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COMPARATIVE STUDY OF THE ACTION OF LONG-TERM ADMINISTRATION OF NICOTINIC ACID, NICOTINAMIDE, AND DIETHYLNICOTINAMIDE (NIKETHAMIDE) ON THE RAT LIVER MONO-OXYGENASE SYSTEM

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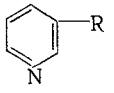
KEY WORDS: liver; microsomal mono-oxygenases; nicotinic acid; nicotinamide; diethylnicotinamide.

Among products of biotransformation of nicotinamide and N-diethylnicotinamide (nikethamide), mainly their N-oxides in the heterocyclic ring are found in the urine of man and animals [2, 3]. Amino acids also are found on incubation of these substances with animal liver microsomes [4]. In different species of animals, formation of the N-oxide of diethylnicotinamide is most stable. For nicotinic acid, oxidation of this type has not been found in microsomes [3, 4]. According to data in the literature [11] nicotinamide can interact with cytochrome P_{450} of liver microsomes to form a type II enzyme–substrate complex.

The data given above may indicate that a cytochrome P_{450}-dependent system participates in biotransformation of nicotinamide and diethylnicotinamide. Meanwhile nicotinamide, like nicotinic acid, is a substrate for synthesis of NADPH [3], essential for its function.

In connection with the facts described above, itwas decided to undertake a comparative study of activity of the mono-oxygenase system of membranes of the endoplasmic reticulum of rat liver cells during repeated injections of nicotinic acid (I), nicotinamide (II), and diethylnicotinamide (III) into the animals:



R: I — $COOH$; II — $CONH_2$; III — $CON(C_2H_5)_2$.

EXPERIMENTAL METHOD

Experiments were carried out in the spring and summer on 56 noninbred male rats weighing 130-170 g. Nicotinic acid, nicotinamide, and diethylnicotinamide were injected into the stomach through a tube once daily for 45 days in equimolar doses: 50, 50, and 73 mg/kg respectively. Control rats received the same volume of water.

The animals were decapitated 24 h after the last injection. The liver microsomal fraction was obtained by the method in [1]. The levels of cytochromes b_5 and P_{450} [9], and activity of NADPH- and NADH-oxidases [5] and oxidoreductases were determined spectrophotometrically on the Specord UV Vis instrument (East Germany). The oxidoreductase function of NADPH-specific flavoprotein (NADPH-ferricytochrome c-oxidoreductase), the middle component of the NADPH-dependent chain of NADH-specific flavoprotein and cytochrome b_5 (NADH-ferricyto-

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TABLE 1. Changes in Activity and Content of Enzymes of NADPH- and NADH-Dependent Redox Chains of Membranes of Endoplasmic Reticulum of Rat Liver Cells and Rate of Xenobiotic Metabolism after Administration of Nicotinic Acid, Nicotinamide, and Diethylnicotinamide (M ± m)

Parameter	Control (100%)	Nicotinic acid	Nicotinamide	Diethylnicotinamide
NADPH-cytochrome c-reductase, μmoles/min/mg	0,20±0,02	0,18±0,01 (88,5)	0,16±0,01 (80,9)	0,26±0,02 (128,7)*
NADPH-nitrotetrazolium reductase, nmoles/min/mg	24,29±1,92	22,81±1,90 (93,9)	23,75±2,13 (97,8)	51,22±3,92 (210,9)*
Cytochrome P_{450}, nmoles/mg	0,85±0,05	0,87±0,06 (102,4)	0,87±0,05 (102,4)	1,36±0,04 (160,0)*
Oxidation of NADPH, nmoles/min/mg	5,67±0,40	4,77±0,25 (84,1)	6,17±0,41 (108,8)	8,69±0,76 (153,3)*
NADH cytochrome c reductase, μmoles/min/mg	0,79±0,06	0,81±0,07 (102,0)	0,52±0,04 (65,8)*	0,96±0,09 (121,0)
NADH-ferricyanide reductase, μmoles /min/mg	3,42±0,23	3,76±0,29 (110,1)	3,47±0,20 (101,5)	4,63±0,19 (135,5)*
Cytochrome b_5 nmoles/mg	0,57±0,02	0,54±0,02 (94,7)	0,57±0,02 (100,0)	0,74±0,01 (129,8)*
Oxidation of NADPH, nmoles/min/mg	3,42±0,22	3,38±0,24 (98,8)	3,43±0,25 (100,3)	2,54±0,18 (74,3)*
N-demethylation of ethylmorphine:				
nmoles/min/mg	10,26±1,54	10,38±1,22 (101,2)	9,56±0,90 (93,2)	16,15±1,79 (157,4)*
nmoles/min/nmole P_{450}	12,07±1,31	11,93±1,49 (98,8)	10,99±0,85 (91,1)	11,88±1,56 (98,4)
N-demethylation of ethylmorphine				
nmoles/min/mg	7,24±1,18	7,53±0,85 (104,0)	7,18±0,95 (99,2)	12,16±1,39 (168,0)*
nmoles/min/nmole P_{450}	8,52±1,00	8,66±0,99 (101,6)	8,25±1,01 (96,8)	8,94±1,21 (104,9)
p-Hydroxylation of aniline				
nmoles/min/mg	0,67±0,11	0,64±0,05 (95,5)	0,81±0,06 (120,9)	0,95±0,09 (141,8)
nmoles/min/nmole P_{450}	0,79±0,12	0,74±0,08 (93,7)	0,93±0,11 (117,7)	0,70±0,08 (88,6)

Legend. Changes (in percent) given in parentheses. *P < 0.05.

chrome b_5-oxidoreductase), was established by NADPH-cytochrome c-, NADPH-nitrotetrazolium-, NADH-ferricyanide-, and NADH-cytochrome c-reductase reactions [1].

