EXPERIMENTAL INVESTIGATION OF THE MECHANISM OF ACTION OF RESERPINE

I. S. Chekman

UDC 615.214.22.015

Reserpine (2.5 mg/kg) reduces the catecholamine concentration in the myocardium and at the same time inhibits cytochrome c oxidase and succinate dehydrogenase activity but does not affect the concentration of ATP, ADP, and AMP. Unithiol reduces the exhausting effect of reserpine on the noradrenalin concentration in the rat myocardium. It is concluded that reserpine upsets the bond between biogenic amines and ATP.

An important aspect of the pharmacodynamics of reserpine, which is responsible for its hypotensive and neuroplegic effects, is its ability to reduce the content of adrenalin, noradrenalin, and serotonin in the tissues [1, 2, 6, 7, 12, 13]. However, the mechanism of the exhausting effect of reserpine of the tissue catecholamine reserves has not yet been explained. According to Brodie and Beaven [8] it is due to the blocking of the active transport system maintaining a certain level of biogenic monoamines in the adrenergic neutron and functioning at the expense of energy of high-energy phosphorus compounds, predominantly ATP, which is formed [5, 10] as a result of biological oxidation. The cytochrome system is an important link in the respiratory chain which transfers electrons to oxygen, thereby completing the cycle of biological oxidation.

The object of the present investigation was to compare the effect of reserpine on the catecholamine content and the level of adenine nucleotides and to discover how this effect is combined with the action of reserpine on the key enzymes of the respiratory chain determining the intensity of biological oxidation and of ATP formation.

TABLE 1. Effect of Reserpine (2.5 mg/kg) on Cytochrome-Oxidase and Succinate Dehydrogenase Activity and Noradrenalin Level in Rat Myocardium

Sample	Time after injection	Cytochrome c oxidase (in i.u./mg protein)	Succinate dehydrogenase (in μg formazan/mg protein in 30 min)	Total catecholamine content (in μg noradrenalin/g fresh tissue)
Control	—	0,24±0,04	709±89	1,19±0,09
Reserpine	40 min	0,204±0,08 P=0,05	490±85 P<0,02	0,82±0,06 P<0,01
Control	—	0,23±0,01	707±20	1,2±0,05
Reserpine	2 h	0,203±0,009 P=0,05	666±21 P>0,05	0,65±0,09 P<0,05
Control	—	0,35±0,017	1 291±95	1,6±0,15
Reserpine	24 h	0,31±0,01 P>0,05	1 148±93 P>0,05	0,74±0,06 P<0,05

Department of Pharmacology and Central Research Laboratory, Kiev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Cherkes.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 3, pp. 59–61, March, 1972. Original article submitted September 22, 1971.

TABLE 2. Effect of Reserpine on ATP, ADP, AMP, Inorganic Phosphorus and Noradrenalin Concentration in Rabbit Myocardium

Sample	ATP	ADP	AMP	Total adenine nucleotides
	μ moles /g tissue			
Control	2,31±0,1	1,39±0,17	0,52±0,04	4,31±0,27
Reserpine (2.5 mg/kg)	2,51±0,3	1,35±0,09	0,58±0,06	4,46±0,38
Control	2,01±0,14	1,27±0,08	0,75±0,11	4,02±0,2
Reserpine (0.25 mg/kg daily for 10 days)	1,58±0,27 P=0,05	1,17±0,07	1,06±0,11 P=0,05	3,81±0,27

Table 1. (Continued)

Sample	$\frac{ATP}{AMP}$	Inorganic phosphorus (in mg %)	Total catecholamine concentration (in μg noradrenalin/g fresh tissue)
Control	4,44±0,25	45,26±1,9	1,33±0,6
Reserpine (2.5 mg/kg)	4,3±0,5	50,8±0,9 P=0,05	0,53±0,1 P<0,05
Control	2,68±0,1	39,3±1,4	—
Reserpine (0.25 mg/kg daily for 10 days)	1,48±0,2 P<0,05	48,9±2,0 P<0,05	—

EXPERIMENTAL METHOD

The first group of experiments was carried out on rats weighing 160-200 g. The catecholamine content was determined in heart muscle by Osinskaya's method of fluorescence analysis with a number of modifications [6] and expressed in micrograms of noradrenalin base per gram fresh tissue. Activity of cytochrome oxidase (1.9.3.1) was determined by a colorimetric method and expressed in indophenol units (i.u.) per milligram protein per minute [14]. Succinate dehydrogenase (13.99.1) activity was determined by reduction of neotetrazolium chloride to colored formazan and expressed in micrograms formazan per milligram protein [9]. Sulfhydryl groups were determined by amperometric titration [3].

In the second group of experiments on rabbits reserpine (2.5 mg/kg) was injected intramuscularly, and 3 h later the content of ATP, ADP, and AMP was determined by paper electrophoresis followed by spectrophotometry at 260 and 290 nm [11]. Inorganic phosphorus was determined by Delory's method in V. A. Grigor'eva's modification and compared with the catecholamine level. Protein was determined by Lowry's method. Since the indices studied showed fluctuations, a control was set up for each series.

