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Studies on the subcellular distribution of [^{3}H]reserpine

It is well established that reserpine can deplete tissues of their catecholamine content. Since existing chemical methods could not detect reserpine in the tissues at a time when the amine content was still quite low, a "hit and run" or irreversible damage hypothesis was formulated to explain reserpine's mechanism of action. However, the development of [^{3}H]reserpine enabled workers (Shepard, Tsein & others, 1958; Maggiolo & Haley, 1964) to demonstrate trace quantities for up to five days post injection. Evidence is available indicating that the depletion induced by reserpine *in vivo* is, in all probability, due to a blockade of amine uptake by the adrenergic nerve granules. (Bertler, Hillarp & Rosengren, 1961; Stitzel & Lundborg, 1967). It is possible, therefore, that the subcellular site of action of reserpine may coincide with a similar subcellular binding of this depleting agent. The present experiments were conducted to test this hypothesis.

Male Swiss-Webster mice, 18–20 g, were injected with [^{3}H]reserpine, 600 μg/kg (approximately 155 μCi/kg) intravenously. The animals were killed by a blow on the head either 15 or 60 min post injection, the hearts removed and homogenized in an ice bath with a Teflon pestle, in 0·25M sucrose containing 0·005M phosphate buffer, pH 7·4 and 0·001 M $MgCl_2$. Nuclear and mitochondrial fractions were obtained by centrifuging in the cold for 10 min at 600 and 8000 *g*, respectively. The 8000 *g* supernatant was then spun at 105,000 *g* for 60 min in a Spinco model L ultracentrifuge to provide a microsomal and a high speed supernatant fraction. Each fraction was analysed for its [^{3}H]reserpine content by a modification of the method of Manara (1967). All sediments were resuspended twice in 10 volumes of acetone. The acetone was evaporated under a stream of nitrogen and the dried material was redissolved in chloroform. Ten volumes of chloroform were added to the high speed supernatant fraction, shaken for 15 min and then centrifuged at 600 *g* for 10 min to

Table 1. *Subcellular distribution of* [^{3}H] *reserpine in the mouse heart at* 15 *and* 60 *min periods after injection*

Fraction	15 min*	60 min*	% decrease
Nuclear	385 ± 23	143 ± 10	64
Mitochondrial	86 ± 12	30 ± 2	65
Microsomal	49 ± 3	19 ± 1	61
Supernatant	17 ± 3	8 ± 2	55

* Each value represents the mean content of [^{3}H]reserpine (ng/g) ± s.e. The means are based upon at least 10 experiments.

Table 2. *A comparison of the reserpine and lipid content of each subcellular fraction*

Subcellular fraction	Reserpine content % 15 min	60 min	Lipid content %
Nuclear	71	71	77
Mitochondrial	16	15	12
Microsomal	10	9	8
Supernatant	3	4	4

break any emulsion formed. Aliquots of all the chloroform extracts were chromatographed on silica gel thin-layer plates to separate reserpine from its major metabolites.

[^{3}H]Reserpine was found in all subcellular fractions within 15 min after its intravenous administration (Table 1). The concentration was highest in the nuclear fraction> mitochondrial> microsomal> supernatant. The microsomal fraction, i.e. that fraction containing the catecholamine storage granules, did not have an unusually high proportion of [^{3}H]reserpine.

Although no unique pattern of distribution was apparent 15 min after injection, it was felt that study of the disposition of [^{3}H]reserpine at two time periods might unmask a specific binding. The results of this experiment are presented in Tables 1 and 2. There was a rapid decrease in [^{3}H]reserpine content in all fractions between 15 and 60 min. The rate of decline, however, was similar in all fractions, and therefore the percentage distribution of [^{3}H]reserpine was identical at both time intervals. Thus reserpine does not appear to have a specific affinity for the amine storage particles. Independently of these studies, Alpers & Shore (1969) also reported an inability to localize [^{3}H]reserpine in a single subcellular compartment.

The observation that some redistribution of [^{3}H]reserpine (Alpers & Shore, 1969; Wagner & Stitzel, unpublished observations), but not [^{3}H]noradrenaline (Stitzel & Lundborg, 1967), occurs during homogenization prompted us to examine the role physical-chemical factors play in determining reserpine's localization. From the evidence in Table 2 it appears that *in vivo* distribution of [^{3}H]reserpine may be accounted for solely on the basis of its high lipid solubility since its disposition closely parallels the total lipid content of each fraction.

In summary the physiological disposition of [^{3}H]reserpine in sympathetically innervated tissue appears to be primarily determined by its high lipid solubility. Subcellular distribution studies emphasized that reserpine is not uniquely associated with the noradrenaline-containing storage granules. However, the large amount of reserpine bound to lipid may mask a smaller more specific binding.

The authors wish to thank Mr. David McClure for his excellent technical assistance. This work was supported by Grants GM 16433–01 and GRS 5–SO1–FR 05433–07 from the U.S. Public Health Service and NDEA Fellowship No. 67–11809.

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October 3, 1969

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