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# BIOSYNTHESIS OF RESERPINE-LINE SUBSTANCES IN THE MYOCARDIUM AND OTHER TISSUES OF MAN AND ANIMALS

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Reserpine-like substances were found to be synthesized in homogenates, microsomes, and cytosol from various human and animal tissues with the utilization of acetate, methyl groups of S-adenosylmethionine, tryptophan, reserpine, etc., as precursors. It is suggested that endogenous reserpine-like substances participate in the autoregulation of the levels of free and bound biogenic amines. KEY WORDS: endogenous reserpine-like substances; biosynthesis; biogenic amines.

Certain pharmacological agents are known to be able to reduce the reserves of biogenic amines in neuron terminals. These substances include, for example, guanethidine, 6-hydroxydopamine, and reserpine [3]. The last of the three has been well studied and is an alkaloid of indole type produced by certain species of tropical plants. Reserpine induces exhaustion of catecholamine depots as a result of blockade of the Mg-ATP-activated system responsible for their uptake at the level of adrenergic reserve granules [4, 6]. Exogenous reserpine, administered to an animal, has been shown [5] to bind with specific receptors on the membranes of catecholamine-containing granules in nerve endings and in the adrenals.

The writers showed previously that the human and animal myocardium contains endogenous substances similar to but not identical with reserpine [1].

The object of this investigation was to continue the study of the pathways of biosynthesis of respine-like substances of animal origin, to which the name "enderpines" or "RP-fraction" was given.

## EXPERIMENTAL METHOD

The substances to be studied were isolated from animal tissues by the method described previously [1]. The intensity of fluorescence of the enderpines was measured on the Aminco–Bowman (USA) instruments. Tissue homogenates (ground with quartz sand for 5 min) and also freshly isolated cytosol and microsomes the rat liver were used as the enzyme systems. For the cytosol and microsomes the incubation system [7] suggested previously was used; its composition was as follows (2 ml): 6 $\mu$M ATP, 0.4 $\mu$M coenzyme A, 4 $\mu$M $MgCl_2$, 2 $\mu$M NaF, 0.4 $\mu$M dithiothreitol in 0.2 M Tris-HCl buffer, pH 7.2, protein concentration 0.5 mg/ml. The controlled enzyme reaction was started by the addition of 200 $\mu$l of the given labeled substrates and in-

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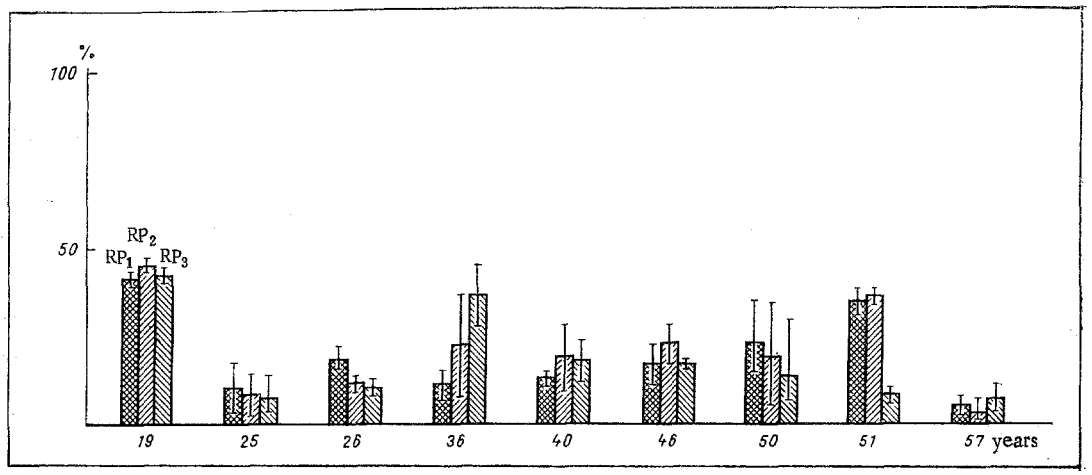


