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Effect of 6-Aminonicotinamide on the activity of hexokinase and lactate dehydrogenase isoenzymes in regions of the rat brain

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Abstract. Changes in the activity of hexokinase and lactate dehydrogenase isoenzymes in the three brain regions and heart were studied in the 6-Aminonicotinamide-treated rats. Drug administration decreased the particulate hexokinase and lactate dehydrogenase activity, but increased the soluble hexokinase.

Keywords. Hexokinase; lactate dehydrogenase; isoenzymes; brain; glycolysis.

Introduction

In animal tissues, nicotinic acid is mostly found in the nicotinamide moiety of the two coenzymes, NAD^+ and $NADP^+$, which are involved in the anaerobic breakdown of glucose, citric acid cycle, and amination of glutamic acid (Sternberg and Phillips, 1959). 6-Aminomcotinamide (6-AN) an antimetabolite of nicotinamide when administered to animals, forms an inactive analogue of NADP causing effects mainly on the nervous system (Herken, 1968b; Herken *et al*., 1974).

Investigations on the effect of 6-AN on the pathways of glucose utilization in brain (Lange *et al*., 1970; Hothersall *et al*., 1981) showed that the compound primarily influences the pentose phosphate pathway, leading to the accumulation of 6-phosphogluconate which in turn decreases glycolytic flux by inhibiting phosphogluco-isomerase.

The brain largely depends on glucose for its energy metabolism. Earlier reports on 6-AN-treated rats showed significant changes in the levels of glucose, glucose-6-phosphate, lactate, ATP etc. (Lange *et al*., 1970; Hothersall *et al*., 1981). The purpose of the present study was to understand further the effects of 6-AN on glucose metabolizing pathways in the brain. The key position of hexokinase and lactate dehydrogenase in the cellular metabolism of glucose makes the study of these enzymes of great interest. In the present work, isoenzymes of these enzymes were studied. Heart tissue was also studied for comparison.

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Abbreviations used: 6-AN, 6-Aminonicotinamide; TPF, total particulate fraction.

Materials and methods

Albino rats of Wistar strain weighing between 180-200 g were used. 6-AN dissolved in 0·9 % saline was injected (*i. p.* 35 mg/kg body wt.) to each rat. The animals were killed at specified time intervals after injection. The control rats were injected 0·9% saline and the enzyme activities were determined at the same time post injection.

Preparation of homogenates

Rats were killed by cervical dislocation and the cerebral hemispheres, cerebellum, brain stem and the heart were excised immediately and weighed. Tissue homogenates (1:10) were prepared in isotonic sucrose medium as described earlier (Kaur *et al*., 1983). The extracts were centrifuged at 12,000 *g* for 40 min and the supernatant was used as the soluble fraction(s). The pellet was washed once and resuspended in the same medium to obtain total particulate fraction (TPF).

Enzyme assay

Hexokinase activity was estimated essentially according to the method of Sharma *et al.* (1963) as modified by Gumaa and McLean (1972). The TPF was treated with Triton-X-100 (final concentration 0·5%) in the cold for 30-60 min to liberate latent and bound enzymes. For the estimation of the activity of isoenzymes, aliquots of each fraction were heated at 45°C for 1 h (Grossbard and Schmike, 1966). This process destroyed hexokinase Type II completely and the substraction of the activity of the heated fraction from the total gave the activity of Type II isoenzyme. Lactate dehydrogenase activity was assayed by the method of Bergmeyer and Bernt (1974) and gel electrophoresis was done by the method of Ornstein (1964). One unit of activity for hexokinase and lactate dehydrogenase was defined as the amount required to form one μmol of NADPH or NAD^+, respectively per min at 25°C.

Results

Changes in hexokinase activity in the brain regions and the heart

The total hexokinase in TPF from the cerebral hemispheres showed a significant decrease in activity after 8 and 16 h of drug (6-AN) administration, whereas, the soluble hexokinase showed an increase. Hexokinase Type I isoenzyme in TPF decreased ($P < 0{\cdot}01$) and this was accompanied by an increase in the soluble fraction enzyme ($P < 0{\cdot}01$) at 16 h. A significant decrease in the activity of hexokinase Type II in TPF was observed at 8 and 16 h ($P < 0{\cdot}02$ and $P < 0{\cdot}05$ respectively). The hexokinase associated with soluble fraction increased at 16 h after drug administration. The results are presented in table 1 and figure 1A.

The activity of the total hexokinase from the cerebellum in TPF did not change after the drug administration. However, the soluble enzyme showed a significant increase at 16 h. Hexokinase Type I in both the subcellular fractions did not change. The activity of hexokinase Type II was 67–80% of the control levels at 2, 8 and 16 h of drug treatment.

Table 1. Effect of 6-AN on total hexokinase from TPF and soluble fraction in different regions of the brain and the heart.

