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Stress and the maintenance of nicotinamide-induced hepatic nicotinamide adenine dinucleotide elevation by chlorpromazine

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IT IS KNOWN that the administration of nicotinamide, a precursor of the pyridine moiety of NAD, will result in a transient elevation of hepatic NAD levels in the mouse and rat. Burton *et al.*[1] observed that the administration of sedative doses of chlorpromazine or reserpine to mice 4 hr prior to the administration of nicotinamide results in prolongation of the nicotinamide-induced elevation of hepatic NAD. The administration of chlorpromazine or reserpine alone did not produce a detectable change in hepatic NAD levels compared to saline-treated controls. Similar results have been obtained with derivatives of chlorpromazine and reserpine such as deserpidine and promazine.[1, 2] A positive correlation has been observed between the tranquilizing activity of these compounds and their ability to prolong nicotinamide-induced hepatic NAD elevation. Burton *et al.*[2] have also shown that non-tranquilizing sedatives such as meprobamate, phenobarbital, and ethanol are unable to maintain elevated hepatic NAD levels even though doses were employed which maintained the mice in a comatose state.

It has been suggested that the relationship between reserpine or chlorpromazine and hepatic NAD levels after nicotinamide administration may be implicated in the mechanism of tranquilization or, at least, may be a reflection of a common biochemical action, since nontranquilizing congeners of these drugs are without effect in modifying NAD levels.

The pituitary–adrenal axis has also been demonstrated to exert an influence upon the level of hepatic NAD; the most pronounced effects are observed after the administration of nicotinamide. The administration of nicotinamide to hypophysectomized or adrenalectomized rats results in a much greater and more prolonged elevation in hepatic NAD levels than in intact controls.[3] Since both chlorpromazine and reserpine have been shown to produce adrenal ascorbic acid depletion and plasma corticosterone elevation,[4] it was decided to investigate the effect of other treatments which influence the pituitary–adrenal axis upon nicotinamide-induced hepatic NAD elevation.

METHODS

Male Holtzman rats (120–220 g) were maintained in communal cages housing 12–15 animals with free access to laboratory chow and water. After receipt from the supplier, rats were acclimatized for at least 1 week prior to use as experimental subjects. Hepatic NAD levels were determined spectrophotometrically by the procedure described by Ciotti and Kaplan[5] for the Racker alcohol dehydrogenase assay. This procedure is specific for determination of oxidized NAD. Adrenal ascorbic acid levels were determined spectrophotometrically by an adaptation[6] of the method described by Sullivan and Clarke.[7]

All drugs were administered 4 hr prior to nicotinamide (500 mg/kg) and were dissolved in distilled water in sufficient concentration so that injection of 1 ml solution/kg body wt. provided the desired dosage. Nicotinamide, chlorpromazine hydrochloride (25 mg/kg), and chlorpromazine sulfoxide hydrochloride (25 mg/kg) were injected i.p. ACTH (250 mU/rat) was administered i.v. via the

lateral tail vein. Hind leg ligation stress was carried out by tying a rubber band around one hind leg 4 hr prior to nicotinamide injection. The ligature was removed at the time of nicotinamide administration. In some experiments tolerance to the effects of chlorpromazine was studied after administration of this drug for 4 days (15 mg/kg daily) with a challenging dose of 25 mg/kg on the fifth day.

Adrenal ascorbic acid determinations were performed on 6 rats from each group 2 hr after drug pretreatment or stress in order to estimate the degree of pituitary–adrenal activation elicited. Nicotinamide was administered to the remaining animals in each group 4 hr after drug pretreatment or stress. Hepatic NAD levels were determined at 12 and 24 hr after nicotinamide administration.

The significance of differences between treatment and control means was determined by using the t statistic derived by Dunnett.[8]

RESULTS AND DISCUSSION

Data in Table 1 indicate that pretreatment with a single dose of chlorpromazine results in a significant ($P < 0.025$) elevation of nicotinamide-stimulated hepatic NAD levels and a significant ($P < 0.001$) decrease in adrenal ascorbic acid levels. Conversely, repeated pretreatment with chlorpromazine

TABLE 1. EFFECT OF CHLORPROMAZINE PRETREATMENT ON HEPATIC NAD AND ADRENAL ASCORBIC ACID IN THE RAT 12 HR AFTER NICOTINAMIDE ADMINISTRATION