Fig. 1. Level of reserpine-like fractions or enderpines ($RP_1$, $RP_2$, $RP_3$) in myocardia of persons of different ages dying suddenly. Level of enderpines determined from their fluorescence, expressed as percentages of intensity of fluorescence of 0.005 M reserpine in chloroform measured at 515 nm peak with wavelengths of excitation of 270 nm. Result calculated per gram wet weight of tissue.

cubation at 37°C (3 h). The specific radioactivity and substrate concentrations were: 4[methyl-$^3$H]-S-adenosylmethionine 7.5 Ci/mmole (1.6 nmole/ml), Na[U-$^{14}$C]-acetate 58 mCi/mmole (215 nmole/ml), L-histidine-[ring-2-$^{14}$C] 44 mCi/mmole (114 mmole/ml), tryptophan-$^3$H (totally labeled) 2 Ci/mmole (5 nmole/ml), L-tyrosine-$^{14}$C (U) 507 mCi/mmole (4.9 nmole/ml), N-formyl-L-methyl-$^{14}$C-methionine 56 mCi/mmole (223 nmole/ml), reserpine benzoyl-$^3$H (G) 0.15 mCi/mmole (82 nmole/ml), DL-[7-$^{14}$C]-adrenalin-DL-bitartrate 33 mCi/mmole (380 nmole/ml). Radioactive adrenalin and reserpine were used only for incubation with tissue homogenates.

The enzymic reaction in cell-free systems was stopped by the addition of $HClO_4$ to a final concentration of 0.4 N, the sample was incubated at 37°C for 18 h, the total volume was made up to 10 ml with 0.4 N $HClO_4$, after which the enderpines were purified by the standard method [1] with fourfold rechromatography of the enderpines in the final stages of their isolation. Prepurified enderpines from bovine liver were used as the carriers at the start of the chromatogram. Radiohomogeneous fractions were scraped from the chromatograms, transferred to scintillation fluid (xylol, 0.3% 2,5-diphenyloxazole) and counted in a Mark 3 instrument (USA).

## EXPERIMENTAL RESULTS

The quantity of enderpines and the ratio between their fractions in the myocardia of persons of different stages dying suddenly (time of autopsy up to 6 h) varied considerably both within the same myocardium and in different subjects (Fig. 1). This may perhaps be connected with the biosynthesis, storage, and utilization of enderpines. This hypothesis was confirmed by investigations of the incorporation of the various radioactive precursors into all three fractions ($RP_1$, $RP_2$, and $RP_3$) of enderpines.

As a rule the background radiation of the flasks, chromatographic plates, and unlabeled fractions of enderpines in these experiments amounted to 20-40 cpm. When radioactive precursors were used, the radioactivity of the flasks with scrapings of fractions of enderpines ranged from 400 to 18,000 cpm for homogenates and from 200 to 1000 cpm for cell-free systems. Incorporation of label into enderpines of the microsomes was obtained for tryptophan ($RP_1$, $RP_2$, and $RP_3$), S-adenosylmethionine ($RP_3$), acetate ($RP_1$, $RP_2$), histidine ($RP_3$), and N-formylmethionine ($RP_3$). For tryptophan, in the enderpines of the cytosol, incorporation took place into fractions $RP_1$, $RP_2$, and $RP_3$, for S-adenosylmethionine into $RP_1$, for acetate into $RP_1$, for histidine into $RP_3$, for tyrosine into $RP_1$, and for N-formylmethionine into $RP_3$.

