EFFECT OF ADENOSINE ON CYCLIC NUCLEOTIDE LEVELS IN BRAIN STRUCTURES

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Many recent investigations have established the important role of adenosine in the regulation of various systems of the body: the heart, brain, lymphocytes, mast cells, and so on. Most adenosine in the brain is contained in the neurons, and it is considered to act as a neuromodulator and neurotransmitter in the mammalian CNS [2, 6, 8].

It has been shown that adenosine, acting on specific nerve cell receptors, can inhibit activation of neurons predominantly by means of a presynaptic mechanism, by inhibiting the release of other neurotransmitters: noradrenalin, acetylcholine, dopamine, GABA, etc. The inhibitory influence of adenosine on neurotransmitter secretion has been linked with blockade of calcium entry into synaptosomes [5, 7]. It was shown in 1979 [3] that adenosine and its analogs can modulate adenylate cyclase activity and thus change the cAMP level in neurons. During the last few years it has been shown with the aid of radio-ligands that adenosine influences adenylate cyclase through at least two subtypes of receptors: through A_1 it inhibits adenylate cyclase, and through A_2 it stimulates it [6, 9]. The highest density of adenosine receptors is found in the cerebellum, hippocampus, cortex, and subthalamic nuclei. However, there is no information in the literature on the action of adenosine on guanylate cyclase or on cGMP metabolism in nerve tissue.

The aim of this investigation was to study the effect of adenosine on cAMP and cGMP levels in the cerebral cortex, hippocampus, hypothalamus, and cerebellum.

EXPERIMENTAL METHOD

Experiments were carried out on 96 male Wistar rats weighing 200-220 g. Thirty minutes before decapitation the animals were given an intramuscular injection of the test preparations (in near-physiological concentrations): adenosine in doses of 0.5 and 5 mg/kg (Reanal, Hungary), dipiridamol in a dose of 0.05 mg/kg (Germed, East Germany), and their combinations. In the control series the rats were given an injection of physiological saline. The brain was removed from the decapitated animals and the frontal cortex, hippocampus, hypothalamus, and cerebellum were isolated and frozen immediately in liquid nitrogen. Proteins were precipitated by 6% TCA. After centrifugation at 2500 rpm the TCA was removed from the supernatant by threefold extraction with water-saturated ether. Concentrations of cyclic nucleotides in the samples were determined by the competitive binding method using test kits from "Amersham International" (England) and expressed in picomoles/mg wet weight of brain tissue.

EXPERIMENTAL RESULTS

On intraperitoneal injection of adenosine in doses of 0.5 and 5 mg/kg it was found to raise the cAMP level in the cerebral cortex and hippocampus. Meanwhile, in a dose of 5 mg/kg, it lowered the cAMP level in the cerebellum. Dipiridamol, a blocker of adenosine reuptake, in a dose of 0.05 mg/kg, combined with adenosine, led to an increase in the cyclic nucleotide concentration in the cortex, hippocampus, and hypothalamus. In the cerebellum, however, only ipiridamol alone raised the cAMP level (Table 1; Fig. 1). The results thus show that exogenous adenosine alters the cAMP concentration in the brain. The fact that adenosine differed in its effect on the cAMP concentration in the different CNS structures is evidently explained by the presence of different types of adenosine receptors on them, a fact confirmed by data in the literature [2, 6, 8].

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TABLE 1. Effect of Adenosine on Cyclic Nucleotide Levels in Brain Structures (in pmoles/mg tissue; M ± m)

Preparation	Dose, mg/kg	Number of experiments	cAMP				cGMP			
			cortex	hippocampus	hypothalamus	cerebellum	cortex	hippocampus	hypothalamus	cerebellum
Control	—	16	1,9±0,4	1,9±0,14	2,8±0,23	8,1±1,5	0,09±0,02	0,02±0,01	0,04±0,007	0,3±0,06
Adenosine	0,5	16	4,4±0,4*	3,7±0,6*	2,4±0,2	10,9±3,5	0,045±0,008*	0,09±0,03*	0,026±0,005*	0,25±0,03
	5	16	3,7±0,9*	3,0±0,4*	3,3±0,9	5,5±2,5*	0,06±0,006*	0,10±0,03*	0,04±0,004	0,26±0,01
Adenosine + dipiridamol	0,5	16	6,0±1,6*	6,2±1,9*	4,2±1,7*	9,0±2,0	0,09±0,02	0,13±0,01*	0,05±0,006	0,28±0,05
	5	16	4,9±1,9*	3,1±0,6*	3,3±0,9	11,3±3,6	0,16±0,04*	0,06±0,01*	0,07±0,01	0,22±0,04
Dipiridamol	0,05	16	5,8±0,3*	3,5±0,6*	3,2±0,3	12,8±3,4*	0,09±0,02	0,18±0,02*	0,02±0,003*	0,28±0,04

Legend. *p < 0.05 compared with control.

