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FURTHER STUDIES ON THE NATURE OF PERSISTENT RESERPINE BINDING: EVIDENCE FOR REVERSIBLE AND IRREVERSIBLE BINDING*

S. NORN† and P. A. SHORE

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

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Abstract—Tritium-labeled reserpine of high specific activity was injected into rats, and drug levels measured in heart, spleen, adrenal glands, and small intestine. All tissues showed a first-order reserpine decline terminating about 24–30 hr after injection. After this time there was a semi-permanent binding persisting for many days. Experiments in which high doses of unlabeled reserpine were given 18 or 30 hr after the labeled drug reveal that at the 18 hr time a portion of the drug is reversibly bound, while at the 30 hr time the drug is irreversibly bound. It is suggested that the reversibly bound phase is associated with blockade of the intraneuronal amine carrier system, while the irreversible phase is associated with prolonged alteration of the granular amine storage mechanism.

A PREVIOUS publication from this laboratory described the specific and persistent binding of small quantities of reserpine in adrenergically innervated tissues, and demonstrated in rat heart a high correlation between the degree of norepinephrine (NE) depletion and the concentration of [^{3}H]reserpine present 18 hr after administration of the labeled drug.[1] Based on measurements of reserpine concentrations at 6, 18 and 30 hr after administration, a half-life of about 18 hr was calculated for the sojourn of reserpine in heart and spleen. It was concluded that the persistent depletion of NE by reserpine is maintained by the physical presence of minute quantities of highly bound reserpine. Based also on these measurements and on the estimated 35-day life span of amine granules in the rat,[2] it was further suggested that, contrary to the proposal of other investigators,[2] amine storage granules are reutilized following reserpine disappearance.

In order to investigate further the long-term disposition of reserpine in tissues, and to shed light on the question as to whether reserpine presence may be even more prolonged than that predicted by the earlier studies from this laboratory, we obtained [^{3}H]reserpine of even higher specific activity than used previously.

Experiments utilizing this material reveal that, in the rat, the period of first-order decline of reserpine in various tissues is succeeded at about 24–30 hr after drug administration by a period of many days in which reserpine concentrations remain relatively unchanged. Evidence is also presented that a major portion of the reserpine present during the first-order phase represents drug which is reversibly bound, while the more permanent phase seen most clearly after 30 hr represents functionally irreversibly bound reserpine.

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† NIH International Postdoctoral Fellow.

It is concluded that both phases of reserpine sojourn are important—the reversible phase being associated with inhibition of an amine concentrating mechanism, while irreversibly bound reserpine is associated with alteration of the amine storage system over the life span of the storage granule. The results supply further evidence for the existence of both a functionally important "available NE" pool and a less important storage pool.

METHODS AND MATERIALS

Radioactive reserpine (575 mc/m-mole) with ^3H in positions 2 and 5 of the trimethoxybenzoyl moiety was obtained from the New England Nuclear Corp. Small quantities of the benzene solution as needed were evaporated to dryness under a stream of nitrogen in a small test tube, redissolved in 50 μl of glacial acetic acid, and diluted with four parts of distilled water. The solution (0·1 ml/100 g) was injected intravenously via the tail vein into female Sprague–Dawley rats weighing 160–190 g. Unlabeled reserpine was Serpasil (Ciba). [^3H]reserpine in tissues was measured as described previously.[1]

RESULTS AND DISCUSSION

Reserpine concentrations in various tissues at various times

As shown in Fig. 1 and Table 1, for the period between 6 and 24 hr following injection of 200 μg/kg, the reserpine content of heart, spleen, adrenal gland and small intestine declined in a manner generally similar to that seen in the previous study following the injection of 25 μg/kg.[1] Equilibrium half-time for this first-order phase of reserpine decline was about 8–10 hr. Eighteen hr after injection of 200 μg/kg, the reserpine content in heart and spleen was about the same as that reported in the earlier study. This finding demonstrates again the relative saturability of reserpine

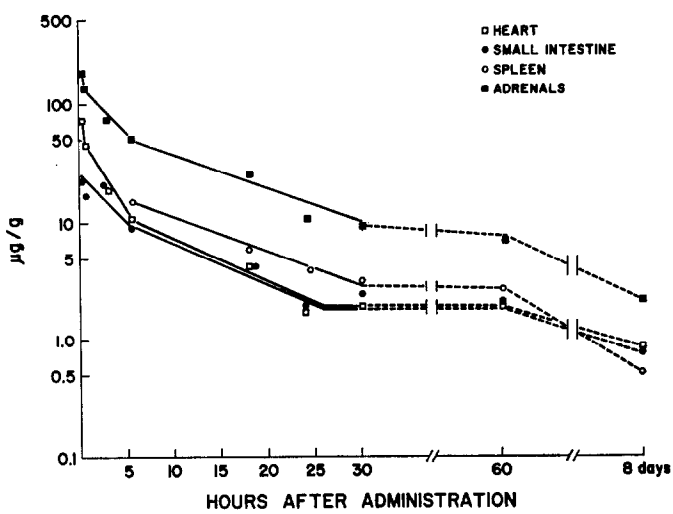

FIG. 1. Reserpine concentrations in several rat tissues at various times after 200 μg/kg i.v. Each point represents the mean of three to ten experiments as described in Table 1.

