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Failure to affect tissue reserpine concentrations by alteration of adrenergic nerve activity*

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RECENT studies[1,2] have affirmed the persistent presence of minute quantities of bound reserpine in several organs, and it has been shown that a correlation exists at certain times between the concentration of bound reserpine and the degree of norepinephrine (NE) depletion.[1] More recently, we have presented evidence pointing to the existence of two types of reserpine binding in rat tissues, a reversible phase lasting about 24–30 hr, and an irreversible phase persisting for many days.[3] It was suggested that the former is associated with the relatively short-lived inhibition of the granule amine carrier mechanism, while the latter is associated with irreversible alteration of the granule storage mechanism for the life span of the storage granule. The present study was designed to investigate the possibility that reserpine bound in adrenergic nerve terminals might be released by adrenergic nerve activity. Such information could help shed light on the site of reserpine binding.

In order to gain direct evidence that bound reserpine is localized in adrenergic nerve terminals, rats were pretreated with 6-hydroxydopamine, a compound which, in high dosage, leads to destruction of adrenergic nerve terminals in several organs.[4] Some of the rats were then given intravenous [^{3}H]reserpine, 200 μg/kg (572 or 424 mc/m-mole), and were killed 18 hr later. [^{3}H]reserpine concentrations were measured in heart and small intestine as described elsewhere,[1] and compared with those of control rats not pretreated with 6-hydroxydopamine. The same tissues of other rats given 6-hydroxydopamine were analyzed for their NE content[5] and compared with those of untreated controls. As shown in Table 1, 6-hydroxydopamine decreased the NE content of the tissues by about 70 per cent and the [^{3}H]reserpine content by about 60 per cent. These results suggest that the bulk, if not all, of bound reserpine is localized in adrenergic nerve terminals.

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TABLE 1. EFFECT OF 6-HYDROXYDOPAMINE ON RAT TISSUE CONCENTRATIONS OF NOREPINEPHRINE AND [^{3}H]RESERPINE*

Tissue	Norepinephrine (μg/g ± S.E.)			[^{3}H]reserpine (ng/g ± S.E.)		
	Control (4)	Treated (8)	Decrease (%)	Control (8)	Treated (8)	Decrease (%)
Heart	1·1 ± 0·1	0·30 ± 0·03	73	4·2 ± 0·5	1·5 ± 0·1	64
Small intestine	0·9 ± 0·1	0·30 ± 0·01	67	4·5 ± 0·7	1·9 ± 0·1	58

* Rats were given 6-hydroxydopamine, 66 mg/kg i.v., twice on days 1 and 7. On day 14 some were killed and the NE content in treated and control rats was measured. Other treated and control rats were given [^{3}H]reserpine, 200 μg/kg i.v., killed 18 hr later, and [^{3}H]reserpine concentrations measured. The number of rats is given in parentheses.

TABLE 2. EFFECT OF PHENOXYBENZAMINE AND INSULIN ON RAT TISSUE CONCENTRATIONS OF [^{3}H]RESERPINE*

Tissue	[^{3}H]reserpine (ng/g ± S.E.)		
	Control (5)	Phenoxybenzamine (5)	Insulin (5)
Heart	2·2 ± 0·2	2·7 ± 0·2	2·3 ± 0·2
Adrenals	23·3 ± 2·3	21·1 ± 1·7	22·6 ± 1·9
Small intestine	2·3 ± 0·3	2·4 ± 0·2	2·2 ± 0·3
Spleen	3·9 ± 0·4	4·9 ± 0·3	4·9 ± 0·7

* Rats were given [^{3}H]reserpine, 200 μg/kg. Some were then given phenoxybenzamine, 25 mg/kg i.p., 6 hr later, while others were given insulin (regular), 100 U/kg s.c., 18 hr later. Convulsions in the latter were controlled by i.p. glucose injections as necessary. [^{3}H]reserpine tissue concentrations were measured 24 hr after administration of the labeled drug. Differences between control and phenoxybenzamine-treated or insulin-treated rats are not significant. The number of rats is given in parentheses.

