EFFECT OF NIKETHAMIDE ON CONTENT OF PYRIDINE NUCLEOTIDES IN RABBIT HEART MUSCLE

N. O. Stepanyan and L. A. Tseitlin

UDC 615.711.44-092:612.173.1

The mean NAD content in rabbit heart muscle was 367, of NAD·H_2 164, of NADP48, and NADP·H_2 78 μg/g fresh tissue. Subcutaneous injection of nikethamide (125 mg/kg) increased the total NAD and NAD·H_2 content on the average by 24% but had no effect on the content of NADP and NADP·H_2 in the heart muscle.

* * *

The mechanism of action of nikethamide on myocardial metabolism has not yet been adequately investigated despite the wide use of the drug in the treatment of heart diseases.

Administration of nikethamide lowers the glycogen content in the rat myocardium and, depending on the dose given, stimulates or inhibits individual enzyme systems of glycolysis [1]. According to Coper, administration of nikethamide to animals increases NAD-kinase activity in the brain and liver [8].

Since nikethamide is a structural analog of nicotinamide, and since the latter has a significant effect on the energy metabolism of the myocardium, increasing in particular the content of the oxidized form of NAD [3], it was interesting to study the effect of nikethamide on the content of pyridine nucleotides in the heart muscle.

EXPERIMENTAL METHOD

Experiments were carried out on female rabbits weighing 2.4-2.8 kg kept on a normal diet. The animals were divided into a control and an experimental group. The experimental animals received nikethamide by subcutaneous injection in a dose of 125 mg/kg. The animals were sacrificed 12 h after injection of the drug and the content of pyridine nucleotides investigated in the heart after preliminary freezing in liquid nitrogen. Proteins were removed with trichloroacetic acid and the NAD content in the filtrates determined by reduction with alcohol-dehydrogenase and subsequent spectrophotometric determination of the reduced dinucleotide in the SF-4 spectrophotometer at 340 mμ [2]. NADP was determined in the supernatants after precipitation of protein with perchloric acid. The perchloric acid was removed with KOH and the precipitate of potassium perchlorate washed in the cold with distilled water and the washing waters were added to the main supernatant. To determine the NADP content, the method of enzymic reduction of NADP by glucose-6-phosphate dehydrogenase and subsequent spectrophotometric determination of the reduced triphosphopyridine nucleotide at 340 mμ was used.

To determine reduced forms of the pyridine coenzymes we developed a method based on the fact that if weakly alkaline extracts are kept, spontaneous oxidation of the reduced forms takes place [4, 14, 16].

To determine reduced forms of the nucleotides, a weighed sample of tissue was placed in preliminarily heated test tubes with a mixture containing 0.1 M Na_2CO_3 and 3.3×10^{-2} M cysteine, and kept for 3 min in a boiling water bath with continuous stirring. The cooled samples were neutralized with malic acid to pH 7.3 and centrifuged at 15,000 rpm for 7 min. The residue was washed twice with 0.05 M Na_2CO_3 and neutralized to pH 7.3, and the washing waters were added to the main supernatant and allowed to stand at room temperature for 20 h. At the end of this time the samples were again centrifuged and the content of NAD·H_2 and NADP·H_2 in them determined by enzymic methods.

EXPERIMENTAL RESULTS AND DISCUSSION

The mean NAD content in the myocardium of healthy rabbits was 367 μg/g fresh tissue, the NAD·H_2 content 164, NADP 48, and NADP·H_2 78 μg/g fresh tissue.

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. 66, No. 7, pp. 51-53, July, 1968. Original article submitted March 27, 1967.

The NADP/NADP·H_2 ratio in the heart was much higher than in other organs (0.62 in the heart and from 0.08 to 0.3 in the liver [6, 7]). The total content of oxidized forms of pyridine nucleotides in the rabbit heart was 63% of the total content of all forms of pyridine nucleotides. In the heart, just as in other organs, the content of oxidized forms of pyridine nucleotides was greater than that of the reduced, so that the ratio $\frac{NAD + NADP}{NAD \cdot H_2 + NADP \cdot H_2}$ was greater than 1.

Administration of nikethamide in a dose of 125 mg/kg increased the content of oxidized and reduced forms of NAD (to 469 and 187.5 μg/g fresh tissue respectively), but had no effect on the content of oxidized and reduced forms of triphosphopyridine nucleotides (48.5 and 79 μg/g fresh respectively). The total contents of oxidized and reduced forms of NAD were increased under the influence of nikethamide on the average by 24%. The NAD/NAD·H_2 ratio was also increased slightly by nikethamide (from the normal value of 2.2 up to 2.5).

Data in the literature on the ratio between the contents of pyridine coenzymes in the myocardium are few in number and contradictory in nature [5, 10, 12, 13]. Disagreement between results given by individual authors for the content of pyridine coenzymes in different organs and tissues can be explained by the fact that both reduced and oxidized forms of these compounds are highly sensitive to enzymic and nonenzymic destruction. Their extraction from the tissues thus requires a strict control over the experimental conditions. Even in an alkaline medium, it was found that NADP·H_2 undergoes oxidation by heme compounds, and to prevent this, cysteine was added to the fluid undergoing extraction [5, 15-17].

The views of Burch and Lowry [5] on the content of NADP labile with respect to the action of acids in various organs and tissues are now much less rigidly held [11, 16]. According to some authors, high values of NADP are the results of nonenzymic oxidation of NADP·H_2 by heme pigments or by free flavins [9]. Our experiments also show that when weakly alkaline (pH 7.3) extracts of heart muscle are stored for 20-24 h, total nonenzymic oxidation of reduced forms of pyridine coenzymes takes place.

LITERATURE CITED

1. K. I. Rubchinskaya, Ukr. Biokhim. Zh., No. 4, 553 (1965).
2. S. E. Severin, L. A. Tseitlin, and T. N. Druzhinina, Biokhimiya, No. 1, 145 (1963).
3. S. E. Severin and L. A. Tseitlin, Vopr. Med. Khimii, No. 3, 300 (1964).
4. J. A. Bassham, L. M. Brit, R. Hems, et al., Biochem. J., 73, 491 (1959).
5. H. B. Burch, O. H. Lowry, and P. von Dippe, J. Biol. Chem., 238, 2838 (1963).
6. P. Caiger, R. K. Morton, O. H. Filsell, et al., Biochem. J., 85, 351 (1962).
7. G. S. Christie and R. N. Le Page, Biochem. J., 84, 25 (1962).
8. H. Coper, Arch. Exp. Path. Pharmak., 236, 59 (1959).
9. A. Guiditta and A. Vitale-Neugebauer, Biochim. Biophys. Acta, 110, 32 (1965).
10. G. E. Glock and P. McLean, Biochem. J., 61, 388 (1955).
11. H. W. Heldt, H. Grief, M. Klingenberg, et al., J. Biol. Chem., 240, 4659 (1965).
12. K. B. Jacobson and N. O. Kaplan, J. Biol. Chem., 226, 603 (1957).
13. L. A. Jebeikin and S. Weinhouse, J. Biol. Chem., 213, 271 (1955).
14. O. H. Lowry, J. V. Passonneau, and M. K. Rock, J. Biol. Chem., 236, 2756 (1961).
15. F. N. Minard and C. H. Hahn, J. Biol. Chem., 238, 2474 (1963).
16. D. Neubert, H. Schulz, and R. Hoehne, Biochim. Biophys. Acta, 92, 610 (1964).
17. H. Shichi, T. Kamiryo, and S. Funahashi, Biochim. Biophys. Acta, 99, 381 (1965).