

CONTENT OF PYRIDINE NUCLEOTIDES IN RAT
MUSCLES AFTER ADMINISTRATION OF CORTICOTROPIN
AND HYDROCORTISONE

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UDC 612.744.1.015.1:577.
155.2.013].014.46:615.357.453

The content of pyridine nucleotides was investigated in the gastrocnemius muscles of rats after intraperitoneal injection of corticotropin (1.5 units/100 g body weight) and hydrocortisone (5 mg/100 g body weight). The content of the reduced forms of the pyridine nucleotides was reduced 3 h after a single injection of hydrocortisone, but increased after daily injections of the same dose of hydrocortisone for 10 days. Administration of corticotropin, once or daily for 10 days, did not significantly increase the content of pyridine nucleotides.

KEY WORDS: gastrocnemius muscles; pyridine nucleotides; corticotropin; hydrocortisone.

Glucocorticoids play an active part in the metabolism of the pyridine coenzymes of dehydrogenases concerned with the anaerobic and aerobic conversion of carbohydrates and the tricarboxylic acid cycle [11]. Administration of glucocorticoids in vivo inhibits the activity of some NAD-dependent dehydrogenases [4, 7, 8, 11, 12, 14, 16, 17]. After injection of cortisone or hydrocortisone, the concentration of NAD and $\text{NAD} \cdot \text{H}_2$ in the mitochondria and supernatant obtained from rabbit liver and kidney cells is reduced [1]. Previous investigations [2, 3] showed that injection of hydrocortisone (5 mg/100 g body weight) into rabbits and rats lowers the ATP concentration in the muscles and heart, and the content and relative proportions of the pyridine coenzymes are known to play an important role in ATP synthesis.

The object of this investigation was to study the effect of corticotropin and hydrocortisone on the content of oxidized and reduced pyridine nucleotides in the skeletal muscles.

EXPERIMENTAL METHOD

Experiments were carried out on 54 male rats weighing 180-200 g. In the first three series the drugs were injected once only, in the next three series once a day for 10 days. In series I and IV the rats were given an intraperitoneal injection of isotonic NaCl solution (0.5 ml/100 g body weight), in series II and V they received hydrocortisone (5 mg/100 g), and in series III and VI corticotropin (ACTH; 1.5 units/100 g). The rats were decapitated 3 h after injection of the hormones and the gastrocnemius muscle taken for analysis. The content of oxidized ($\text{NAD} + \text{NADP}$) and reduced ($\text{NAD} \cdot \text{H}_2 + \text{NADP} \cdot \text{H}_2$) pyridine nucleotides was determined in a homogenate of the muscle by a fluorimetric method [13]. The standard for fluorimetry was a solution of N'-methylnicotinamide in a concentration of 10 $\mu\text{g}/\text{ml}$. The concentration of nicotinamide coenzymes was calculated in $\mu\text{g}/\text{g}$ wet weight of tissue. Allowance was made for the fact that the method determines the total of the oxidized or reduced pyridine nucleotides. However, animal tissues contain mainly NAD and $\text{NAD} \cdot \text{H}_2$ and very little NADP and $\text{NADP} \cdot \text{H}_2$.

Department of Biophysics, Central Scientific-Research Laboratory, Kiev Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR, A. I. Cherkes.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 78, No. 9, pp. 44-46, September, 1974. Original article submitted November 27, 1973.

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TABLE 1. Content of Pyridine Nucleotides (in $\mu\text{g/g}$ wet weight of tissue) in Gastrocnemius Muscles of Rats 3 h after a Single Injection of Corticotropin (1.5 units/100 g body weight) and Hydrocortisone (5 mg/100 g) ($M \pm m$)

| Nucleotides | Control | Corticotropin | Hydrocortisone |
|--|------------------|-----------------|------------------|
| | 1 | 2 | 3 |
| NAD+NADP | 254,0 \pm 17,0 | 282,0 \pm 8,4 | 223,0 \pm 16,2 |
| NAD \cdot H ₂ + NADP \cdot H ₂ | 145,0 \pm 10,6 | 166,0 \pm 8,3 | 108,0 \pm 10,8 |
| NAD + NADP+ | | | $P_{1-3} < 0,05$ |
| NAD \cdot H ₂ + NADP \cdot H ₂ | 414,0 \pm 16,7 | 448 \pm 8,8 | 331,0 \pm 26,8 |
| | | | $P_{1-3} < 0,05$ |
| NAD + NADP | 1,90 \pm 0,13 | 1,75 \pm 0,13 | 2,13 \pm 0,15 |
| NAD \cdot H ₂ + NADP \cdot H ₂ | | | |