Pretreatment	Hepatic NAD (μg NAD/g liver ± S.E.M.)	Adrenal ascorbic acid (mg/100 ml ± S.E.M.)
Experiment 1: pretreatment followed by nicotinamide (500 mg/kg, i.p.)		
Saline	1128 ± 78 (12)*	505 ± 33 (6)
Chlorpromazine	1502 ± 80 (12)	238 ± 32 (6)
Repeated chlorpromazine	1166 ± 109 (12)	360 ± 28 (6)
Experiment 2: animals received indicated treatment only (no nicotinamide)		
Untreated (basal levels)	493 ± 43 (24)	480 ± 21 (10)
Repeated chlorpromazine	554 ± 14 (12)	356 ± 17 (6)

* Number of animals per group.

had no significant effect upon hepatic NAD elevation in response to nicotinamide. Adrenal ascorbic acid levels were not depleted to the same extent after repeated chlorpromazine administration when compared to a single injection of chlorpromazine. Repeated chlorpromazine administration had no significant effect upon basal hepatic NAD levels. These results thus suggest that the stress from a single dose of chlorpromazine may be implicated in the mechanism of prolongation of elevated levels of hepatic NAD after nicotinamide administration. It is known that tolerance to the adrenal ascorbic acid depleting action of chlorpromazine occurs readily in the rat.[9] Yuwiler *et al.*[10] have shown that the administration of a single dose of chlorpromazine (25 mg/kg) to the rat results in adrenocortical activation, as evidenced by a significant decrease in adrenal ascorbic acid and an increase in plasma corticoids. Significant elevation of plasma corticoid levels may persist for as long as 17 hr after the administration of a single dose of chlorpromazine.

Table 2 compares the effect of chlorpromazine and hind limb ligation as stressors upon adrenal ascorbic acid levels and hepatic NAD elevation. Both chlorpromazine and hind limb ligation produce

TABLE 2. EFFECT OF CHLORPROMAZINE AND HIND LIMB LIGATION ON HEPATIC NAD AND ADRENAL ASCORBIC ACID IN THE RAT 12 HR AFTER NICOTINAMIDE ADMINISTRATION

Pretreatment	Hepatic NAD (μg NAD/g liver ± S.E.M.)	Adrenal ascorbic acid (mg/100 ml ± S.E.M.)
Saline	1228 ± 172 (9)*	498 ± 20 (6)
Chlorpromazine	1706 ± 151 (9)	309 ± 51 (6)
Hind limb ligation	1703 ± 268 (9)	358 ± 24 (6)

* Number of animals per group.

stress as evidenced by a significant ($P < 0.005$) degree of adrenal ascorbic acid depletion as well as an ($P < 0.1$) elevation in hepatic NAD levels.

If the stress-producing effect of chlorpromazine or hind limb ligation is involved in the mechanism of elevation of hepatic NAD levels, a similar effect should be produced by ACTH, but not by chlorpromazine sulfoxide, which lacks sedative effects and does not produce activation of the pituitary–adrenal axis.[11] Table 3 indicates that ACTH administration is also able to potentiate nicotinamide-induced elevation of hepatic NAD, but chlorpromazine sulfoxide had no significant effect. There

TABLE 3. EFFECT OF ACTH, CHLORPROMAZINE, AND CHLORPROMAZINE SULFOXIDE PRETREATMENT ON HEPATIC NAD AND ADRENAL ASCORBIC ACID IN THE RAT 12 HR AFTER NICOTINAMIDE ADMINISTRATION

Pretreatment	Hepatic NAD (μg NAD/g liver $\pm$ S.E.M.)	Adrenal ascorbic acid (mg/100 ml $\pm$ S.E.M.)
Saline	1144 $\pm$ 109 (9)*	377 $\pm$ 45 (6)
ACTH	1981 $\pm$ 149 (9)	272 $\pm$ 14 (6)
Chlorpromazine	1883 $\pm$ 133 (9)	200 $\pm$ 12 (6)
Chlorpromazine sulfoxide	1253 $\pm$ 104 (9)	429 $\pm$ 8 (6)

* Number of animals per group.

appears to be little difference between the effects of hind limb ligation, chlorpromazine administration, and ACTH administration upon nicotinamide-induced elevation of hepatic NAD. When the effects of chlorpromazine, ACTH, or hind limb ligation upon nicotinamide-induced hepatic NAD elevation are measured at 24 hr, significant ($P < 0.005$) elevations above saline controls are seen (Table 4). There is still evidence of adrenal ascorbic acid depletion in those groups receiving chlorpromazine ($P < 0.1$) or subjected to hind limb ligation ($P < 0.05$).

