PHARMACOLOGY

Protective Effect of Intracerebroventricular Injection of Adenosine Agonists during Total Cerebral Ischemia

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Adenosine and its analogs N^6-cyclohexyladenosine (CHA) and N-ethylcarboxamideadenosine (NECA) are highly effective when injected into the lateral ventricle in very low doses (by 1-2 orders lower than subcutaneously). The protective effect of A-agonists is considered to be mediated by a central mechanism and realized via A-receptors of the brain: the effects of low doses of A-agonists are mediated by A_1-receptors, whereas higher doses exert an extra A_2-effect.

Key Words: *A-receptor agonists; antihypoxants; cerebral ischemia*

Adenosine and other A-agonists are characterized by a high cerebroprotective effect (CPE) in cerebral ischemia, as has been shown in experimental occlusion of cerebral arteries, in *in vitro* reproduction of neuronal ischemia [8,11,13,14], and in total ischemia of the brain [3,4], in which CPE was due not to an increase in the cerebral bloodflow, but rather to protection of the neurons themselves [3]. Similar results were observed in other models [8,13]. This is in line with the data on a direct effect of A-agonists on the brain, reducing its activity [2,6,8,11,13]. However, the hypothesis on the central mechanism of A-agonist CPE is still to be directly confirmed in experiments [10].

We studied A-agonist CPE for intracerebroventricular injection and compared it with that for subcutaneous injection.

MATERIALS AND METHODS

Experiments were carried out with 255 white mice of both sexes weighing 18-20 g. Adenosine manufactured by Reanal (Hungary), CHA synthesized in the laboratory of A. M. Yurkevich (Vitaminy Research and Production Amalgamation, Moscow), and NECA synthesized in the laboratory of M. Yu. Lidak (Institute of Organic Synthesis, Riga, Latvia) were used. The agents were injected into the right lateral cerebral ventricle in the following manner: the skin was cut from the upper part of the head and a microsyringe needle was inserted to a depth of 3 mm at a point 1-2 mm to the right of the chiasma of the cranial; 10 μl of solution was then injected (the method was recommended by N. K. Popova and I. P. Voronova). The adequacy of the method was repeatedly confirmed by injection of stain. The duration of gasping (agonal respiration) after decapitation was an indicator of brain resistance to total ischemia [3,4]. Since gasping duration did not conform to the normal distribution [4], the results were analyzed using the nonparametric Wilcoxon-Mann-Whitney *U* test. ED_{50} was assessed by probit analysis after Litchfield-Wilcoxon-Roth [1].

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RESULTS

CPE of all three A-agonists increased in the course of the first hour postinjection (Fig. 1). Adenosine

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activity then noticeably decreased, whereas CHA CPE remained stable for the next 3 h. The time course of NECA CPE was more intricate: at low doses (3.2-16 nmol/kg) it was maintained at the same level for 1 to 6 h, whereas at higher doses (32-96 nmol/kg) the maximal effect was observed 3 h after injection, which then moderately decreased over 6 h (Fig. 1). On the whole the time course of A-agonist CPE, when injected intracerebroventricularly (the present study) and subcutaneously [4] is similar: a brief effect of adenosine, a longer one of CHA, and the longest with a delayed peak of NECA. However, intracerebroventricular injection of adenosine analogs attests to an approximately twice as rapid development and an even more rapid abolishment of CPE. This appears to be due to elimination of the slow process of A-agonist penetration and accumulation in the brain.

The dose curves of all three A-agonists reflect saturation, which is attained for adenosine at 19 μmol/kg, for CHA at 18 nmol/kg, and for NECA at 16 nmol/kg in an hour and at 32 nmol/kg (at a higher level) in 3 h (Fig. 2). It is evident that adenosine is much inferior to CHA and NECA in activity (its dose curve is shifted to the right by 3 orders). These differences seem to be due to a more rapid metabolism of adenosine and to the metabolic resistance of CHA and NECA [2,6,8]. The comparative efficacies of CHA and NECA are dose-dependent. At lower doses (4.5 to 10 nmol/kg) CHA activity is 1.5 times higher ($p<0.05$-0.01, Fig. 2) and, hence, the ED_{50} value for CHA is 3.2 times lower than for NECA (Table 1). At higher doses (32-96 nmol/kg) the NECA effect increases and is 1.5 times higher than that of CHA ($p=0.01$). This manifests itself when NECA is injected 3 h (maximal effect) but not 1 h before total ischemia (Figs. 1 and 2).

Intracerebroventricular injection is much more effective than subcutaneous injection: the dose curves are markedly shifted to the left for all three A-agonists (Fig. 2) and equally effective doses are 1-2 orders lower. ED_{50} values for subcutaneous injection are 190 times higher for CHA and 13 times higher for NECA compared with the doses for intracerebroventricular injection (Table 1).