N-demethylation activity (type I substrate — amidopyrine, ethylmorphine) was determined by Nash's method [8], and p-hydroxylation activity (type II substrate — aniline) by the method of Kato and Gillette [6].

To determine the character and degree of binding of the substances with the oxidized form of cytochrome P_{450} they were added to the microsomal fraction in a concentration of 2 mM. Differential spectra and the value of K_S (dissociation constant of the enzyme–substrate complex) for nicotinamide and diethylnicotinamide were determined by the method of Kato et al. [7].

EXPERIMENTAL RESULTS

After internal administration of nictonic acid (50 mg/kg) for 45 days the oxidoreductase activity of components of NADPH- and NADH-dependent electron-transport chains, the content of cytochrome P_{450} and b_5, and the rate of N-dealkylating and p-hydroxylating reactions in rat liver microsomes were unchanged. Under the influence of nicotinamide (50 mg/kg) the rate of p-hydroxylation of aniline was increased (by 20%), but not significantly. However, after administration of diethylnicotinamide in an equimolar dose (73 mg/kg) and for the same period, the content of cytochromes P_{450} and b_5 in the microsomes and activity of NADPH-cytochrome c- and NADPH-nitrotetrazolium reductases, and the rate of oxidation of NADPH were increased by 60, 30, 29, 111, and 53%, respectively (Table 1). An increase (by 36%) in the reductase function of the initial stage of the NADPH-dependent redox chain of the microsomes also was increased, but oxidation of NADPH was inhibited under these circumstances.

The rate of N-demethylation of amidopyrine and ethylmorphine and also of p-hydroxylation of aniline was increased under the influence of diethylnicotinamide by 57, 68, and 42%, respectively.

Experiments *in vitro* showed that addition of diethylnicotinamide, like nicotinamide, to the microsomes led to the formation of enzyme–substrate complexes with cytochrome P_{450} of type II. The degree of affinity of diethylnicotinamide for the hemoprotein is twice that of nicotinamide (K_S is 0.59 and 1.05 mM, respectively).

Repeated intragastric administration of diethylnicotinamide into rats thus causes induction of enzymes of the monooxygenase system of endoplasmic reticulum membranes of liver cells. Diethylnicotinamide differs from nicotinic acid and nicotinamide by its stronger lipophilicity, on account of the presence of $-CH_2-CH_3$ groups in its molecule [10], which gives it greater

affinity for cytochrome P_{450}. This enzyme–substrate interaction evidently also lies at the basis of the inductive action of diethylnicotinamide.

The results suggest that repeated administration of nikethamide in clinical practice can lead to weakening of its intrinsic pharmacotherapeutic action and also to a change in the therapeutic effect and toxicity of substances in whose metabolism the mono-oxygenase system of the liver participates.

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LIVER MITOCHONDRIAL DNA TURNOVER IN RATS OF DIFFERENT AGES

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KEY WORDS: aging; liver; mitochondrial DNA; turnover

One of the principal characteristics of aging is a decrease in the intensity of energy metabolism [1, 7]. This decrease may be due to functional insufficiency of the mitochondria. The number of mitochondria in the liver of old animals has been found to decrease, while at the same time the increase in size, so that the ratio of the area of the mitochondrial membranes expressed per unit area of cell or per unit area of mitochondrion falls [14], as also does the activity of several mitochondrial enzymes [15]. These changes lead to a decrease in the adenosine phosphate pool and a decrease in the ATP content and ATP/ADP ratio in the liver of old rats [3]. A search for the causes of these changes in energy metabolism led the writer to postulate age changes in biogenesis of mitochondria. In particular, it was shown that although the rates of synthesis and breakdown of mitochondrial proteins, coded by the nuclear genome, remain unchanged during aging, the rates of these same processes as regards proteins coded by the mitochondrial genome fall in old age [2, 4]. The rate of replication of mitochondrial DNA (mtDNA) in the liver of old rats also decreases [12].

Since cellular homeostasis is maintained by coordination between synthesis and breakdown of macromolecules, the aim of this investigation was to determine the velocity constants of mtDNA turnover in the liver of rats of different ages. Since the mitochondrial population is heterogeneous as regards its sedimentation behavior, morphology, and function [4, 10, 11, 13], fractions of heavy and light mitochondria also were investigated.

EXPERIMENTAL METHOD

Mature (7 months) and old (28 months) female Wistar rats received an intraperitoneal injection of ^{3}H-methylthymidine (USSR origin) in a dose of 400 μCi/100 g body weight. The animals

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