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Protective Effect of Adenosine in Total Brain Ischemia

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Adenosine has been shown to be an effective antihypoxic drug in experimental hypercapnic [1-4], hypobaric [1-3,15], hemic, and tissue hypoxia. However, clinicians are most interested in local hypoxia caused by blood flow impairment (especially cerebral and cardiac) [6]. Some adenosine analogs have protective effect in brain ischemia [8]; however, this effect is not found in hypertensive animals [13]. The effects of adenosine agonists on brain blood flow are varied [8,10].

To investigate the effects of adenosine and other substances, total brain ischemia in decapitation was chosen as a study model and used first to study hypoxia biochemistry [9] and then antihypoxic drug effects [7,11,12]. The duration of gasping is considered to be dependent on the respiratory center neuronal status rather than on brain blood flow[7]. This model made it possible to demonstrate that adenosine is an effective brain-protective drug in total brain ischemia, superior to other agents studied earlier.

MATERIAL AND METHODS

The experiments were conducted on white mice weighting 18 to 23 g. The following drugs were used: nimodipine (Bayer Leverkusen, Germany), haloperidol and cavinton (Gedeon Richter, Hungary), piracetam (Polfa, Poland), ethomersol (Organic Chemistry Institute, Kiev), γ-hydroxybutyrate (Pharmacological Institute, Russian Academy of Medical Sciences), 2'-deoxyadenosine (Sigma, USA), pharmacopeic phenazepam (Russia), and other drugs (Reanal, Hungary). Most of the drugs were injected subcutaneously in aqueous solution; cavinton, dissolved in 2% Twin solution, was injected intraperitoneally. The dose used was 10 ml/kg. After decapitation, gasping duration was recorded by stopwatch until the mouth did not open. Since gasping timing patterns were unknown, the results were evaluated simultaneously according to both the conventional F, t, and d tests and the nonparametric U test of Wilcoxson-Mann-Whitney.

RESULTS

Within 30 min to 1 h after injection adenosine markedly increased gasping duration, the maximum value being observed 1 h following injection (the average increase was 33.7 sec. or 192% compared to the control, Table1). Other nucleosides and nucleotides were also studied. 2'-deoxyadenoside and ITP did not show any protective

Table 1. Effect of Nucleosides and Nucleotides on Gasping time in Mice (M±m)

Agents 1,12 mmol/kg 60 min before ischemia induction	Effect, sec	Range, sec	n	Compared to control		Compared to adenosine	
				Pt(d)	Pu	Pt(d)	Pu
Control	17,5±0,3	14-24	59	-	-	-	-
Adenosine	51,2±1,1	44-60	19	<<0,001	<<0,001	-	-
2'-deoxyadenoside	22,9±3,50	15-35	5	>0,2	>0,2	<<0,001	<0,001
Inosine	25,5±3,0	18-38	6	<0,05	<0,002	<<0,001	<0,001
5'-AMP	44,2±3,8	37-58	5	<0,01	<0,001	<0,05	<0,1
ADP	46,2±3,8	38-54	5	<0,001	<0,001	<0,1	<0,05
ATP	36,6±2,0	32-42	5	<0,001	<0,001	<<0,001	<0,001
ITP	16,3±1,3	14-19	6	>0,4	>>0,2	<<0,001	<0,001

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TABLE 2. Effect of various drugs on gasping time in mice (M±m)

Drugs	Dose, mg/kg	Time of injection min	Effect, sec	Range sec	n	Compared to control		Compared to adenosine	
						Pt(d)	Pu	Pt(d)	Pu
Control	-	30-90	17,5±0,3	14-24	59	-	-	-	-
A. Increasing brain blood flow:									
Cavinton	5	30	16,8±0,4	16-18	5	>0,4	>>0,2	<<0,001	<0,001
Flunarisine	50	30	19,6±0,5	18-21	5	<0,05	<0,1	<<0,001	<0,001
		90	18,4±0,5	18-20	5	>0,3	>0,2	<<0,001	<0,001
Nimodipine	0,1	30	15,2±0,7	13-17	5	<0,02	<0,05	<<0,001	<0,001
	1	90	18,0±1,1	15-20	4	>0,6	>>0,2	<<0,001	<0,001
B. Decreasing brain blood flow:									
Phenazepam	1	30	29,6±1,7	27-33	5	<0,01	<<0,001	<<0,001	<0,001
Haloperidol	1	30	19,2±1,4	15-23	5	<0,1	0,2	<<0,001	<0,001
	5	30	36,8±3,0	30-46	5	<0,01	<<0,001	<<0,001	<0,002
gamma-hydroxybutyrate	300	30	29,8±1,4	27-35	5	<0,01	<<0,001	<<0,001	<0,001
	1000	30	44,0±2,2	39-50	5	<<0,001	<<0,001	<0,01	0,01
		180	36,7±4,5	25-55	6	<0,01	<<0,001	<0,05	<0,01
	2000	180	41,8±2,0	35-46	5	<<0,001	<<0,001	<0,001	<0,01
C. Other drugs:									
Piracetam	500	30	17,0±0,8	14-20	8	>0,4	>>0,2	<<0,001	<<0,001
		90	17,0±0,3	16-18	5	>0,2	>>0,2	<<0,001	<0,001
	1000	120	18,5±0,6	17-20	4	>0,3	>>0,2	<<0,001	<0,002
Ethomersol	50	30	18,5±0,6	15-22	10	>0,1	>>0,2	<<0,001	<<0,001
		90	17,8±1,2	15-22	5	>0,1	>>0,2	<<0,001	<0,001

