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EFFECT OF 6-AMINONICOTINAMIDE ON MONOAMINE OXIDASE AND Na^+K^+ATPase ACTIVITY IN DIFFERENT REGIONS OF RAT BRAIN

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Abstract—The monoamine oxidase activity in the cerebral hemispheres decreased significantly after 2, 4, 8 and 16 hr of 6-amino-nicotinamide (35 mg/kg body weight, i.p.) administration. In the cerebellum, the MAO activity was not affected significantly. In the brain stem, however, a significant increase was observed after 2 hr of drug administration followed by a gradual decrease at later time intervals. The Na^+K^+ATPase activity in the cerebral hemispheres was increased significantly at 2 and 4 hr of 6-aminonicotinamide administration. This was followed by a gradual decrease at later time intervals. In the cerebellum, like monoamine oxidase, the Na^+K^+ATPase did not change significantly. The brain stem showed a decrease at 2 hr of drug administration, followed by a significant increase at 4 hr and then a gradual decrease to near control values.

6-Aminonicotinamide (6-AN) is an antimetabolite of NADP. Exchange reactions catalysed by NADP glycerohydrolase (EC 3.2.2.6) can cause the incorporation of 6-AN into nicotinamide nucleotides to form inhibitory analogues, which causes a marked inhibitory effect on 6-phosphogluconate dehydrogenase (EC 1.1.1.44) [1] and the resultant accumulation of 6-phosphogluconate causes a secondary blockade of the glycolytic pathway by inhibiting phosphoglucoisomerase (EC 5.3.1.9) [2]. The drug not only interferes with the pentose phosphate pathway and glycolytic pathway, but also the catecholamine synthesis, reductive steps of lipogenesis and cholesterol genesis and detoxification of H_2O_2 formed in catecholamine degradation [3].

The reasons for studying monoamine oxidase (EC 1.4.3.4) and Na^+K^+ATPase (EC 3.6.1.3) during 6-AN administration are as follows: (a) 6-AN causes an inhibition of pentose phosphate pathway, thus making the NADPH unavailable for the tetrahydropteridine cofactors of tyrosine hydroxylase resulting in deranged biosynthesis of catecholamines [4]; (b) catecholamines have been shown to affect the Na^+K^+ATPase activity in brain homogenate in a biphasic manner, i.e. activation at low concentration and inhibition at high concentration [5]. Since catecholamines are direct substrates of monoamine oxidase, the role of MAO in the regulation of Na^+K^+ATPase is important. Also the inhibition of MAO-A and MAO-B by clorgyline and deprenyl affects the uptake of dopamine, noradrenalin and serotonin in the rat brain synaptosomal fraction [6].

The present investigation attempts to study the monoamine oxidase and the Na^+K^+ATPase activity from various regions of the rat brain following 6-aminonicotinamide administration and to discuss the correlation between altered catecholamine levels owing to their deranged biosynthesis.

MATERIALS AND METHODS

Chemicals. Kynuramine dihydrobromode, 4-hydroxyquinoline, adenosine triphosphate, ouabain, Tris and 6-aminonicotinamide were obtained from Sigma (U.S.A.). All the other chemicals were obtained from BDH (Pool, U.K.) and were of analytical grade.

Experimental animals. Adult rats of Wistar strain weighing between 200 and 220 g were used for the experiments.

6-Aminonicotinamide administration. 6-AN was injected intraperitoneally (35 mg/kg body weight) to the rats. Since 6-AN was dissolved in physiological saline (0.9% NaCl), control rats were also injected with the same amount of saline. The rats were sacrificed 2, 4, 8 and 16 hr after the drug administration.

Preparation of homogenates. Homogenates were prepared and differential centrifugation performed as described earlier [7].

Assay of enzyme activities. Monoamine oxidase and Na^+K^+ATPase activities were assayed according to the method of Catravas *et al.* [8] and Zaheer *et al.* [9] as described earlier [7, 10].

Protein determinations. Protein was determined according to the method of Lowry *et al.* [11].