Reserpine (Rausedil, 0.25%) was injected intramuscularly in a dose of 2.5 mg/kg 40 min and 2 and 24 h before decapitation of the animals, or in a dose of 0.25 mg/kg daily for 10 days. To study the mechanism of action of reserpine, unithiol was used as a donor of sulfhydryl groups; it was given in doses of 80 or 400 mg/kg body weight 30 min before the injection of reserpine.

EXPERIMENTAL RESULTS

As Table 1 shows, 40 min after injection of reserpine cytochrome c oxidase and succinate dehydrogenase activity was reduced and there was a tendency for the noradrenalin concentration in the rat myocardium to fall. After 2 h the changes in the activity of the respiratory enzymes were in the same direction, and there was a marked decrease in the tissue catecholamine reserves. The noradrenalin level in the myocardium 24 h after injection of reserpine was still considerably (68%) below the control value, and the activity of the respiratory enzymes showed a tendency toward recovery. These observations are in agreement with those obtained by Sun et al. [16] who showed by a histochemical method that reserpine inhibits the activity of cytochrome c oxidase and succinate dehydrogenase.

Having completed the analysis of the mechanism of the effect of reserpine on these enzymes and remembering that succinate dehydrogenase is an SH-containing enzyme, the next step was to study changes in the number of functionally active sulfhydryl groups of proteins in the rat myocardium under the influence of reserpine. Experiments showed that reserpine, 2 h after its injection, reduced the number of functionally

active sulfhydryl groups in the myocardium of these animals. These results suggest that a component of the mechanism of the catecholamine-liberating action of reserpine is its blocking of SH-groups of the active center of succinate dehydrogenase, which participates in electron transport in the respiratory chain. This was confirmed by the results of experiments using unithiol as a donor of sulfhydryl groups. Its administration (80 mg/kg) slightly reduced the exhausting effect of reserpine on the catecholamine concentration in the rat myocardium, while in a dose of 400 mg/kg it almost completely inhibited the development of this effect.

According to the literature, in the adrenergic neuron noradrenalin and adrenalin are bound with ATP, and reserpine, which disturbs the storage of sympathetic nervous system mediators, reduces their content in the tissues. It was therefore decided to compare the control of ATP, ADP, and AMP with the catecholamine level. The results are summarized in Table 2. They show that the content of ATP, ADP, and AMP in the myocardium of rabbits receiving reserpine in a dose of 2.5 mg/kg was unchanged while the level of inorganic phosphorus was increased. Meanwhile the noradrenalin concentration in the myocardium fell considerably. Only after prolonged (10 days) administration of reserpine was a tendency observed for the content of ATP to fall and of AMP to rise, accompanied by statistically significant changes in the inorganic phosphorus level, probably on account of inhibition of oxidative phosphorylation [15]. However, this is not an essential factor in the mechanism of the exhausting effect of reserpine on the tissue catecholamine depots, for in acute experiments there was a marked decrease in the noradrenalin concentration but no change in the ATP level.

After administration of reserpine for 10 days the ATP/AMP ratio was reduced. The increase in AMP may have some part to play in the development of increased sensitivity of the tissues to sympathetic mediators during prolonged reserpinization.

It can be concluded from the results of these investigations that reserpine reduces the catecholamine concentration in the tissues by disturbing the bonds between these biogenic amines and ATP. Its action on cytochrome oxidase and succinate dehydrogenase plays an important role in this process.

LITERATURE CITED

1. N. B. Vysotskaya, É. M. Matlina, V. V. Men'shikov, and K. M. Khalimova, in: Biogenic Amines [in Russian], Moscow (1967), p. 162.
2. V. V. Zakusov and N. V. Kaverina, The Pharmacology of Monoaminergic Substances [in Russian], Moscow (1970).
3. S. M. Nistratova, Thiol Compounds in Medicine [in Russian], Kiev (1959), p. 11.
4. V. O. Osinskaya, Biokhimiya, No. 4, 537 (1957).
5. V. P. Skulachev, Relationship of Oxidation to Phosphorylation in the Respiratory Chain [in Russian], Moscow (1962).
6. F. P. Trinus, Author's Abstract of Doctoral Dissertation, Kiev (1956).
7. I. S. Chekman, Vrach. Delo, No. 10, 5 (1970).
8. B. Brodie and M. Beaven, Med. Exp. (Basel), 8, 320 (1963).
9. E. Kun and L. Abrood, Science, 109, 144 (1949).
10. A. Lehninger, The Mitochondrion [Russian translation], Moscow (1966).
11. T. Sato, S. Thomson, and W. Danforth, Analyt. Biochem., 5, 542 (1963).
12. A. Schaepdryver, Actual. Pharmacol. (Paris), p. 226 (1963).
13. P. A. Shore, Pharmacol. Rev., 14, 532 (1962).
14. W. Straus, J. Bio. Chem., 217, 733 (1954).
15. A. Schwartz and K. Lee, Nature, 188, 948 (1960).
16. S. C. Sun. R. S. Sohal, H. L. Colcolough, et al., J. Pharm. Exp. Ther., 161, 210 (1968).