Region	Fraction	Control	Time after 6-AN injection (h) 2	8	16
			units/g		
Cerebral hemispheres	TPF	6·8 ± 0·15	7·24 ± 0·07	6·1 ± 0·08[c]	5·1 ± 0·2[e]
	S	3·0 ± 0·11	3·1 ± 0·01	3·1 ± 0·1	3·8 ± 0·14[d]
Cerebellum	TPF	3·6 ± 0·27	3·6 ± 0·03	3·1 ± 0·04	4·0 ± 0·17
	S	2·8 ± 0·15	2·9 ± 0·11	2·8 ± 0·14	3·6 ± 0·11[c]
Brain stem	TPF	2·8 ± 0·16	3·0 ± 0·08	2·4 ± 0·23	2·8 ± 0·09
	S	2·5 ± 0·22	3·0 ± 0·03	2·6 ± 0·14	2·7 ± 0·13
Heart	TPF	1·6 ± 0·29	1·3 ± 0·02	0·96 ± 0·06	1·9 ± 0·17
	S	3·4 ± 0·17	2·7 ± 0·08[b]	3·7 ± 0·25	4·0 ± 0·16[a]

Each value is a mean ± SEM of four or more experiments, done in triplicates.
TPF, Total Particulate Fraction; S, Soluble fraction.
[a] $p < 0{\cdot}05$; [b] $p < 0{\cdot}02$; [c] $p' < 0{\cdot}01$; [d] $p < 0{\cdot}002$ and [e] $p < 0{\cdot}001$.

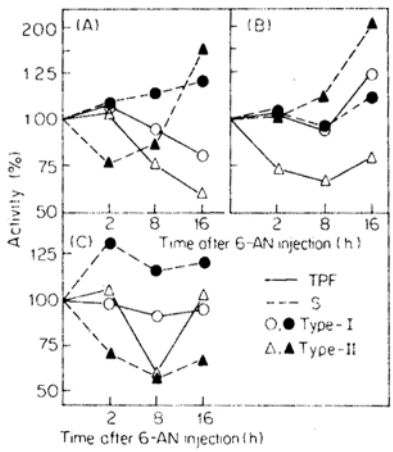

Figure 1. Percent activities of hexokinase Type 1 and Type II isoenzymes in TPF and soluble fraction from (A) cerebral hemispheres (B) cerebellum and (C) brain stem, after 6-AN treatment. Absolute activities of hexokinase Type I and Type II isoenzymes in TPF were 5·5 ± 0·2 and 1·3 ± 0·01; 2·8 ± 0·3 and 0·7 ± 0·1 and 2·3 ± 0·2 and 0·5 ± 0·04 and in soluble fraction were 2·3 ± 0·1 and 0·72 ± 0·05; 2·3 ± 0·1 and 0·5 ± 0·06 and 1·9 ± 0·2 and 0·65 ± 0·08 from the cerebral hemispheres, cerebellum and the brain stem respectively. Each value is a mean ± SEM of 4 or more experiments, done in triplicates.

The changes however were not significant. Hexokinase Type II in soluble fraction increased at 16 h ($P < 0{\cdot}02$) after injection. The results are presented in table 1 and figure 1B.

Total hexokinase activity in the TPF and soluble fraction and Type I isoenzyme in the TPF from the brain stem showed only marginal changes at all the three intervals of time after drug administration. Soluble fraction hexokinase Type I increased to 132% at 16 h. After 8 h, hexokinase Type II activity in both the subcellular fractions decreased significantly ($P < 0{\cdot}01$ and $P < 0{\cdot}05$ respectively). The results are presented in table 1 and figure 1C.

The total hexokinase activity in TPF from the heart tissue was significantly decreased after 8 h, whereas the soluble fraction enzyme showed a decrease at 2 h

followed by a gradual rise in the activity upto 16 h. A significant decrease in hexokinase Type I from TPF occurred at 8h *(P* < 0·02) with a subsequent increase at 16 h ($P < 0{\cdot}01$). Hexokinase Type I in the soluble fraction showed increase at 8 and 16 h ($P < 0{\cdot}02$ and $P < 0{\cdot}01$ respectively). Type II TPF hexokinase increased at 2 and 16 h ($P < 0{\cdot}05$ and $P < 0{\cdot}02$ respectively). Soluble Type II showed a decrease at 2h ($P < 0{\cdot}01$). The results are presented in table 1 and figure 2.

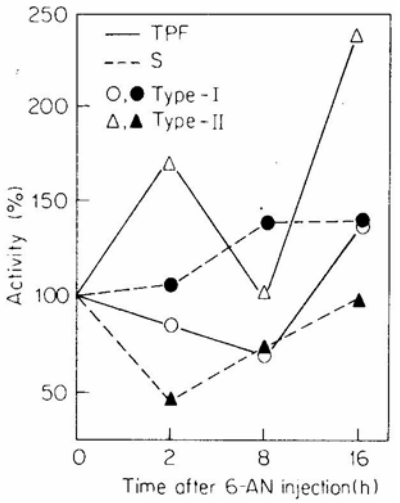

Figure 2. Activity of hexokinase Type I and Type II isoenzymes in TPF and soluble fraction from the heart following 6-AN treatment. Absolute activities of hexokinase Type I and Type II are 0·95 ± 0·06 and 0·3 ± 0·04 and 1·8 + 0·1 and 1·6 + 0·1 and in TPF and soluble fraction respectively.

