CONTENT OF OXIDIZED AND REDUCED FORMS OF NAD IN THE BRAIN TISSUE AND LIVER OF RATS IN NORMAL CONDITIONS AND AFTER ADMINISTRATION OF IPRONIAZID

E. V. Sharkova

UDC 615.782.12-092:/612.82+612.35/.015.11:577.158.8.013

It has recently been reported [3] that in Wilson's experiments the intraperitoneal injection of iproniazid was followed by a decrease in the content of NAD* in the brain tissue and liver. The mechanism of the action of iproniazed on the contest of the pyridine coenzymes has not been studied.

The extensive used of iproniazid in chemical practice and the great importance of NAD in a wide range of biochemical reactions illustrate the importance of the explanation of the effect of iproniazid on processes associated with NAD conversions. For this purpose, the first essential was to reproduce the phenomenon described by Wilson. This was the object of the present investigation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 200-250 g. Iproniazid was dissolved in physiological saline and injected intraperitoneally in a volume of 1 ml containing a dose of 100-800 mg/kg body weight. Rats not receiving iproniazid served as controls.

The brain and liver of the experimental animals were frozen with liquid nitrogen (for analysis of the brain, the head was frozen intact). The tissue for investigation, in a frozen state, was ground in a motar and weighed samples of tissue were prepared from the powder thus obtained. One weighed sample was treated with four times its volume of cold 5% TCA and homogenized in a glass homogenizer. After centrifugation at -4° and 12 000 RPM for 10 min, the NAD concentration was determined in the protein-free supernatant by the specific reaction of its reduction in the presence of alcohol dehydrogenase, ethanol, and semicarbazide at pH 8.1-8.2 [8]. The measurements were made in the SF-4 spectrophotometer at λ= 340 mμ.

The second weighed sample was transferred to a test tube with four times (for the liver) and twice (for the brain) its volume of a hot 0.2M 50% alcoholic solution of Na_2CO_3, and heated on a boiling water bath for 20 min. The contents of the tube were then cooled on ice. The tissue residue was removed by centrifugation at -4° and 15 000 RPM for 20 min. Since the loose sediment adsorbed NAD-H_2, it was washed three times with an alcoholic solution of sodium carbonate (0.5 ml each time). The washings were added to the main supernatant and the resulting solution was neutralized in the cold with a 1 M solution of malic acid to pH 7.5 with constant mixing. After preliminary oxidation of the NAD-H_2 with PMS, the determination was carried out in the usual way – spectrophotometrically with alcohol dehydrogenase [11]. For this purpose, 0.4 ml of a 10^{-3}M solution of PMS was added to 2 ml of the supernatant, neutralized to pH 7.5. After 20 min the proteins were precipitated by the addition of 0.6 ml of 26% TCA. The concentration of NAD was determined in the protein-free supernatant.

EXPERIMENTAL RESULTS

The table shows that the content of NAD in the brain tissue and liver of the rats was considerably greater than the content of NAD-H_2. The ratio of NAD-H_2/NAD for the liver was 0.7 and for the brain 0.4. The table also shows that in the experiment performed on the animals between November and January, the

*NAD – oxidized nicotinamide-dinucleotide; NAD-H_2 – reduced nicotinamide-dinucleotide; TCA – trichloroacetic acid; PMS-phenazine metasulfate.

Laboratory of Biochemistry, Institute of Pharmacology and Chemotherapy, Academy of Medical Sciences of the USSR, Moscow (Presented by Active Member of the Academy of Medical Sciences of the USSR, S. E. Severin). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 5, pp. 49-51, May, 1967. Original article submitted July 23, 1965.

Content of NAD and NAD-H_2 in the Brain Tissue and Liver of Rats (in μg/g Moist Weight of Tissue)

Date of experiments	Liver		Brain	
	NAD	NAD-H_2	NAD	NAD-H_2
November-December, 1964	446±7 (6)	—	209±6 (5)	—
January, 1965	444±5 (3)	326±10 (4)	206±4 (5)	75±3 (7)
February, 1965	501±92 (7)	319±78 (7)	202±7 (3)	—

Note. The number of experiments is shown in parentheses

individual variations in the content of NAD and NAD-H_2 in both the liver and brain tissue were small. On the other hand, in the experiments carried out in February, much greater variations were found in the content of NAD and NAD-H_2 in the liver.

The results of estimation of the content of dinucleotides after administration of iproniazid may be divided into two groups. In the series of experiments carried out between November and January (when the individual variations in the NAD content in the liver (550 μg/g), but not in the brain tissue. In the experiments conducted in February, because of the considerable individual variations in the content of dinucleotides in the liver, no changes could be detected after injection of iproniazid, regardless of the doses of iproniazid given—from 100-800 mg (when the dose injected was 100 mg the NAD content was 550 μg, when the dose injected was 600 mg the NAD content was 543 μg).

The results of the determination of the content of NAD and NAD-H_2 in the brain tissue and liver of the rats given in this paper are in good agreement with those reported in the literature [1, 2, 4-7, 9, 10].

The considerable individual variations in the content of NAD and NAD-H_2 in the liver discovered in the experiments carried out in February were probably associated with incipient seasonal changes.

So far as the effect of iproniazid on the NAD content in the liver and brain tissue is concerned, the decrease in the NAD content described by Wilson after injection of iproniazid [3] could not be confirmed. In the experiments of Wilson and co-workers, it was noted that in normal rats the NAD content in the brain tissue and liver was very high (800-900 μg/g for the liver and 450 μg/g for the brain). Injection of iproniazid caused a decrease in the content of the dinucleotide to the levels characteristic of the control animals in the present experiments. The discrepancy between the present results and those obtained by Wilson may possibly be attributable, on the one hand, to differences in the diet (a high content of nucleotides in the tissues suggests the use of a diet rich in vitamin PP—nicotinamide), and on the other hand, to the fact that Wilson carried out his experiments on pure line animals.

Hence, the results of these experiments show that following intraperitoneal injection of iproniazid into albino rats obtained from the same visarium and not belonging to a pure line, no decrease in the NAD content took place, in contrast to Wilson's observations, in either the brain tissue or the liver, while in the experiments conducted in January, on the other hand, a slight decrease in the NAD content was observed in the liver.

LITERATURE CITED

1. F. C. Brown, Canad. J. Biochem., 42, 489 (1964).
2. H. B. Burch and P. V. Dippe, J. Biol. Chem., 239, 1898 (1964).
3. R. M. Burton, in the book: Inhibition in the Nervous System and GABA, London (1960), p. 185.
4. R. M. Burton and N. O. Kaplan, Arch. Biochem. Biophys., 101, 139 (1963).
5. G. E. Glock and P. Mclean, Biochem. J., 61, 385 (1955).
6. O. H. Lowry and J. V. Passonnean, J. Biol. Chem. 238, 2474 (1961).
7. F. N. Minerd and C. H. Hahn, J. Biol. Chem., 239, 2746 (1963).
8. E. Racker, J. Biol. chem., 184, 315 (1950).
9. M. A. Spirtes and H. J. Eichell, Arch. Biochem. Biophys., 53, 308 (1954).
10. M. A. Spirtes and S. W. Milstein, Endocrinology, 72, 829 (1963).
11. V. Stollar, Biochem. Biophys. Acta, 44, 245 (1960).