60. C. P. Slichter, Principles of Magnetic Resonance, New York (1963), pp. 64-81.
61. T. S. Stone, T. Buckman, P. L. Nordio, et al., Proc. Natl. Acad. Sci. USA, 54, 1010 (1965).
62. V. Tauber, H.-J. Wemman, M. Panzer, et al., Arzneimittel.-Forsch, 36, 1089 (1986).
63. M. S. Taveras, Invest. Radiol., 19, 247 (1984).
64. P. Thevenin, Neuroradiology, 24, 133 (1983).
65. H. S. Weinman, R. C. Brasch, W. R. Press, et al., Lancet, 1, 484 (1984).
66. H. S. Weinman, R. C. Brasch, W. R. Press, et al., Am. J. Roentgenol., 142, 619 (1984).
67. G. E. Wesbey, R. C. Brasch, and B. L. Engelstad, Radiology, 149, 175 (1983).
68. D. J. Widder, W. L. Greif, K. J. Widder, et al., Radiology, 151, 44 (1985).
69. G. L. Wolf and E. S. Fobben, Invest. Radiol., 19, 324 (1984).
70. G. L. Wolf, K. R. Burnett, E. S. Goldstein, and P. M. Joseph, Contrast Agents for Magnetic Resonance Analysis, New York (1985), pp. 231-266.

SYNTHESIS OF N^6-CYCLOHEXYLADENOSINE AND INVESTIGATION OF ITS PHARMACOLOGICAL PROPERTIES IN COMPARISON WITH ADENOSINE

V. V. Eliseev, O. V. Travkin,
N. O. Chistyakova, A. G. Ovchinnikova,
and V. M. Prokopenko

UDC 615.22:547.857].012.1
+615.22:547.857].015.4

Adenosine plays an important part in the regulation of the cardiovascular system. Attempts have been made to use this compound as a hypotensive and antiadrenergic drug [3, 5, 8]. The capacity of adenosine to slow down the atrioventricular conduction and to produce dilatation of peripheral vessels is caused by its interaction with A_1 and A_2 purine receptors. The effect of exogenous adenosine is extremely short-lasting as a result of its rapid conversion to inosine under the influence of plasma and tissue adenosine deaminase (ADA). For the study of purine receptors use is made of synthetic ligands that are resistant to the action of ADA and have high affinity for the receptor structure. In carrying out the pharmacological analysis, N^6-cyclohexyladenosine (CHA) is very often used.

The purpose of this study is to investigate the influence of CHA on the arterial pressure (AP) and the heart rate (HR) in rats in the cases of intravenous and peroral administration. The effect of CHA is compared with that of adenosine.

EXPERIMENTAL (CHEMICAL)

For the preparation of CHA, 0.7 g (0.0058 mole) of cyclohexylamine hydrochloride is added to a mixture consisting of 5 ml 40% sodium hydroxide and 10 ml of chloroform. The chloroform layer is separated, dried over a mixture of waterfree magnesium sulfate and the base for two days, and filtered. To the filtrate is added 10 ml of dry DMF, 2 ml of dry triethylamine, and 1 g (0.0035 mole) of 6-chloropurine riboside [6]. The mixture obtained is stirred at 35-40°C for 36 h and then evaporated under vacuum at 40-45°C. The oily residue is dissolved in 10 ml of acetone and 80 ml of dry ether is added. The ethereal solvent is decanted, to the residue is added 20 ml of absolute ethanol, and the mixture is set aside at 0-3°C for 15 h. The precipitated crystals of CHA are washed with cold absolute alcohol, then with dry ether, dried under vacuum at room temperature, and recrystallized from 95% ethanol.

Calculated and found elemental values for C, H, and N did not differ more than 0.3%. Mass spectrum. M^+ 349 (7.5%). PMR spectrum (Tesla-80, DMSO-d6, internal standard TMS): C_2H 8.45 ppm (s), C_8H 8.2 ppm (s), H-I^1 5.95 ppm (d), CH_2 2.0-1.4 ppm (m)..

Laboratory of Organic Synthesis (L. E. Alekseeva, director) and Laboratory of Pharmacology (V. V. Eliseev, director) of the All-Union Scientific Research Technological Institute of Antibiotics and Medically Prescribed Enzymes, Leningrad. Translated from Khimiko-farmatsevticheskii Zhurnal, Vol. 23, No. 5, pp. 549-551, May, 1989. Original article submitted March 22, 1988.

0091-150X/89/2305-0369 $12.50

TABLE 1. Hydrolysis of Adenosine and CHA under the Influence of ADA and the Inhibitory Effect of CHA

Compound	Hydrolysis of the substrates		Inhibitory effect	
	Δ E	% hydrolysis	Δ E	% hydrolysis
Adenosine	0,282	100	0,282	0
CHA	0	0	0,255	9,6

EXPERIMENTAL (BIOLOGICAL)

