EFFECT OF ADIPHENINE IN VITRO ON ABSORPTION OF NORADRENALIN BY ISOLATED NERVE ENDINGS AND SYNAPTIC VESICLES

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The central nicotinic cholinolytic adiphenine in experiments in vitro, unlike its analogue the muscarinic cholinolytic benactyzine, causes noradrenalin to accumulate in isolated nerve endings and synaptic vesicles.

Besides their common cholinolytic effect [1, 2], there is also a substantial difference in the mechanism of action of muscarinic and nicotinic cholinolytics on the CNS: the former exhaust the noradrenalin (NA) reserves in the brain whereas the latter increase them [3]. The writers [4] have shown that exhaustion of the reserves of mediators in the CNS by the muscarinic cholinolytic benactyzine takes place as a result of their liberation from nerve endings and, in particular, from synaptic vesicles.

In the investigation described below the effect of the nicotinic cholinolytic adiphenine was studied on NA storage in isolated nerve endings (synaptosomes) and in synaptic vesicles.

EXPERIMENTAL METHOD

Synaptosomes and synaptic vesicles were isolated from the rat brain by Whittaker's method [5]. All sucrose solutions contained the monoamine oxidase inhibitor nialamide (10^{-4} M). The brain from six to eight animals was taken for the experiment. The isolated synaptosomes or synaptic vesicles were diluted in 10 volumes of 0.32 M sucrose and centrifuged for 15 min at 20,000 g. The residue was resuspended in Krebs–Ringer solution (10 ml) containing 0.18 M glucose to maintain isotonicity. Exogenous NA was added to the sample as a carrier (20 μg/ml), the sample was divided into two halves (control and experimental), and adiphenine solution was added to the latter up to a concentration of 50 μg/ml. The samples were incubated for 15 min at 37°C, cooled, and centrifuged for 15 min at 20,000 g. The NA concentration in the supernatant and residue was determined spectrophotometrically by the method of Euler et al. [6, 7].

EXPERIMENTAL RESULTS AND DISCUSSION

In the presence of adiphenine the NA concentration in the nerve endings was significantly increased (Table 1). Meanwhile the total NA concentration and its concentration in the incubation medium of the experimental samples were a little lower than in the control. Consequently, it can be concluded from these results that the increase in the NA concentration in the synaptosomes is attributable to the inhibitory action of adiphenine on enzymes (notably monoamine oxidase) responsible for the metabolic conversions of NA.

The effect of adiphenine on the NA concentration in the synaptic vesicles was much stronger than in the intact synaptosomes (Table 1): the NA concentration in the vesicles of the experimental samples was 1.5 times higher than in the control.

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TABLE 1. Distribution of NA in Fraction of Synaptosomes (I) or Synaptic Vesicles (II) and Incubation Medium (in μg per sample)

Fraction	[C_1] NA of incubation medium		[C_2] NA in synaptic structures	
	control	expt.	control	expt.
I				
$M \pm m$	19,4±0,29	18,3±0,21	1,66±0,02	2,01±0,03
% of control		94		121
P		<0,05		<0,001
II				
$M \pm m$	22,7±0,3	19,7±0,3	0,59±0,06	0,91±0,07
% of control		87		155
P		<0,05		<0,025

$[C_1]+[C_2]$		$K=\frac{[C_2]}{[C_1]+[C_2]} \cdot 100\%$		n
control	expt.	control	expt.	
21,1±0,32	20,3±0,25	7,8	9,9	5
	96			
	>0,05			
23,3±0,3	20,6±0,6	2,5	4,4	2
	91			
	>0,05			

Legend: n) number of experiments; [C] concentration; K) index of absorption of NA.

By contrast with the muscarinic cholinolytic benactyzine, its active analogue, the nicotinic cholinolytic adiphenine, thus does not cause the liberation of NA but increases its accumulation by the presynaptic structures. Consequently, the increase in the NA concentration in the whole brain found previously during the action of adiphenine [3] is the result of deposition of the mediator in the synaptic vesicles of nerve endings.

It follows from these experiments that adiphenine (and also, evidently, other nicotinic cholinolytics) can justifiably be regarded as a presynaptic blocking agent of adrenergic mediation in the CNS.

LITERATURE CITED

1. S. V. Anichkov, in: Textbook of Pharmacology [in Russian], Part 1, Leningrad (1961), p. 158.
2. S. G. Kuznetsov and S. N. Golikov, Synthetic Atropine-like Substances [in Russian], Leningrad (1962).
3. S. S. Krylov and E. A. Snegirev, in: Biogenic Amines in Clinical Medicine [in Russian], Moscow (1970), p. 260.
4. N. R. Elaev, M. O. Karryeva, and M. P. Podosinovikova, Farmakol. i Toksikol., 35, 632 (1972).
5. V. P. Whittaker and M. N. Sheridan, J. Neurochem., 12, 363 (1965).
6. U. S. von Euler and J. Floding, Acta Physiol. Scand., 33, Suppl. 45 (1955).
7. U. S. von Euler and S. Lishaiko, Acta Physiol. Scand., 45, 122 (1959).