activity, inosine was 4 times active as adenosine while adenosine phosphates showed a lower protective effect (57-85% of the adenosine effect).

Two possible explanation of the findings could be offered: 1) the adenosine derivatives are the "raw material" for cellular macroerg synthesis, 2) the effect is mediated through specific receptors. The first hypothesis is not consistent with the marked differences found in the protective activity of adenine compounds, adenosine and adenosine phosphates being the only compouns with high protective effect, whereas inosine (riboxin), used for macroerg synthesis in the cell and for this reason employed in medical practice, has been shown to be virtually ineffective. The second hypothesis supports the experimental findings. There are known to be adenosine A-receptors, ATP P-receptors (both are found on the outer surface of the plasma membrane), and P-sites on the inside of the plasma membrane [5,14]. P-receptor involvement is hardly possible because of lower ATP protective effect (by 43%) compared to adenosine. P-site involvement is ruled out because of the total inactivity of 2'-deoxyadenosine [5,14]. The maximum protective effect of adenosine suggest A-receptor involvement. It is common knowledge that adenosine phosphates are rapidly dephosphorylated in the body to adenosine, which accounts for many of their effects [14].

For comparison we studied the protective effect of 8 other substances using other brain ischemia models [6,11,12]. It is obvious that the drug increasing brain blood flow (cavinton, nimodipine in a dosage of 1 mg/kg) as well as piracetam and ethomersol do not increase gasping duration, while nimodipine in a dose of 0.1 mg/kg even somewhat decreases gasping duration (by 13%). The slight protective effect of flunarisine (+12%) is significant according to the t but not the U test if administered 30 minutes before brain ischemia is induced; however, this effect is not observed when the drug is injected 90 min before ischemia is induced.

Unlike the above drugs, such agents as phenazepam, haloperidol, and hydroxybutyrates (decreasing brain blood flow, especially narcotic doses of the two last-mentioned drugs) do show a protective effect, but apparently lower one as compared to adenosine (the maximum values being 69, 110, and 151%, respectively, compared to the control value, or 36, 57, and 79% of the adenosine effect). The adenosine cerebroprotective effect is at least 2 to 4 times as high as that

of 30 various drugs were studied earlier by using the gasping model. The most effective drugs, such as high-dose amitriptiline, pentobarbital, diazepam, etc. (those which decrease locomotor activity [7]) increased gasping time only by 36-74% (8-16 sec.) [7,11,12]. The evidence suggests that adenosine and its analogs can be regarded as most promising cerebroprotective drugs in brain ischemia.

The effect of adenosine is probably to be attributed to a decrease of neuron activity due to reduced O_2 consumption and body temperature drop [3] and/or due to the inhibited release of excitatory amino acids [8] and other neurohormones, such as catecholamines.

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Protective Effect of 2,3-Butanedione Monoxime on the Myocardial Ischemia in Rats

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The recovery of heart pump function after cardioplegic arrest depends considerably on the degree of preservation of high-energy phosphates in the myocardial tissue. One of the ways to protect the ischemized myocardium consists in the reduction of cell ATPase activity. In this respect 2,3-butanedione monoxime (BDM) may be of the great value due to its ability to reduce the sensitivity of contractile proteins to Ca-ions as well as its direct inhibitory effect on the myosin-actin interaction [1,2,6,7]. BDM has been shown to be able to slow considerably the reduction of the content of high-energy phosphates in the rat heart under conditions of hypoxia [10].

Table 1. ATPase Activity of Myocytes (μmole P_i/min·μg protein) Isolated from Myocardial Bioptates in Control and in Experiments with BDM (M±m)

Conditions	before cardioplegia		after reperfusion	
	Ca, Mg-ATPase	Mg-ATPase	Ca, Mg-ATPase	Mg-ATPase
control n=6	0,11±0,01	0,05±0,00	0,12±0,03	0,06±0,01
BDM n=4	0,12±0,01	0,05±0,00	0,12±0,00	0,05±0,00

The purpose of the present work was to determine whether BDM possesses

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