in the modern view, determines the reserves of available mediator in a nerve ending. This is in agreement with the decrease in the number of synaptic vesicles observed under the influence of armin, obtained during a study of the ultrastructure of motor endings in the rat diaphragm [1].

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LUMINESCENCE-HISTOCHEMICAL STUDY OF THE EFFECT OF ATROPINE AND DIMETHOXYDICHLOROVINYL PHOSPHATE ON THE CATECHOLAMINE CONCENTRATION IN SOME RAT BRAIN STRUCTURES

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Facts indicating a close functional connection between the cholinergic and catecholaminergic mediator systems of the brain have now been obtained [2, 3, 9, 10]. However, not all aspects of this phenomenon have been equally well studied. In particular, further investigation is required into the effect of substances exciting and blocking conduction of the nervous impulse in cholinergic synapses on catecholamines (CA), for this is hindering our understanding of the mechanisms which lie at the basis of their action on the CNS.

The object of this investigation was a quantitative luminescence-histochemical study of the effect of the cholinolytic atropine and the anticholinesterase agent dimethoxydichlorovinyl phosphate (DDVP) on the content of dopamine (DA) and noradrenalin (NA) in various rat brain structures.

EXPERIMENTAL METHOD

Experiments were carried out on 40 male albino rats weighing 180-200 g. Atropine sulfate or DDVP was injected in doses of 5 and 10 mg/kg respectively, into the animals intraperitoneally. Physiological saline was injected into control rats. CA were detected by the method of Loren et al. [13], for which purpose the brain of the animal, anesthetized with hexobarbital, was perfused 30 min after the injection with 150 ml of a solution (0-4°C) of the following composition: glyoxylic acid 2%, paraform 1%, magnesium sulfate 25%, in 0.1 M phosphate buffer, pH 7.4. After the end of perfusion the brain was quickly removed and frozen in solid CO_2. Sections 30 μ thick were dried in a jet of warm air (90°C) for 5 min and mounted in polystyrene. The sections were examined in the "Luman-I" luminescence microscope (the light source was a DRSh-250-2M

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TABLE 1. Effect of Atropine and DDVP on Intensity of Luminescence (in relative units) of CA-Containing Brain Structures (M ± m)

Mediator	Structure	Control (n = 20)	Atropine (n = 10)	DDVP (n = 10)
DA	Caudate nucleus	1,00	1,39±0,13*	1,58±0,14*
	Adjacent nucleus	1,00	1,41±0,12*	1,44±0,16*
	Region A-10	1,00	0,80±0,09*	1,08±0,12
	Substantia nigra	1,00	0,64±0,06*	0,99±0,18
DA+NA	Septal nucleus	1,00	0,88±0,11	1,38±0,03*
NA	Nucleus of terminal fibers	1,00	1,28±0,14	1,11±0,18
	Locus coeruleus	1,00	0,82±0,06*	1,29±0,07*

*P < 0.05.

mercury vapor lamp). The CA content in the brain structures was estimated from the intensity of luminescence of the objects studied, which was determined by means of a microspectrophotometric attachment to the SFN-10 microscope. The results were subjected to statistical analysis by the method of correlated series, for which purpose two rats each from the control group and two subjected to the action of the drugs took part simultaneously in the experiment. A change in the CA content was expressed as a percentage of the corresponding control, taken as 100%.

EXPERIMENTAL RESULTS

Visual examination of the preparations showed bright green luminescence in structures containing CA. The structures were identified by reference to atlases of the rat brain [1, 11]. The regions of location of bodies of DA- and NA-ergic neurons (the substantia nigra, region A-10 after Dahlström and Fuxe [7], the locus coeruleus) and structures in which telodendrons of CA-ergic neurons ramify (caudate nucleus, adjacent nucleus, lateral septal nucleus, inferior nucleus of terminal fibers) were studied. Preliminary investigations showed that reserpine, injected intraperitoneally in a dose of 5 mg/kg after 24 h reduces the intensity of luminescence in the caudate and adjacent nuclei and in the nucleus of terminal fibers by 85-95%, in the septal nucleus completely, and in region A-10, the substantia nigra, and locus coeruleus by 65-75%, evidence of the specific character of the luminescence [12].

Atropine and DDVP changed the intensity of luminescence in most structures studied (Table 1).

It will be clear from Table 1 that the intensity of luminescence in the caudate and adjacent nuclei increased after injection both of atropine and of DDVP, an antagonist of its action on cholinergic structures, evidence of accumulation of DA in these structures. Atropine slightly reduced the DA concentration in the neuron bodies in region A-10 and the substantia nigra. The organophosphorus compound DDVP, in the dose used, had no effect on the DA content in these structures. Neither atropine nor DDVP caused a significant change in the NA content in the inferior nucleus of terminal fibers. Antagonistic relations were found between atropine and DDVP in their action on NA located in neuron bodies in the locus coeruleus: Atropine significantly lowered whereas DDVP increased the intensity of luminescence measured above the neurons of that structure, reflecting corresponding changes in the NA content. Reciprocal relations in the action of the muscarinic cholinolytic and anticholinesterase drug on the content of this neuromediator are probably evidence that activity of NA-ergic structures of the brain is mainly under the direct control of cholinergic systems.

In experiments with injection of both atropine and DDVP, changes in the DA content in the caudate and adjacent nuclei were in the same direction. This may be indirect evidence that interaction between cholinergic and dopaminergic mediator systems is based not on one, but on several mechanisms. In that case injection of substances with opposite actions on cholinergic structures could ultimately lead to identical changes in the DA content.

This hypothesis is supported by experimental data according to which cholinergic drugs can modify the activity of DA-ergic structures by their direct action on DA-containing neurons [5, 8, 14] and indirectly through

neuron circuits including other mediator systems, such as the GABA-ergic system [6], and they may also affect the rate of DA turnover in synapses, through a change in the acetylcholine concentration in brain structures [4] and, finally, they may interfere with the metabolism of this neurotransmitter [3].

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