TABLE 1. RESERPINE CONTENT (ng/g) OF VARIOUS ORGANS AFTER 200 μg/kg RESERPINE

Time after administration	Heart	Small intestine	Spleen	Adrenals
30 min	45·7 ± 2·8 (3)	17·1 ± 2·1 (3)	—	143·0 ± 8·6 (3)
3 hr	18·9 ± 2·9 (3)	21·6 ± 3·3 (3)	—	77·7 ± 9·0 (3)
6 hr	11·3 ± 1·8 (6)	8·9 ± 0·9 (6)	14·8 ± 3·6 (3)	54·5 ± 8·4 (6)
18 hr	4·3 ± 0·5 (8)	4·5 ± 0·7 (8)	6·2 ± 0·9 (3)	27·0 ± 2·5 (8)
24 hr	1·8 ± 0·2 (10)	2·0 ± 0·2 (10)	4·0 ± 0·5 (10)	10·5 ± 1·7 (10)
30 hr	1·9 ± 0·1 (6)	2·5 ± 0·2 (6)	3·3 ± 0·3 (3)	9·3 ± 0·8 (6)
60 hr	1·9 ± 0·2 (5)	2·1 ± 0·2 (5)	2·9 ± 0·2 (5)	7·6 ± 1·0 (5)
8 days	0·9 ± 0·1 (3)	0·8 ± 0·2 (3)	0·6 ± 0·1 (3)	2·3 ± 0·2 (3)
13 days	1·3 ± 0·1 (3)	0·8 ± 0·0 (3)	0·6 ± 0·1 (3)	2·3 ± 0·3 (3)

[^{3}H]Reserpine was injected i.v. into rats which were killed at various times and the several organs analyzed for [^{3}H]reserpine as described in Methods. Figures denote means ± S.E.M. Figures in parentheses denote number of experiments.

binding sites reported previously.[1] There was also seen in the various tissues, a period of up to about 6 hr following injection, of a more rapidly declining phase which presumably represents nonspecific binding and redistribution of the injected drug.

Of great interest was the observation that, about 24–30 hr after reserpine administration, a new phase of much more persistent reserpine binding was evident. Thus about the same reserpine concentrations were found in the various organs at 60 hr as at 30 hr. Analysis of these same organs 8 and 13 days after injection showed considerable concentrations of reserpine remaining. Because of the limited [^{3}H]reserpine supply and the long time periods involved, it was not possible to accurately gauge the half-life of reserpine in this phase.

A similar set of experiments was carried out using a much larger dose of reserpine. [^{3}H]reserpine (200 μg/kg) was combined in a syringe with 1·8 mg/kg unlabeled reserpine. After intravenous injection of this total dose of 2 mg/kg, reserpine concentrations were measured at various times in tissues on the basis of a new specific activity of 57·5 mc/mM. The results, shown in Table 2 demonstrate that except for

TABLE 2. RESERPINE CONTENT (ng/g) OF VARIOUS ORGANS AFTER 2 mg/kg RESERPINE

Time after administration (hr)	Heart	Small intestine	Spleen	Adrenals
1	307 (232, 382)	128 (152, 103)	462 (372, 552)	1293 (1000, 1585)
2	128 (114, 142)	70 (71, 69)	229 (254, 203)	534 (454, 613)
6	83 (99, 66)	42 (31, 54)	148 (100, 195)	397 (304, 490)
18	13·8 ± 4·3 (4)	9·3 ± 1·4 (4)	33·7 ± 12 (4)	61 ± 11 (4)
30	5·4 ± 0·5 (5)	5·3 ± 0·4 (5)	19 ± 2·5 (5)	31 ± 2·5 (5)
60	3·5 (3·3, 3·6)	3·7 (3·3, 4·0)	11 (9, 12)	26 (22, 30)

[^{3}H]Reserpine, 200 μg/kg, and unlabeled reserpine, 1·8 mg/kg, were mixed and injected i.v. into rats which were killed at various times and the several organs analyzed for [^{3}H]reserpine as described in Methods. Total reserpine content was calculated on basis of one-tenth specific activity of the radioactive drug. Figures denote means ± S.E.M. with number of experiments or individual values in parentheses.

higher initial reserpine concentrations, similar kinetics obtained as in the previous experiment. Thus, even though ten times the previous dose was injected, 30 hr later the residual reserpine was only about two to three times that seen following the lower dose. Of greater interest was the finding that, again, little further decline occurred between 30 and 60 hr.