Changes in lactate dehydrogenase from the brain regions and the heart

Lactate dehydrogenase activity in the cerebral hemispheres decreased significantly at 8 and 16 h after drug administration. The enzyme activity in the cerebellum and the brain stem showed minimal changes after 6AN injection. In the heart, lactate dehydrogenase activity showed a significant increase at 16 h. The results are presented in table 2.

The gel electrophoretic pattern of the isoenzymic forms of lactate dehydrogenase are presented in figure 3. A significant decrease in the Type III and IV isoenzymes in the

Table 2. Effect of 6-AN on lactate dehydrogenase in different regions of the rat brain and the heart.

		Time after 6-AN injection (h)		
Region	Control	2	8	16
		units/g		
Cerebral hemispheres	27·7 ± 1·08	25·2 ± 0·14	18·3 ± 0·6[c]	24·2 ± 0·44[b]
Cerebellum	19·8 ± 1·6	20·2 ± 0.14	18·1 ± 0·44	22·9 ± 1·3
Brain stem	16·2 ± 1·4	13·1 ± 0.33	12·9 ± 0·49	15·5 ± 0·54
Heart	109·6 ± 5·1	104·2 ± 5.3	115·8 ± 2·8	129·6 ± 6·5[a]

Each value is a mean ± SEM of four or more experiments, done in triplicates.
[a] $p < 0{\cdot}05$; [b] $p < 0{\cdot}02$ and [c] $p < 0{\cdot}001$.

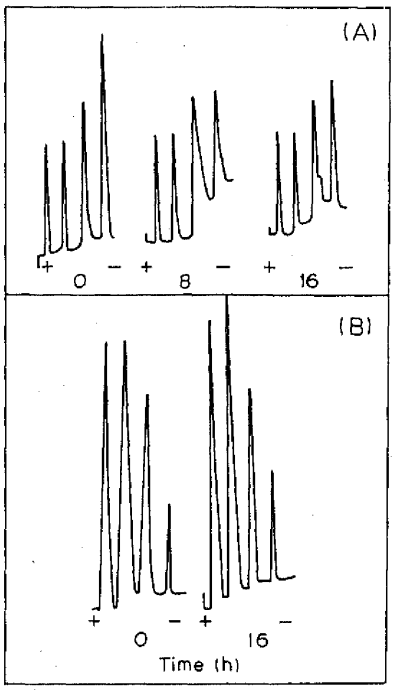

Figure 3. Densitometric tracings of the Polyacrylamide gel electrophoresis of lactate dehydrogenase isoenzymes in (A) cerebral hemispheres and (B) heart from 6-AN treated rats. (C) Control at 8 and 16 h after drug treatment in the cerebral hemispheres and the heart.

cerebral hemispheres was observed at 8 and 16 h after 6-AN treatment, whereas, in the heart significant increase in the amount of all the four isoenzymes was observed.

Discussion

Significant neurological symptoms have been reported after administration of 6-AN to rats. Herken *et al.* (1974) showed that after 6-8h of drug administration, the antimetabolite became fixed cellularly in the brain, which was unable to act as a hydrogen carrier in oxidation-reduction reactions, utilizing NADP. In the present experiments, significant effects of 6-AN on the enzyme activities were seen after 8 h of drug treatment.

A significant decrease in the particulate hexokinase was observed, with an increase in the soluble form but with no significant change in the total hexokinase. Glucose-6-phosphate level in the brain after 6-AN treatment increases considerably as compared with controls (Herken *et al*., 1974; Lange *et al*., 1970). From the results of *in vitro* and *in vivo* experiments, it has been suggested that a soluble-bound hexokinase equilibrium is very much sensitive to the metabolite control, in particular to glucose-6-phosphate (Wilson, 1980). Copley and Fromm (1967) reported that bound hexokinase has a high K_i for glucose-6-phosphate and a lower K_m for ATP as compared to the soluble form, suggesting that the bound form of hoxokinase in the brain is the more active form. The present results suggest that the more active form (bound) of hexokinase in the rat brain

is converted to a less active form (soluble) under conditions of reduced cerebral energy metabolism.

The decrease in the hexokinase activity in the heart tissue may be due to the inhibition of insulin release by 6-AN. Ammon and Steinke (1972) showed that in pancreatic islets, decreased activity of the pentose phosphate pathway and reduced NADPH level induced by 6-AN, are associated with a reduced insulin response to glucose.

6-AN significantly decreased the lactate dehydrogenase activity in the brain. The evidence for an inhibition in the lower segment of the glycolytic pathway in 6-AN-treated rats is evident from the changes in the metabolite profile of this pathway. Lange *et al.* (1970) and Hothersall *et al.* (1981) reported a decline in pyruvate and lactate levels of 6-AN treated rats. The enzyme activity decrease observed in the present work may be due to substrate deficiency.

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