TABLE 4. EFFECT OF ACTH, CHLORPROMAZINE, AND HIND LIMB LIGATION ON HEPATIC NAD AND ADRENAL ASCORBIC ACID IN THE RAT 24 HR AFTER NICOTINAMIDE ADMINISTRATION

Pretreatment	Hepatic NAD (μg NAD/g liver $\pm$ S.E.M.)	Adrenal ascorbic acid (mg/100 ml $\pm$ S.E.M.)
Saline	538 $\pm$ 23 (9)*	454 $\pm$ 21 (6)
Chlorpromazine	1603 $\pm$ 75 (9)	378 $\pm$ 28 (6)
ACTH	855 $\pm$ 46 (9)	451 $\pm$ 40 (6)
Hind limb ligation	1147 $\pm$ 83 (9)	360 $\pm$ 26 (6)

* Number of animals per group.

Chlorpromazine, hind limb ligation, or ACTH administered 4 hr prior to nicotinamide potentiates the nicotinamide-induced elevation of hepatic NAD measured 12 or 24 hr later. Each of these procedures causes activation of the pituitary–adrenal axis as indicated by adrenal ascorbic acid depletion. Nicotinamide itself was found to be a stressor by the same criterion. The ability of the rat to respond to nicotinamide injection with the typical stress response may play an important role in the hepatic NAD elevation caused by nicotinamide.

It is suggested that the activity of chlorpromazine in maintaining nicotinamide-induced hepatic NAD elevation may be due primarily to the effect of this drug upon the pituitary–adrenal axis and does not necessarily represent a component of its central nervous system activity.

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Nitrofurans and enzyme systems of the neonatal animal

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THE UDP GLUCURONYL transferase enzyme system is poorly developed in the human newborn. This has led to impaired detoxification of drugs which are excreted as glucuronide conjugates[1, 2] and to an increase in neonatal hyperbilirubinemia when drugs which interfere with the enzyme function are used.[3, 4] Fouts and Adamson[5] have shown that a number of drugs that are metabolized in the adult by enzymes found in the hepatic microsomes are not metabolized by the livers of newborn rabbits. The present study is concerned with the effect of certain nitrofurans on glucuronyl transferase of rat liver homogenate and with a comparison of the ability of maternal and neonatal liver and kidney preparations of rats and rabbits to degrade three nitrofurans: nitrofurantoin, 1-[(5-nitrofurfurylidene)amino] hydantoin; furazolidone, 3-[(5-nitrofurfurylidene)amino]-2-oxazolidinone; and nifuradene, 1-[(5-nitrofurfurylidene)amino]-2-imidazolidinone.

METHODS

Formation of bilirubin glucuronide was studied in a system similar to that used by Lathe and Walker.[6] Novobiocin and chloramphenicol, known inhibitors of glucuronyl transferase[3, 4] and the sodium salt of nitrofurantoin were added as solutions in alkaline KCl.[7] Because of limiting solubility, furazolidone and nifuradene were added as 1 mg dry powder per flask, and their concentrations were determined spectrophotometrically[8] after incubation in control samples from which the bilirubin had been omitted.

For the nitrofuran degradation studies, the liver and kidney samples were removed rapidly from neonatal and maternal rats after death by decapitation. The maternal rats were stock animals about 11 months of age. The neonatal rats ranged from 2 hr to 3 days in age. Tissues were placed in chilled 0·067 M phosphate buffer, pH 7·4 and processed as rapidly as possible. Livers and kidneys from each litter of rats were pooled. Attempts to slice the neonatal tissue resulted in a mince, and the corresponding maternal tissue was subdidvided to a similar surface area. The incubation medium was 0·067 M phosphate buffer, pH 7·4, containing 100 mg glucose/100 ml (control) or a solution of nitrofuran (22–46 mg/l.) in the control medium. Maternal tissue weights were matched to neonatal tissue weights for each experiment. Liver samples ranged from 320–429 mg/flask and were incubated in 2·5 ml of the appropriate medium. Kidney samples ranged from 143–277 mg and were incubated