TABLE 2. Content of Pyridine Nucleotides (in $\mu\text{g/g}$ wet weight of tissue) in Gastrocnemius Muscles of Rats after Repeated (10 days) Injections of Corticotropin (1.5 units/100 g body weight) and Hydrocortisone (5 mg/100 g) ($M \pm m$)

| Nucleotides | Control | Corticotropin | Hydrocortisone |
|--|-----------------|------------------|-------------------|
| | 1 | 2 | 3 |
| NAD + NADP | 260,0 \pm 7,3 | 290,0 \pm 17,6 | 362,0 \pm 14,6 |
| | | | $P_{1-3} < 0,001$ |
| NAD \cdot H ₂ + NADP \cdot H ₂ | 130,0 \pm 6,0 | 158,0 \pm 14,3 | 157,0 \pm 11,7 |
| NAD + NADP+ | | | $P_{1-3} < 0,1$ |
| NAD \cdot H ₂ + NADP \cdot H ₂ | 390,0 \pm 7,0 | 448,0 \pm 26,0 | 519,0 \pm 26,0 |
| | | $P_{1-3} < 0,1$ | $P_{1-3} < 0,001$ |
| NAD + NADP | 2,0 \pm 0,12 | 1,88 \pm 0,16 | 2,3 \pm 0,14 |
| NAD \cdot H ₂ + NADP \cdot H ₂ | | | |

EXPERIMENTAL RESULTS AND DISCUSSION

The experiments showed that rat gastrocnemius muscles contain mainly the oxidized forms of the pyridine nucleotides. This proportion was found not only in the control rats, but also after injection of corticotropin and hydrocortisone (Tables 1 and 2). Other workers have observed the same relative content of oxidized and reduced pyridine nucleotides in the muscles of intact rabbits and rats [5].

The content of pyridine nucleotides showed a tendency to rise 3 h after a single injection of ACTH (Table 1), whereas the ratio of oxidized to reduced forms showed a tendency to fall. After injection of hydrocortisone the content of the reduced forms fell by $37 \pm 16 \mu\text{g/g}$ and the total by $83 \pm 35 \mu\text{g/g}$ and the total by $83 \pm 35 \mu\text{g/g}$. The content of the oxidized forms also showed a characteristic tendency to fall.

After a single injection of corticotropin into the rats the changes in the content of pyridine nucleotides in the muscles were thus very small, whereas after injection of hydrocortisone a decrease in their content was observed mainly on account of reduced forms. After injection of corticotropin for 10 days the content of oxidized and reduced pyridine nucleotides showed a tendency to rise. Their total also rose slightly – by $58.0 \pm 26.8 \mu\text{g/g}$. The ratio of NAD + NADP to NAD \cdot H₂ + NADP \cdot H₂ remained almost unchanged (Table 2). After injection of hydrocortisone for 10 days the content of pyridine nucleotides rose: of oxidized by $102.0 \pm 16.4 \mu\text{g/g}$, of reduced by $27.0 \pm 14.0 \mu\text{g/g}$, and of both together by $129.0 \pm 26.7 \mu\text{g/g}$ (Table 2).

After repeated injections of ACTH the content of pyridine nucleotides in the muscles thus rose slightly. After repeated injections of hydrocortisone, but not after a single injection, the content of the oxidized forms of the pyridine nucleotides in the rats' muscles increased.

The decrease in the concentration of reduced pyridine coenzymes in the muscles after a single injection of hydrocortisone may indicate their more rapid oxidation or, more likely, their slow formation as a result of a decrease in the activity of NAD-dependent dehydrogenases. For instance, injection of hydrocortisone is stated to inhibit the activity of dehydrogenases of the Krebs cycle in the heart, liver, kidneys, diaphragm, brain, and thymus [4, 7, 8, 11, 12, 14, 16, 17]. The increase in the content of the oxidized forms during prolonged administration of hydrocortisone points to stimulation of the synthesis of pyridine nucleotides. Glucocorticoids are known to induce tryptophan-pyrrolase activity [15].

A single injection of hydrocortisone, which disturbs electron transport in the respiratory chain, leads to a decrease in the content of the reduced forms, whereas its prolonged administration, which stimulates synthesis, leads to an increase in the content of the oxidized forms of the pyridine nucleotides.

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