subsequent to the submission of this paper, findings have been reported which suggest that large doses of reserpine also may enhance the permeability of heart mitochondria to norepinephrine,[13] a finding consistent with previously observed changes in mitochondrial architecture as revealed by electron microscopic studies.[14]

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