COMPARATIVE STUDY OF THE EFFECT OF NICOTINAMIDE AND DIETHYLNICOTINAMIDE ON MONOOXYGENASE, AND GLUCURONYL- AND GLUTATHIONE-CONJUGATING SYSTEMS OF THE LIVER

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Microsomal monooxygenases and transferases are responsible for phases of biotransformation of xenobiotics by catalyzing biochemical conversions of foreign substances in man and animals. Metabolites formed by cytochrome P-450 acquire nucleophilic groups, which make possible the subsequent formation of conjugates, a process catalyzed by transferases. Since all conjugates (with rare exceptions) are nontoxic, the products of cytochrome P-450-dependent biotransformation of xenobiotics may be more toxic than the original compounds [11]. Besides the formation of metabolites of xenobiotics, the functioning of microsomal monooxygenases is accompanied by generation of superoxide radicals, hydrogen peroxide, and organic peroxides.

In this connection, in order to predict the possible consequences of the action of inducers of these enzymes it is important to know the degree to which they stimulate reactions of microsomal oxidation, compared with their action on conjugation processes.

We give below data on the effect of nicotinamide and diethylnicotinamide on activity of the cytochrome P-450-dependent hydroxylating complex, compared with their action on activity of UDP-glucuronosyl- and glutathione-S-transferases.

EXPERIMENTAL METHOD

In the experiments of series I (on 38 male rats weighing 180-220 g) nicotinamide (50 mg/kg, subcutaneously) or an equimolar amount (75 mg/kg) of diethylnicotinamide was administered in the course of 5 days. Control rats received the corresponding volume of water. The animals were decapitated 24 h after the last injection of the substances, the liver was perfused with cold 1.15% KCl solution, and the microsomal and cytosol fractions were isolated [3]. Concentrations of cytochromes b_5 and P-450 [13], the velocity of N-demethylation of ethylmorphine [12] and of *p*-hydroxylation of aniline [8], and activity of UDP-glucuronosyl-transferase [7] were determined in the microsomes. Activity of glutathione-S-transferase was determined in the microsomes and cytosol [6] and activity of UDP-glucose dehydrogenase in the cytosol [14] were determined. Activity of UDP-glucuronosyl transferase was judged by the decrease in the level of the reaction substrate *p*-nitrophenol (λ = 400 nm) in the medium, and glutathione-S-transferase function by the formation of a conjugate of glutathione with 1-chloro-2,4-dinitrobenzene (λ = 340 nm) in the medium. The protein content in the microsomes and cytosol was determined as in [9].

In the experiments of series II the effect of nicotinamide and diethylnicotinamide (doses and schedule of injection the same as for the experiments of series I) on activity of glucuronyl conjugation in vivo was investigated. For this purpose, in one group of animals (30 male rats weighing 180-210 g), after injection of the preparations, changes in the concentration of free and bound (with endogenous substrates) glucuronic acid [15] were determined in urine collected over a period of 15 h, and in

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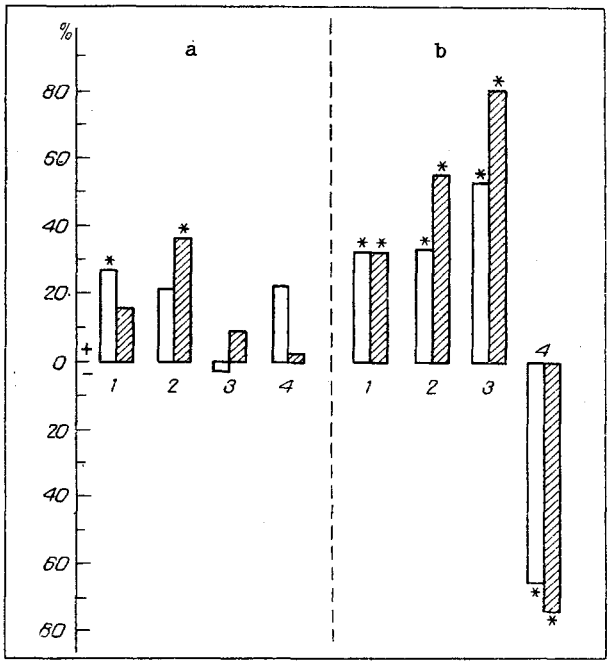

Fig. 1. Changes in activity of monooxygenase (a) and glucuronyl- and glutathione-conjugating (b) systems in the liver of rats receiving nicotinamide (unshaded columns) or diethylnicotinamide (shaded columns), compared with control, taken as 100%. a: 1) Cytochrome b_5, 2) cytochrome P-450; 3) N-demethylation of ethylmorphine; 4) hydroxylation of aniline; b: 1) cytosol glutathione-S-transferase; 2) microsomal glutathione-S-transferase; 3) UDP-glucuronosyl transferase; 4) duration of narcotic action of chloral hydrate. *$p <$ 0.05.

another group (78 male mice weighing 18-22 g) the duration of sleep (the side position of the animals after injection of chloryl hydrate (300 mg/kg, intraperitoneally), inactivation of which in vivo is effected mainly by its conjugation with glucuronic acid [10], were investigated.