RESULTS AND DISCUSSION

The monoamine oxidase activity in the 12,000 *g* pellet from the cerebral hemispheres, decreased significantly after 2, 4, 8 and 16 hr of 6-AN administration, with P values <0.01, <0.02, <0.02 and <0.02 respectively. In the cerebellum, the MAO

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Table 1. Effect of 6-aminonicotinamide on monoamine oxidase activity in different regions of rat brain

	Hours after 6-AN administration	Cerebral hemispheres	Cerebellum	Brain stem
12,000 *g* pellet	Control	40.2 ± 2.2	37.4 ± 1.2	34.1 ± 2.1
	2	20.0 ± 1.7*	32.4 ± 1.8	44.8 ± 2.8‡
	4	25.0 ± 1.0†	25.4 ± 1.8	40.5 ± 1.0
	8	25.0 ± 2.0†	35.0 ± 0.4	39.8 ± 1.0
	16	20.4 ± 0.8*	33.4 ± 1.4	35.0 ± 0.5
12,000 *g* supernatant	Control	31.8 ± 4.0	30.4 ± 0.6	32.4 ± 0.8
	2	30.4 ± 2.5	30.0 ± 0.4	35.2 ± 0.2
	4	28.4 ± 1.0	29.0 ± 0.5	35.2 ± 0.5
	8	28.2 ± 1.0	28.5 ± 0.6	34.0 ± 0.3
	16	27.3 ± 0.8	28.2 ± 0.6	33.0 ± 1.0

Activities are expressed as μmoles/90 min/g. Each value is a mean ± S.E.M. of four or more separate experiments.
* $P < 0.01$; † $P < 0.02$; ‡ $P < 0.05$ (Student's *t* test).

activity was not affected significantly. In the brain stem, however, a significant increase was observed, followed by a gradual decrease at later time intervals. In the 12,000 *g* supernatant, the MAO activity followed an almost similar pattern to that seen in the 12,000 *g* pellet enzyme, in all three brain regions. The changes, however, were not significant (Table 1).

The 12,000 *g* pellet Na^+K^+ATPase activity in the cerebral hemispheres was increased significantly at 2 hr ($P < 0.02$) and 4 hr ($P < 0.05$) of 6-AN administration. At later time intervals (8 and 16 hr) there was a gradual decrease in the enzyme activity. In the cerebellum, the pellet Na^+K^+ATPase activity did not change significantly. After 16 hr of drug administration, however, the activity was significantly decreased ($P < 0.05$). In the brain stem, a decrease was observed at 2 hr of drug administration, followed by a significant increase ($P < 0.05$) at 4 hr and then a gradual decrease to near control values.

The 12,000 *g* supernatant Na^+K^+ATPase showed an almost identical pattern of changes to that seen in the 12,000 *g* pellet enzyme in all three brain regions. In the cerebral hemispheres, a significant increase ($P < 0.05$) was observed at later time intervals. In the cerebellum, the Na^+K^+ATPase activity was unchanged after 2, 4 and 8 hr of drug administration. However, at 16 hr, the activity decreased significantly ($P < 0.02$). In the brain stem, a decrease was observed at 2 hr, followed by a significant increase, with P values <0.02 and <0.05 at 4 and 8 hr respectively (Table 2).

6-AN affects the brain metabolism in a variety of ways [3]. Of these, the systems utilizing NADP are most important. These systems include: (i) ribose-5-phosphate formation by the oxidative reactions of pentose phosphate pathway; (ii) the reductive steps of lipogenesis and cholestrol genesis; (iii) formation of glumate and GABA; (iv) catecholamine synthesis and other hydroxylation reaction; (v) detoxification of H_2O_2 formed in catecholamine degradation; (vi) the maintenance of glutathione in the reduced forms [3].