Binding affinity of reserpine at 18 *and* 30 *hr*

In the previous communication, it was concluded that reserpine present 18 hr after administration is irreversibly bound.[1] This conclusion was reached on the basis of experiments in which a large dose of unlabeled reserpine given 18 hr after [^{3}H]reserpine did not alter [^{3}H]reserpine concentrations after a further 18-hr period when compared to control experiments in which tissues of rats given [^{3}H]reserpine only were analyzed after 36 hr. As the present study demonstrates a marked difference in the kinetics of reserpine disappearance at 18 hr as compared with 30 hr, it seemed possible that the previous results could have led to an incorrect conclusion. Thus even if unlabeled reserpine injected at 18 hr had displaced some [^{3}H]reserpine it might not have been observed when analysis was performed after another 18-hr period. Accordingly, new experiments were designed to test the affinity of reserpine binding at 18 and 30 hr after administration.

Unlabeled reserpine, 0·5 mg/kg, was injected intravenously 18 or 30 hr after injection of [^{3}H]reserpine, 200 μg/kg. One hr after administration of the unlabeled drug, the animals were killed and [^{3}H]reserpine concentrations measured. As can be seen in Table 3, unlabeled reserpine decreased the [^{3}H]reserpine tissue concentration at the 18 hr time, but not at the 30 hr time. These results suggest strongly that at 18 hr after reserpine injection, a portion of the drug is reversibly bound, while at 30 hr, all of the residual reserpine is irreversibly bound. It may be noted that the large dose of unlabeled reserpine given at the 18 hr time did not decrease the [^{3}H]reserpine below that seen in controls at 30 hr, suggesting that a portion of the 18 hr material is also irreversibly bound.

It has been shown that the gross pharmacological effects of reserpine are relatively short-lived and are more closely related temporally to effects on granular amine uptake

TABLE 3. EFFECT OF UNLABELED RESERPINE GIVEN 18 OR 30 hr AFTER [^{3}H]RESERPINE ON CONCENTRATIONS OF THE LABELED DRUG

	[^{3}H]Reserpine (ng/g ± S.E.M.)			
	18 hr		30 hr	
Tissue	Control	Treated	Control	Treated
Heart	4·2 ± 0·5 (8)	2·2 ± 0·1 (7)	2·0 ± 0·1 (6)	1·7 ± 0·1 (5)
Adrenals	27·0 ± 2·5 (8)	12·3 ± 1·4 (7)	9·3 ± 0·8 (6)	7·7 ± 0·8 (5)
Small intestine	4·5 ± 0·7 (8)	2·4 ± 0·1 (7)	2·5 ± 0·2 (6)	2·1 ± 0·1 (5)
Spleen	6·2 ± 0·9 (3)	4·7 ± 0·4 (7)	3·3 ± 0·3 (6)	3·6 ± 0·4 (5)

Rats received [^{3}H]reserpine (200 μg/kg i.v.). Eighteen or 30 hr later some rats received unlabeled reserpine (0·5 mg/kg i.v.) and were killed 1 hr later. Controls received [^{3}H]reserpine only. Figures in parentheses denote number of experiments.

mechanisms than on the more prolonged amine depletion.[3] These observations have led, in part, to the concept of multiple pools of norepinephrine, an "available" pool and a storage pool.[3–5] The results described in this paper suggest that the reversibly bound reserpine of 24–30 hr sojourn is important in blockade of the amine carrier function which is likely associated with maintenance of the "available" pool, while the irreversibly bound reserpine seen most clearly 30 hr or more after reserpine, but probably present at 18 hr also, is important in the prolonged alteration of amine storage capacity. The prolonged presence of irreversibly bound reserpine supports the hypothesis of Dahlstrom and Häggendal[2] that following reserpine-induced NE depletion, new amine granules must be formed before full restitution of NE content can occur.

It is not clear from these experiments whether reversibly bound reserpine is in part slowly converted to irreversibly bound, or whether both types of binding sites are acted on independently.

These observations thus provide the means to reconcile experiments demonstrating that reserpine acts reversibly on the Mg^{2+}-ATP stimulated granular amine carrier system, yet also acts for weeks to permanently alter amine storage granules so that repletion of NE stores must await new granule formation.[2,3] The present studies also provide further evidence for the duality of an amine concentrating mechanism at the granular level which replenishes the "available" NE pool and an amine storage capacity which serves presumably as a reservoir for the "available" pool.

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