In the experiments of series III (30 male rats weighing 200-250 g) the effect of nicotinamide and of diethylnicotinamide (doses and schedules of injection the same as in the previous series of experiments) on activity of conjugation processes with glutathione was studied in the intact animal. For this purpose, under superficial ether anesthesia, the substrate of glutathione-S-transferase, namely sulfobromphthalein (50 mg/kg) was injected by the jet method into the femoral vein, in the form of a 2.5% solution (in 1% NaCl solution). The concentration of sulfobromphthalein in the blood plasma was determined 10 min after its intravenous injection [2].

EXPERIMENTAL RESULTS

Subcutaneous injection of nicotinamide into rats in a dose of 50 mg/kg for 5 days caused an increase in the cytochrome b_5 concentration in the liver microsomes by 26.7%. A tendency was noted for the concentration of cytochrome P-450 and the velocity of *p*-hydroxylation of aniline to increase. The velocity of N-demethylation of ethylmorphine remained unchanged. After injection of an equimolar quantity of desethylnicotinamide under similar conditions an increase in the concentration of cytochrome P-450 was found in the liver microsomes by 37.0%. The level of cytochrome b_5 and the rate of metabolism of the substrates did not change significantly.

TABLE 1. Changes in Plasma Concentration of Sulfobromphthalein and Excretion of Glucuronic Acid with the Urine after Subcutaneous Injection of Nicotinamide (50 mg/kg) and Diethylnicotinamide (75 mg/kg, $M \pm m$) Daily for 5 Days

Parameter	Control	Nicotinamide	Diethylnicotinamide
Sulfobromophthalein, mg/100 ml plasma	19,13±1,31	17,97±0,29 (94)	14,44±1,33* (75)
Glucoronic acid, mg/portion:			
total	2,97±0,29	2,88±0,21 (97)	3,75±0,52 (126)
bound	1,80±0,18	2,35±0,19* (131)	2,68±0,4* (149)
bound total	0,61	0,81	0,71

Legend. Changes relative to control, taken as 100%. $*p < 0.05$.

More marked changes were observed in activity of the enzyme systems of xenobiotic conjugation. Under the influence of the injected nicotinamide, an increase in glutathione-S-transferase activity of 33.1 and 33% was observed in the cytosol and microsomes respectively, accompanied by an increase of 53.0% in UDP-glucuronosyl transferase. After injection of diethylnicotinamide into rats activity of cytosol and microsomal glutathione-S-transferases and of UDP-glucuronyl transferase rose by 33.1, 54.6, and 80.5% respectively compared with intact animals (Fig. 1).

It was shown in vivo (Table 1) that under the influence of nicotinamide the plasma level of injected sulfobromphthalein showed a tendency to fall, whereas after injection of diethylnicotinamide the fall was significant (by 24.5%). The 15-hourly excretion of glucuronides, determined from the concentration of bound glucuronic acid, in rats receiving nicotinamide and diethyl nicotinamide was higher than in the control by 31 and 49% respectively. Excretion of total glucuronic acid and the volume of urine remained unchanged under these circumstances. The ratio of bound glucuronic acid to its total volume, reflecting the effectiveness of glucuronic conjugation of endogenous substances in the experimental animals, increased from 0.61 to 0.81 (nicotinamide) and 0.71 (diethylnicotinamide) respectively.

The duration of chloral hydrate-induced sleep in mice receiving a preliminary injection of nicotinamide (100 mg/kg) or diethylnicotinamide (75 mg/kg) was shortened by 65 and 75% respectively.

Shortening of the duration of chloral hydrate-induced sleep under the influence of nicotinamide and diethylnicotinamide was evidently connected with acceleration of its conversion in vivo into trichloroethanol-glucuronide, and not with functional antagonism (at the brain level) of these substances, for the chloral hydrate was injected 24 h after the last injection of the substances.

Thus nicotinamide and diethylnicotinamide, if injected subcutaneously in doses of 50 and 75 mg/kg daily for 5 days, increase activity of microsomal monooxygenases, and also of the glucuronyl- and glutathione-conjugating systems in the liver both in vitro and in vivo. More marked changes were observed in activity of enzymes involved in xenobiotic conjugation (especially with glucuronic acid). The enzyme-stimulating action of diethylnicotinamide was stronger than that of nicotinamide.

An increase in the content of cytochromes P-450 and b_5 in the microsomal membranes under the influence of nicotinamide and diethylnicotinamide can evidently be attributed to their substrate induction [1].

An explanation of the stimulating effect of these preparations on activity of xenobiotic conjugation with glucuronic acid and glutathione must take into account the possible role of nicotinamide and diethylnicotinamide in the provision of substrate for UDP-glucuronyl- and glutathione-S-transferases. Support for this view is given by data on the involvement of nicotinamide in the reactions of biosynthesis of UDP-glucuronic acid and glutathione [5]. Diethylnicotinamide evidently also possesses similar properties. In a study of its effect (75 mg/kg, 5 days) on UDP-glucose-dehydrogenase activity in the cytosol fraction of rat liver — the key enzyme of UDP-glucuronic acid biosynthesis — an increase of activity by 154.6% was found. These data are in agreement with the previously established fact of an increase in the content of glycogen [4], used for synthesis of UDP-glucuronic acid, in the hepatocytes of rats receiving diethylnicotinamide for a long time.

The predominantly stimulating action of nicotinamide and, in particular, of diethylnicotinamide on xenobiotic conjugation, compared with their effect on microsomal oxidation, is important in connection with the possible use of these compounds as components of the mechanism aimed at preventing accumulation of foreign substances in the body and promoting their detoxication.

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