MECHANISMS OF ACTION OF NICOTINAMIDE-ADENINE DINUCLEOTIDE IN INSUFFICIENCY OF THE CEREBRAL BLOOD SUPPLY

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Besides its role in the energy metabolism of the brain, nicotinamide-adenine dinucleotide (NAD) has a protective action in hypoxic hypoxia and in experimental disturbances of the cerebral circulation [1, 3], it has now been shown that one of the mechanisms regulating the ammonia level in brain tissue is deamination of NAD and reamination of deamino-NAD by aspartate [6].

Following the trend which has developed in the study of the neurochemical compensatory regulation of the cerebral circulation [4, 5], in the present investigation the effect of NAD was studied on some aspects of ammonia metabolism in brain tissue and the blood supply to the brain when the blood flow is reduced.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats and cats anesthetized with urethane. Unilateral occlusion of the common carotid artery in rats was used as the model of disturbance of the cerebral hemodynamics. The local cortical blood flow was determined quantitatively by the hydrogen clearance method [9] in the modification described in [2]. The regional cerebral blood flow was studied by a radioisotope (^{133}Xe) method [8, 10, 11]. Ammonia was determined by the microdiffusion distillation method [7, 13]. Total glutamate dehydrogenase (GDH) activity was determined spectrophotometrically and expressed in Wroblewski units/mg protein [14]. Protein was determined by Lowry's method [12].

EXPERIMENTAL RESULTS

The results show that 24 h after unilateral ligation of the common carotid artery there was a marked increase in the free ammonia concentration in the rat's brain from 0.49 ± 0.02 mg% in the control to 1.20 ± 0.04 mg% ($P < 0.001$) after ligation of the carotid artery. Intraperitoneal injection of NAD in a dose of 10 mg/kg under these conditions led to a decrease in the excess ammonia concentration to 0.82 ± 0.03 mg% ($P < 0.05$).

Considering modern views that, besides its coenzyme activity, NAD also has catalytic activity toward GDH, one of the key enzyme systems controlling the ammonia level in brain tissue, the action of NAD in the removal of ammonia was studied primarily by investigating the effects of NAD on total GDH activity during cerebral ischemia. The results showed that during deficient brain perfusion GDH activity in the reaction of reductive amination of α-ketoglutarate fell from 0.38 ± 0.01 to 0.22 ± 0.02 μmole NADH/mg protein/min ($P < 0.02$), and this naturally led to inhibition of utilization of ammonia and to its accumulation in excessive concentrations in brain tissue, whereas activity of the enzyme in oxidative deamination was unchanged. After injection of NAD in a dose of 10 mg/kg GDH activity was increased to 0.31 ± 0.08 μmole NADH/mg protein/min ($P < 0.02$), or 81.8% of its initial level; in that way NAD promoted ammonia utilization along the pathway of glutamic acid synthesis. It becomes clear that the neurochemical mechanisms of action of NAD in connection with the removal of excessive concentrations of ammonia from the brain are linked with restoration of GDH activity in the reaction of reductive amination of α-ketoglutarate.

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TABLE 1. Effect of Intravenous Injection of NAD in a Dose of 5 mg/kg on Local Blood Flow through Parietal Cortex in Cats (M ± m; n = 8)

Index	Initial background	Injection of NAD (5 mg/kg)
Local cerebral blood flow, ml/min/100 g	32,9±1,9	40,6±3,2 $P<0,05$
Resistance of cerebral vessels, mm Hg/ml/min/100 g	3,4±0,3	2,2±0,2 $P<0,01$
Systemic arterial pressure, mm Hg	116,3±3,0	102,7±3,7 $P<0,02$

In the next series of experiments the cerebral vasomotor properties of NAD were studied in order to detect its possible role in the vascular mechanisms of compensation of the disturbed cerebral circulation. In experiments with stabilized autoperfusion of the cat's brain through the internal maxillary arteries it was shown that intracarotid injection of NAD in doses starting from 0.2 mg kg was accompanied by a decrease in resistance of the cerebral vessels of the carotid system by 12.4 ± 2.1%, whereas in a dose of 0.5 mg/kg NAD lowered the cerebrovascular resistance by 24.5 ± 2.5%. A considerable reduction of tone of the cerebral vessels (by 44.2 ± 2.8%; $P < 0.05$) was found after intracarotid injection of NAD in a dose of 1 mg/kg, and at the same time the level of the systemic arterial pressure fell.

A fact which attracts attention is that during injection of NAD the first reaction to be observed was that of the cerebral vessels, and not until 10-15 sec later was the systemic pressure observed to fall. Moreover, in small doses NAD reduced cerebrovascular tone without affecting the arterial pressure. This is evidence that the reduction in cerebrovascular tone arising after injection of NAD was not the result of their myogenic autoregulatory reaction in response to a change in the intravascular pressure, but took place on account of the direct effect of NAD on the cerebral vessels.

Experiments using quantitative methods of measuring the cerebral blood flow showed that injection of NAD in a dose of 1 mg/kg directly into the blood stream of the brain was followed by an increase in the regional cortical blood flow from 36.3 ± 3.0 to 48.5 ± 2.7 ml/min/100 g ($P < 0.01$), i.e., by 33%, accompanied by a decrease in cerebrovascular resistance from 3.5 ± 0.3 to 2.0 ± 0.1 mm Hg/ml/min/100 g ($P < 0.01$) and a decrease in the systemic arterial pressure from 124.4 ± 4.1 to 106.8 ± 3.1 mm Hg/ml/min/100 g.

Intravenous injection of NAD in a dose of 5 mg/kg into cats caused an increase in the local cortical blood flow by 20% accompanied by a decrease in the cerebrovascular resistance and systemic arterial pressure (Table 1).

The ability of NAD to increase the blood supply to the brain in intact animals thus revealed served as the basis for a study of the effect of NAD on the local cerebral blood flow in the presence of a deficient blood supply to the brain. The local blood flow 1 h after unilateral ligation of the common carotid artery in rats was found to be reduced from 40.6 ± 1.7 to 27.7 ± 3.1 ml/min/100 g ($P < 0.05$).

A single intraperitoneal injection of NAD in a dose of 10 mg/kg led to recovery of the disturbed blood flow, which was 32.1 ± 2.1 ml/min/100 g, i.e., 87% of its initial level, 20 min after injection of NAD, and reached 94% of its initial level 1 h after injection of NAD.

These new aspects of the action of NAD revealed by this investigation enable it to be regarded as one of the neurochemical components of compensation for deficiency in the blood supply to the brain.

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