Incubation on homogenates of rat myocardium, brain, liver, and kidneys, and also on human hypothalamus, adrenals, and aorta was carried out after addition of 400 $\mu$l of radioactive substrates to the previously ground tissues. After incubation for 3 h at 37°C the enderpines were isolated by the standard method. Incorporation of the label into the enderpine fractions was observed in homogenates of the kidneys and liver (S-adenosylmethionine, $RP_3$), kidneys and heart (tyrosine, $RP_3$), and brain (adrenalin, $RP_1$, $RP_2$, $RP_3$). Adrenalin, tryptophan, and reserpine were incorporated particularly intensively into all fractions of enderpines (up to 5000-18,000 cpm with 10-40 g of homogenate). A wide variation of the rates of incorporation of the precursors dur-

ing repetition of the experiments on the same tissues was characteristic, and was probably due to the metabolic state preceding incubation. Trytophan and, in particular, reserpine had very high rates of incorporation. For instance, the rate of incorporation of tryptophan into all three fractions of rat enderpines was 1-16 pg label per gram wet weight of tissue per hour for brain, 3-20 pg/g/h for heart, 3-17 pg/g/h for kidneys, and 0.5-6 pg/g/h for liver. Incorporation of reserpine into enderpines of rat brain, kidneys, liver, and heart took place a thousand times more intensively, the values being 18-357, 16-208, 5-203, and 32-195 ng label/g tissue/h respectively. Reserpine was also incorporated very intensively into the enderpines of human tissues, especially the hypothalamus (cases of sudden death): for the hypothalamus 148-584 ng label/g tissue/h, adrenals 3-17 ng/g/h, aorta 1-52 ng/g/h (intima 1-46, media + adventitia 5-12).

The rate of incorporation of adrenalin into the kidneys, liver, brain, and heart, pooled from five rats, was 379-2494 cpm for $RP_1$, 634-6355 cpm for $RP_2$, and 1245-18,205 cpm for $RP_3$. Many precursors are thus involved in the biosynthesis of the enderpines, a fact which points to the existence of a large pool of enzymes responsible for the biogenesis of these compounds.

Analysis of mass-spectra of the enderpines has shown [1] that the molecular weight of these compounds is higher than that of reserpine itself. The enderpines evidently consist of "reserpine" and "nonreserpine" moieties. Consequently, only the incorporation of labeled reserpine into the "reserpine moiety" of the enderpines can be interpreted and the localization of the other precursors is unknown.

It follows from the active incorporation of reserpine into all three fractions of enderpines during their biosynthesis that the results of the previous experiments to study incorporation of reserpine into various animal tissues and subcellular structures require a fundamentally different interpretation [5, 8, 9]. To begin with, the unusually long (more than a week) presence of exogenous reserpine in animal tissues is explained [8]. In that particular research, to extract exogenous labeled reserpine a method was used [2] which, in its initial stages, is similar to our own [1], and leads to inaccurate determination of the quantity of reserpine extracted, for it does not allow for enderpines synthesized de novo present in the total extract. Reserpine cannot be eliminated quickly from the body, for it is intensively utilized for the biosynthesis of enderpines, which are natural components of animal tissues. Another important, but unexplained, observation is that reserpine-$^3$H, injected intravenously into mice, is incorporated after 15 min into the subcellular fractions of the myocardium and other tissues, but the fraction of bound reserpine is reduced 60 min later by 60% [9]. In that investigation reserpine was isolated by thin-layer chromatography, so that contamination with enderpines was ruled out. The rapid fall in the level of bound reserpine in this case can also be explained by its incorporation into synthesized enderpines, which were then eliminated in the course of purification of the reserpine. Giachetti et al. [5], in a study mentioned above, after proving interaction between exogenous reserpine and specific receptors of catecholamine-containing granules, postulated that a certain active factor, whose nature is unknown, is implicated in this process. In the present writers' view, this active factor is the biosynthesis of enderpines. It can tentatively be suggested that exogenous reserpine binds with nerve endings of the adrenergic system, where it is modified by enzyme action into enderpine. If this is so, it is the enderpines and not reserpine whose functions are related to depots of biogenic amines. These functions perhaps behave as an autoregulatory system of the levels of functionally active (free) and inactive (bound) biogenic amines.

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