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SHORT COMMUNICATIONS

Inhibition of gamma-aminobutyric acid transaminase with 6-aminonicotinamide in regions of the rat brain

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6-Aminonicotinamide (6-AN) is a potent nicotinamide antagonist and its derivatives are pharmacologically very active in the brain [1, 2]. After administration of 6-AN, remarkable neurological symptoms are observed. The most striking effect is a spastic paralysis which affects mainly the hind limbs [1, 3]. It is not known why 6-AN damages the central nervous system, and the total number of enzymes inhibited by it is also unknown.

Studies have been carried out to analyze the biochemical basis of the neurotoxicity of 6-AN [4]. There is a 400-fold accumulation of 6-phosphogluconate in the central nervous system after 6-AN treatment producing a blockade in the pentose phosphate pathway [2]. Furthermore, the large increase in 6-phosphogluconate in the central nervous system of rats and mice after 6-AN administration leads to an inhibition of glucose-phosphate isomerase and a decrease in the lactate concentration as compared to the untreated controls and, consequently, to a reduction of the glycolytic flux rate [2, 5, 6]. Redetzki and Alvarez-O'Bourke [7], showed that 6-AN is a central nervous system depressant.

In recent experiments using 6-AN as a biochemical tool, Bielicki and Krieglstein [8], found changes in the gamma-aminobutyric acid (GABA) and glutamate concentration in rat brain after treatment with 6-AN. They suggested that these changes could be responsible for some neurological symptoms produced by 6-AN and that the drug appeared to affect the GABA shunt. The amino acid GABA is a putative inhibitory neurotransmitter in both vertebrate and invertebrate nervous systems [9] and is intimately related to the oxidative metabolism of carbohydrates in the central nervous system by means of this shunt [10]. Therefore, we decided to study the effect of 6-AN on gamma-aminobutyric acid transaminase, GABA-T, (4-aminobutyrate:2-oxoglutarate aminotransferase, EC 2.6.1.19), an enzyme of the GABA shunt which is the major degradative pathway for GABA.

Materials and Methods

Experimental animals. Adult rats of Holtzman strain weighing between 200–250 g were used for experimental purposes.

Chemicals. Chemicals used were from Sigma Chemical Co., U.S.A.

6-Aminonicotinamide treatment. 6-AN was injected intraperitoneally (35 mg/kg body weight) to the rats. Since 6-AN was dissolved in physiological saline, control rats were also injected with the same amount of saline.

Preparation of homogenate. Rats were killed by cervical dislocation and brains were excised and chilled immediately. Cerebral hemispheres, cerebellum and brain stem were separated and weighed immediately. Homogenates were prepared (1:10) using a Potter–Elvehjem type homogeniser fitted with a teflon plunger. The homogenising medium contained the following in the final concentrations; 0.25 M sucrose, 20 mM triethanolamine buffer pH 7.4 and 0.1 mM dithiothreitol.

Estimation of GABA-T activity. GABA-T was estimated essentially according to the method of De Boer and Bruinvels [11]. Triton treatment of the homogenate was carried out as follows: one volume of homogenate was added to three volumes of ice cold Triton medium containing a final concentration of 0.67 per cent Triton X-100, 50 mM Tris–HCl pH 8.5 and 5 mM dithiothreitol. Triton treated homogenate was kept ice cold for 1 hour before use. GABA-T activity was measured using a coupled enzyme method which utilizes endogenous succinic semialdehyde dehydrogenase to convert the formed succinic semialdehyde to succinate. The concurrently produced NADH was used as an estimate of GABA-T activity. A unit of GABA-T activity was defined as one μmole of NADH formed at 37° in one hour.

Protein determination. Protein was determined according to the method of Lowry *et al.* [12].

Statistical analysis. To express significance, 2-way analysis of variance was applied. For difference between control and treated groups, Dunnette's multiple comparison test was applied.

Results and Discussion

The effect of 6-Aminonicotinamide on the activity of GABA-T was determined in three regions of the rat brain at 4 hr and 16 hr of drug administration. GABA-T activity was very significantly decreased in all the three regions studied, i.e. cerebral hemispheres, cerebellum and brain stem after 4 hr of drug treatment at which time, spastic paralysis affecting mainly the hind limbs was also observed. Enzyme activity returned to control values in the cerebral hemispheres and brain stem after 16 hr of drug treatment. In the cerebellum, however, GABA-T activity increased above the control values by 41 per cent at 16 hr.

The significant changes in the activity of GABA-T at 4 hr and 16 hr of drug administration in different regions of the brain, are given in Table 1.

The very significant inhibition of GABA-T in the cerebral hemispheres, cerebellum and brain stem at 4 hr of drug administration would lead to increased GABA concentration which could be responsible for the central nervous system depressant and anticonvulsant actions of 6-AN reported by Redetzki and Alvarez-O'Bourke [7].

The significant increase in GABA-T activity (41 per cent above normal values) in the cerebellum at 16 hr of drug treatment would lead to decreased GABA levels confirmed by the reports of Bielicki and Krieglstein [8]. They also reported a decrease in the glycolytic flux rate at 16 hr of drug administration. Decreased GABA levels in the cerebellum at 16 hr may be due to its participation in the normal oxidative metabolism of carbohydrates in brain via the GABA shunt.