TABLE 3. EFFECT OF COLD STRESS ON RAT TISSUE CONCENTRATIONS OF [^{3}H]RESERPINE*

Tissue	[^{3}H]reserpine (ng/g ± S.E.)	
	Control (6)	Cold-stressed (3)
Heart	1·8 ± 0·1	2·0 ± 0·1
Adrenals	10·5 ± 1·7	10·4 ± 2·3
Small intestine	2·0 ± 0·2	2·3 ± 0·3
Spleen	4·0 ± 0·5	4·9 ± 0·2

* Rats were given [^{3}H]reserpine, 200 μg/kg i.v. Six hr later, some were placed in a 5° cold room for 18 hr. Rats were killed 24 hr after administration of the labeled drug. Differences between control and cold-stressed rats are not significant. The number of rats is given in parentheses.

TABLE 4. EFFECT OF GANGLIONIC BLOCKADE ON RAT TISSUE CONCENTRATIONS OF [^{3}H]RESERPINE*

Tissue	[^{3}H]reserpine (ng/g ± S.E.)	
	Control (6)	Chlorisondamine (3)
Heart	1·8 ± 0·2	1·6 ± 0·2
Adrenals	10·5 ± 1·7	8·4 ± 2·0
Small intestine	2·0 ± 0·2	1·9 ± 0·3
Spleen	4·0 ± 0·5	3·6 ± 0·2

* Rats were given [^{3}H]reserpine, 200 μg/kg. Six hr later, some were given chlorisondamine, 5 mg/kg i.p. Rats were killed 24 hr after administration of the labeled drug. Differences between control and chlorisondamine-treated rats are not significant. The number of rats is given in parentheses.

To determine whether adrenergic nerve stimulation could lead to release of bound reserpine, rats were given [^{3}H]reserpine and treated with phenoxybenzamine 6 hr later or with insulin 18 hr later. Both substances are known to induce a marked stimulation of adrenergic nerve activity, but by different mechanisms.[6,7] The rats were killed 24 hr after administration of the labeled reserpine. [^{3}H]reserpine concentrations were measured in several organs and compared with those of controls given labeled drug only. As shown in Table 2, stimulation of adrenergic nerve activity by either phenoxybenzamine or insulin failed to decrease the amount of bound reserpine. In other experiments, subjection of rats to cold stress also failed to decrease [^{3}H]reserpine levels (Table 3).

As it was conceivable that the reserpine treatment had in itself already maximally stimulated adrenergic nerve activity reflexly by lowering peripheral NE concentrations, an experiment was performed to determine whether inhibition of adrenergic nerve activity might actually lead to an increased [^{3}H]reserpine tissue concentration as compared with controls. Accordingly, rats were given the ganglionic blocking drug, chlorisondamine, 6 hr after [^{3}H]reserpine, but, as shown in Table 4, measurement of tissue reserpine concentrations 24 hr after administration revealed no alteration of reserpine levels by ganglionic blockade.

The results described in this paper indicate that most, if not all, of bound reserpine is localized in the adrenergic nerve terminal, presumably at the amine storage granules, since the action of the drug is manifested there. As reserpine levels are not affected by treatments which would either stimulate or inhibit activity in the adrenergic neurone, it would appear that persistently bound reserpine is not attached to a releasable component of the granules such as the soluble protein and dopamine β-hydroxylase demonstrated to be released from adrenal medullary granules after nerve stimulation.[8] The results suggest, rather, that reserpine is bound to the granule membrane, which is not released by neural stimulation,[8] and where the drug persists for the remainder of the life span of the granule membrane. Such a picture is consistent with the prolonged presence of reserpine as described previously.[3]

Department of Pharmacology,
University of Texas,
Southwestern Medical School,
Dallas, Tex. 75235, *U.S.A.*

S. NORN
P. A. SHORE

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