Experiments were carried out with 110 male mongrel white rats weighing 160-180 g. The animals were narcotized with nembutal (50 mg/kg intraperitoneally). CHA and adenosine (product of the firm Reanal, Hungary) were administered into a femoral vein through a cannula or perorally through a gastric tube. CHA was dissolved in 0.02 ml of DMSO and distilled water was added to a volume of 0.2 ml (in case of intravenous administration) or to 0.5 ml (in case of peroral administration). In control experiments the solvent did not influence the parameters of hemodynamics. Adenosine was dissolved in 0.2 and 0.5 ml of water, respectively. The doses of CHA were 3, 10, 30, and 100 μg/kg in case of intravenous administration and 1, 10, 25, 50, 75, and 100 μg/kg in case of peroral administration. The doses of adenosine were: 0.1, 0.25, 2.5, and 5 mg/kg in case of intravenous administration and 25, 50, 100, and 500 mg/kg in case of peroral administration. Each group consisted of 6-7 animals. During the experiment the average AP (AP_{av}) in the femoral vein and the HR were measured periodically. The animals were observed 60 min after the administration of CHA, 5 min after intravenous administration of adenosine, and 180 min after peroral administration of adenosine. According to method [1] were determined the ED_{25} and ED_{50}, causing, respectively, 25 and 50% change in AP_{av} and HR. We studied the possible conversion of adenosine and CHA under the action of ADA that had been isolated from rat hearts, and also the inhibitory action of CHA on the activity of ADA [9]. The reaction mixture contained 0.6 ml of 0.25 M glycine–glycinate buffer (pH 7.4), 0.1 ml of extract of the rat myocardium (heart chambers were homogenized in 0.25 M saccharose solution and centrifuged at 150,000g), and 2.3 ml of distilled water. After equilibrating at 22.5°C, to the mixture was added 0.1 ml of 0.002 M adenosine or CHA. The activity of ADA was judged by the degree of lowering of the absorption at 265 nm over a period of 10 min. To assess the inhibitory action of CHA on ADA we carried out a preliminary incubation of CHA (10^{-5} mole) with tissue extract for 5 min. As substrate for the deamination reaction we used adenosine. The obtained data were statistically processed with Student's t test.

Intravenous administration of adenosine to rats led to a lowering of AP_{av} of short duration and to a decrease in HR. The largest effect was noticed 5-15 sec after administering the compound at a dose of 2.5 mg/kg. AP_{av} was lowered by 72 ± 2% relative to its starting value and HR by 82 ± 2%. Increasing the dose did not lead to an increase in effect. Fifty percent lowering of AP_{av} and HR was attained with an $ED_{50}AP$ of 1.03 mg/kg and $ED_{50}HR$ of 1.00 mg/kg, respectively. By the 5th minute of AP_{av} and HR were normalized. Peroral administration of adenosine led to moderate lowering of AP_{av} during the 45th-180th minute. The maximal effect was found in the 90th minute at a dose of 100 mg/kg: AP_{av} was lowered by 37 ± 9%. Increasing the dose did not cause hypotonia. The effect lasted till the 160th minute. $ED_{50}AP$ was 75 mg/kg. During the whole period of observations, HR did not change for certain at all doses investigated.

The effect of CHA surpassed that of adenosine. On intravenous administration of the compound the largest effect was noticed at the 15th-30th second. The action of the preparation lasted for the whole observation period (60 min). $ED_{50}AP$ and $ED_{50}HR$ were 22 and 12 μg/kg, respectively. On peroral administration a fast, long-lasting but less pronounced effect was observed. A reliable change in HR already occurred in the 5th minute after administration and in AP_{av} in the 15th minute. The maximal change was noticed at a dose of 75 μg/kg; the decrease in AP_{av} in the 30th minute was 30 ± 7% and in HR 54 ± 5%. $ED_{25}AP$ and $ED_{25}HR$ were 49 and 27 μg/kg. The effect of the preparation lasted till the end of observation, 180 min.

During the biochemical evaluation of the compounds as substrates and inhibitors of ADA it was found that CHA, as opposed to adenosine, is stable to deamination (see Table 1). And what is more, at a concentration of 10^{-5} M it partially inhibits ADA.

According to modern ideas, the negative chronotropic and vasodilator effects are caused by their interaction with the A_1 purine receptors of the atrium and the A_2 receptors of the vascular system, respectively [2, 4, 7]. These receptors differ by their sensitivity to various agonists. Thus, CHA and 1-N^6-phenylisopropyladenosine show large affinity for A_1 receptors, and 5'-N-ethylcarboxamidoadenosine for A_2 receptors. As regards adenosine, the sensitivity of both types of receptors is roughly the same [7]. In our experiments CHA showed a larger influence on HR than on AP_{av}, which points to its larger affinity for A_1 receptors and confirms the data obtained by using the method of radioactive ligands. Apparently, CHA is already rapidly and well absorbed in the stomach and its effect is lasting and is reached with small doses. The action of adenosine is found only in the case of intravenous administration, the effect of the compound is of short duration, and is found at doses that are 45-80 times as large.

The hypotensive effect on peroral administration of adenosine manifests itself at a dose 140 times as large as for CHA. The negative chronotropic effect was not found with this method of administration. This is possibly explained by the fact that adenosine is completely metabolized in the gastrointestinal tract and the liver and that its metabolites, for example, inosine, possess the hypotensive activity. It is known that the latter produces a lower hypotensive effect and does not change HR. In our opinion the data obtained point to the advisability of searching for novel medicines among adenosine derivatives.

LITERATURE CITED

1. M. L. Belen'kii, Elements of the Quantitative Evaluation of a Pharmacological Effect [in Russian], 2nd edn., Leningrad (1963).
2. V. V. Eliseev, Khim-farm. Zh., 21, No. 8, 910-919 (1987).
3. R. M. Berne, J. P. DiMarco, and L. Belardinelli, Circulation, 69, 1195-1197 (1984).
4. D. B. Evans, J. A. Schender, and J. A. Bristol, Life Sci., 31, 2425-2432 (1982).
5. A. F. Fukunaga, W. E. Flacke, and B. C. Bloor, Anest. Analg., 61, 273-278 (1982).
6. J. F. Gerster, J. W. Jones, and R. A. Robins, J. Org. Chem., 28, 945-948 (1963).
7. S. J. Haleen and D. B. Evans, Life Sci., 36, 127-137 (1985).
8. A. Sollevi et al., Anesthesiology, 59, 9 (1983).
9. C. Warter et al., J. Biol. Chem., 195, 161-166 (1953).