6-AN has been shown to be an antimetabolite of nicotinamide in pyridine nucleotide synthesis and is incorporated into NADP or NAD, forming 6-amino-NADP or 6-amino-NAD. These abnormally structured nucleotides are unable to act as hydrogen carriers [13]. In addition, the 6-amino analogue of NADP is a competitive antagonist of NADP- or NADPH-dependent oxidoreductases. Since NAD is usually present in considerable excess and only about one tenth of this coenzyme is converted into the derivative containing 6-AN, and because 6-amino-NAD hardly affects NAD-dependent dehydrogenases, the inhibition of NAD-dependent enzymes should be negligible

Table 1. Effect of 6-aminonicotinamide on gamma-aminobutyric acid transaminase in regions of the rat brain

Region of brain	Protein content (mg/g)	Time after administration of 6-aminonicotinamide (35 mg/kg) Zero (μmoles/g/hr)	4 hr (μmoles/g/hr)	P	16 hr (μmoles/g/hr)	P
Cerebral hemispheres	63.3 ± 7.7	21.2 ± 3.6	4.8 ± 0.8	†	17.9 ± 1.9	N.S.
Cerebellum	68.0 ± 6.9	19.2 ± 2.0	3.2 ± 0.0	‡	27.1 ± 2.7	*
Brain stem	62.7 ± 1.3	30.7 ± 0.8	8.4 ± 0.6	‡	27.8 ± 1.4	N.S.

Each value is a mean of 4–6 values.
N.S. not significant.
* $p < 0.05$; versus control.
† $p < 0.01$; versus control.
‡ $p < 0.001$; versus control.

[2, 14]. No change in NAD was detected and only a small decrease in NADH was observed after 6-AN treatment [7, 14]. It was therefore assumed that inhibition of endogenous succinic semialdehyde dehydrogenase used in the coupled assay system for GABA-T estimation was negligible.

The alteration of the GABA shunt by 6-AN allows a new idea of its mechanism of action. Alterations in cerebral content or turnover rate of the components of the GABA system may lead to serious changes in the normal functions of neurons. Studies of the entire GABA shunt using 6-AN are in progress in our laboratory to elucidate the further significance of the GABA system in neuronal cell function.

Summary

The effect of 6-Aminonicotinamide (6-AN) on gamma-aminobutyric acid transaminase was studied in three regions of the rat brain.

Gamma-aminobutyric acid transaminase activity was very significantly decreased in cerebral hemispheres, cerebellum and brain stem after 4 hr of drug treatment. Enzyme activity returned to control values in cerebral hemispheres and brain stem after 16 hr of drug treatment. In the cerebellum, however, gamma-aminobutyric acid transaminase activity increased above control values by 41 per cent at 16 hr.

6-AN seems to affect the GABA shunt and can therefore be used to study this pathway in the central nervous system.

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REFERENCES

1. W. J. Johnson and J. D. McColl, *Science* **122**, 834 (1955).
2. H. Herken, K. Lange and H. Kolbe, *Biochem. biophys. Res. Comm.* **36**, 93 (1969).
3. M. Katsuhiko, S. Asano, J. Katamura and K. Takatori, *Yakugak Zasshi* **95**, 547 (1975).
4. H. Herken, in *Mechanisms of Drug Toxicity*, Proc. 3rd Pharmac. Meeting, Vol. 4, p. 198. Pergamon Press, Oxford (1966).
5. H. Herken, G. Meyer-Estorf, K. Halbhubner and D. Loos, *Naunyn Schmiedeberg's Arch. Pharmac.* **293**, 245 (1976).
6. J. Krieglstein and R. Stock, *Naunyn Schmiedeberg's Arch. Pharmac.* **290**, 323 (1975).
7. H. M. Redetzki and F. Alvarez-O'Bourke, *J. Pharmac. Exp. Ther.* **137**, 173 (1962).
8. L. Bielicki and J. Kreiglstein, *Naunyn Schmiedeberg's Arch. Pharmac.* **294**, 157 (1976).
9. E. Roberts and R. Hammerschlag, in *Basic Neurochemistry* (Eds G. J. Segel, R. N. Albers, R. Katzman and B. W. Agranoff), p. 218. Little, Brown and Company (1976).
10. C. F. Baxter, in *The Nature of Gamma-aminobutyric Acid, Handbook of Neurochemsitry Vol. 3* (Ed. A. Lajtha), p. 289. Plenum Press (1970).
11. Th. De Boer and J. Bruinvels, *J. Neurochem.* **28**, 471 (1976).
12. O. H. Lowry, N. J. Rosebrough, A. H. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1957).
13. L. S. Dietrich, I. M. Friedland and L. A. Kaplan, *J. biol. Chem.* **233**, 964 (1958).
14. H. P. T. Ammon, *Naunyn Schmiedeberg's Arch. Pharmac.* **290**, 251 (1975).