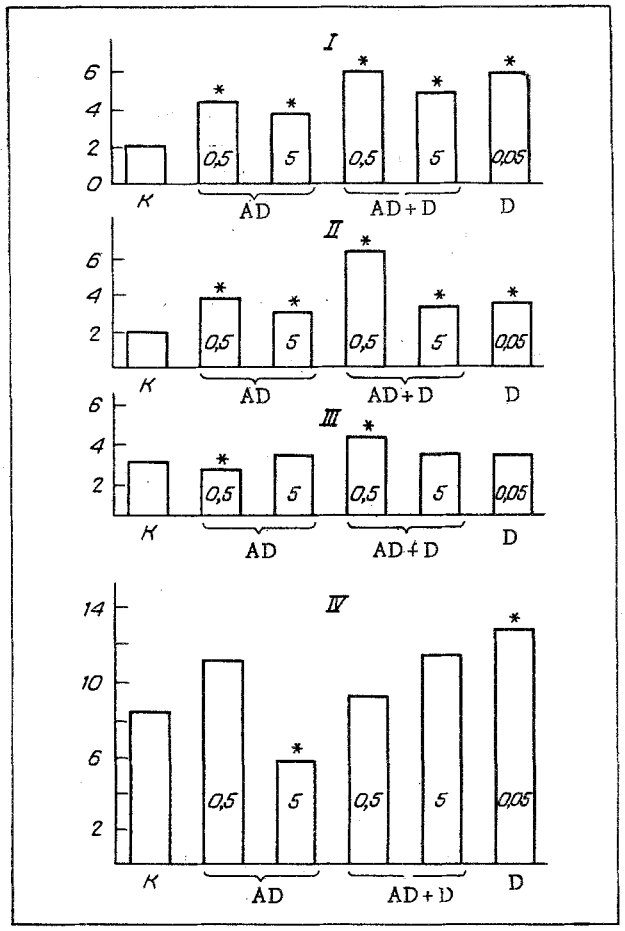

Fig. 1. Effect of adenosine on cAMP level in brain structures. Ordinate, cAMP level (picomoles/mg wet weight of tissue). I) Cerebral cortex, II) hippocampus, III) hypothalamus, IV) cerebellum, K) control; AD) adenosine (0.5 and 5 mg/kg); D) dipiridamol (0.5 mg/kg). *p < 0.05 compared with control.

Investigation of the effect of adenosine on the cGMP level showed that in both doses tested it lowers the level of this cyclic nucleotide in the cerebral cortex. Dipiridamol had no effect, although when given together with adenosine, it had the opposite effect: the cGMP level was significantly raised compared with that following injection of adenosine alone or dipiridamol alone. The effect of these preparations on the cGMP concentration in the hippocampus was expressed as a sharp increase in the cyclic nucleotide concentration, especially under the influence of dipiridamol (Table 1).

The cGMP concentration in the hypothalamic structures was reduced after injection of adenosine in a dose of 0.5 mg/kg. Dpiridamol had the same effect. Adenosine, in a dose of 5 mg/kg, did not affect the level of this nucleotide in the hypothalamus, whereas combined

injection of the preparations had the opposite effect, similar to that observed in the cerebral cortex. In the cerebellum, no significant effect on the cGMP level was discovered by either of the preparations studied. There was only a tendency for the level of this nucleotide to fall under the influence of adenosine. It may be that adenosine influences the bioavailability of calcium through a change in the activity of the guanylate-cyclase system [7].

The experiments showed that cAMP and cGMP at the level of the cerebral cortex have reciprocal relationships with one another, whereas in the hippocampus, hypothalamus, and cerebellum, this is not observed. Adenosine is known to be metabolized quite rapidly in the body by the enzyme adenosine deaminase to inosine and hypoxanthine. Since inosine is a ligand of benzodiazepine receptors [1, 4], and can also bind with adenosine receptors [7, 8], it can be tentatively suggested that the changes observed in the cyclic nucleotide levels are connected with the action both of adenosine itself and of its metabolites.

Thus adenosine, by modifying the activity of the cyclase systems, can interfere with the fine mechanisms of functioning of neuron populations.

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INCREASED Na^+/H^+ EXCHANGE IN ERYTHROCYTES OF HYPERTENSIVE PATIENTS

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In the mid-1970s the first data were obtained to show an increase in passive (i.e., unconnected with Na, K-ATPase function) permeability of the erythrocyte membrane of hypertensive patients [14] and spontaneously hypertensive rats (SHR) [6] for monovalent cations. These observations have now been repeated in several laboratories and they are associated with an increase in the rate of translocation of the following carrier ions: 1) Na^+,K-cotransport (furosemide-inhibited component of transmembrane Na^+ and K^+ transfer); 2) Na^+/Na^+ antitransport, which is activated 2-4-fold by substitution of Li^+ for Na^+, and which is accordingly calculated as the difference between the rate of Li^+ outflow into medium containing and not containing Na^+ [7].

Comparatively recently an Na^+/H^+ antitransport (exchange) system responsible for regulation of the intracellular pH, and blocked by amiloride and its derivative [8], has been identified in the plasma membranes of cells of a number of tissues. It has been shown that the rate of working of this transmitter is increased many times over under the influence of compounds causing activation of protein kinases of tyrosine type (growth factors) or a phosphoinositide response (α-adrenoreceptor agonists, some peptide hormones), and it is accompanied by alkalification of the cytoplasm and in some cases, by DNA duplication and tissue proliferation [11].

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