The changes in the metabolite levels contributed by these pathways as also the anternative pathways of glucose utilization [3] resulting from treatment of rats with 6-AN may cause an inhibitory effect on the MAO activity. The nature of this inhibition whether primary or secondary is not known. However, the *in vivo* effect can be attributed only partially, to the

Table 2. Effect of 6-aminonicotinamide on Na^+K^+ATPase activity in different regions of rat brain

	Hours after 6-AN administration	Cerebral hemispheres	Cerebellum	Brain stem
12,000 *g* pellet	Control	10.2 ± 2.0	8.9 ± 1.2	9.0 ± 1.3
	2	16.4 ± 1.0*	8.4 ± 0.1	6.7 ± 0.2
	4	14.0 ± 1.2†	7.0 ± 0.2	13.5 ± 0.2†
	8	12.0 ± 0.5	7.0 ± 1.0	10.0 ± 1.0
	16	9.0 ± 0.7	6.0 ± 2.0†	7.2 ± 0.6
12,000 *g* supernatant	Control	5.4 ± 1.1	4.5 ± 0.1	4.5 ± 0.4
	2	8.2 ± 1.2†	4.2 ± 1.0	3.8 ± 0.4
	4	8.0 ± 1.4†	4.0 ± 1.0	7.8 ± 0.8*
	8	6.0 ± 0.8	3.0 ± 0.4	7.2 ± 1.2†
	16	5.0 ± 0.1	2.7 ± 0.4*	6.0 ± 0.8

Activities are expressed as μmoles/Pi/g/min. Each value is a mean ± S.E.M. of four separate experiments.
* $P < 0.02$; † $P < 0.05$; † pmoles Pi/g/min (Student's *t* test).

changes in catecholamine levels, owing to deranged catecholamine synthesis as a result of the decrease in the activity of the rat limiting enzyme of the catecholamine biosynthesis, i.e. the tyroxine hydroxylase [4].

A decrease in the monoamine oxidase activity observed in the present experiment may result in the lowering of aldehyde levels. 3-Methoxy-4-hydroxybenzaldehyde, a structural analogue of 3-methoxy-4-hydroxy phenylacetaldehyde (a product of MAO catalysed reaction with dopamine as substrate) was found to affect the rat brain Na^+K^+ATPase in a biphasic manner, i.e. stimulation at lower concentration and inhibition at higher concentrations [12].

Thus, the observed increase in the Na^+K^+ATPase activity in the cerebral hemispheres at 2 hr after drug administration could be due to the following reasons: (a) increase in ATP levels, or (b) due to low levels of aldehyde formed as a result of reduced MAO activity. The first possibility is ruled out since 6-AN does not increase the ATP levels at 2 hr but on the contrary decreases it [13]. It has been shown in the present study that at 2 hr, the MAO activity in the cerebral hemispheres is decreased significantly. With this decrease in the MAO activity, the amounts of aldehyde formed is low and, therefore, it can stimulate the Na^+K^+ATPase activity. In the brain stem, the decrease in the Na^+K^+ATPase activity could be due to an increased MAO activity at 2 hr after the drug administration.

At later time intervals, an overall decrease in the Na^+K^+ATPase activity in all the three brain regions can be attributed to the decrease in the ATP levels following 6-AN administration. Since 6-AN is known to cause neurological symptoms in the rat, like spastic paralysis, this drug may be used as a tool to elucidate the molecular mechanism underlying these neurological artefacts which involve altered catecholamine metabolism.

In summary, the monoamine oxidase activity in the cerebral hemispheres decreased significantly after 2, 4, 8 and 16 hr of 6-aminonicotinamide (35 mg/kg body weight, i.p.) administration. In the cerebellum, the MAO activity was not affected significantly. In the brain stem, however, a significant increase was observed after 2 hr of drug administration followed by a gradual decrease at later time intervals. Na^+K^+ATPase activity in the cerebral hemispheres was increased significantly at 2 and 4 hr of 6-aminonicotinamide administration. This was followed by a gradual decrease at later time intervals. In the cerebellum the Na^+K^+ATPase like monoamine oxidase, did not change significantly. The brain stem showed a decrease at 2 hr of drug administration, followed by a significant increase at 4 hr and then a gradual decrease to near control values.

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