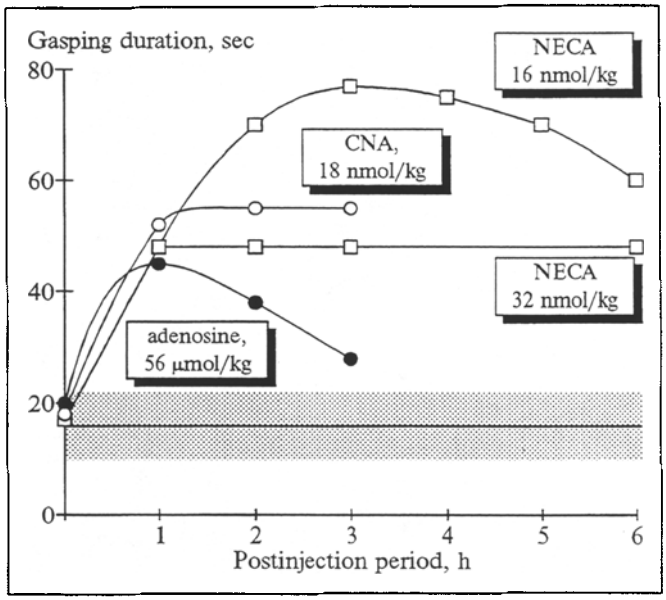

Fig. 1. Time course of cerebroprotective effect of A-agonists for intracerebroventricular injection. Here and in Fig. 2: the horizontal line and hatched zone show the mean value and range of fluctuations in the duration of gasping in the control.

Previously we demonstrated that the substances increasing the cerebral bloodflow do not affect gasping duration, and that agents reducing cerebral activity protect the brain; this gave rise to the idea that CPE of A-agonists has to do with a direct impact on the brain [3]. The much higher activity of A-agonists for intracerebroventricular injection confirms this hypothesis.

The more marked decrease of CHA ED_{50} in comparison with the NECA dose for intracerebroventricular administration in comparison with subcutaneous injection (Table 1) is evidence of a lesser penetration of CHA across the blood-brain barrier. This is in line with the direct evidence of a much lesser penetration into the brain of N6-phenylisopropyladenosine, a close chemical and pharmacological CHA analog [2,6,8], in comparison with NECA [7,12]. Evidently, the higher activity of NECA in comparison with CHA for subcutaneous injection (Fig. 2) is due not to a more significant role of the A_2-receptors, as we previously believed [4], but to their easier penetration into the brain. Another factor may be a greater

TABLE 1. ED_{50} (nmol/kg) of A-Agonists for Various Routes of Administration

Injection	CHA	NECA	NECA/CHA ratio	p
Intracerebroventricular (1)	5.5±1.5	17.8±3.9	3.2	<0.01
Subcutaneous (2)	1047±330	237±62	0.23	<0.05
(2)/(1) ratio	190	13.3		
p	<0.005	=0.001		

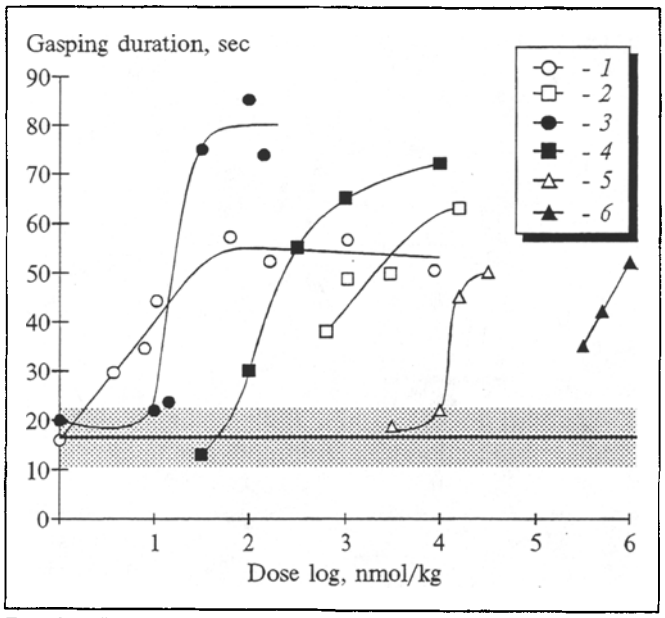

Fig. 2. Dose curves of cerebroprotective effect of A-agonists for intracerebroventricular (icv) and subcutaneous (sc) injection. CHA in 1 h: icv (*1*) and sc (*2*); NECA in 3 h: icv (*3*) and sc (*4*); adenosine in 1 h: icv (*5*) and sc (*6*).

significance for NECA of the peripheral mechanisms, particularly a drop of arterial pressure [8,13]. A higher CPE of low doses of CHA (Fig. 2) and lower ED_{50} values (Table 1) are clearly observed for intracerebroventricular injection, which rules out differences in penetrability of the blood-brain barrier and the peripheral effect. The high A_1-selectivity of CHA [2,6,8,13] permits us suppose that CPE of low A-agonist doses is realized through the A_1-receptors of brain neurons. This agrees with the fact that the majority of direct effects on brain neurons are realized via the A_1-receptors [13]. However, for injection of higher doses it is NECA that proves to be more active (Fig. 2). This may be due to another mechanism of CPE, namely, A_2-receptor stimulation, because CHA is much less affine to them, whereas NECA is affine to them similarly as to A_1 or even more [2,6,8,13].

The different order of CHA and NECA activities for subcutaneous and intracerebroventricular injection necessitates very careful interpretation of experiments with peripheral administration of A-agonists. Neither we [4,5] nor many other authors (e.g. [9]) took this into consideration before.

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