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ALTERATION OF NOREPINEPHRINE METABOLISM BY BARBITURATES

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Abstract—Competitive inhibition of NAD-linked aldehyde dehydrogenase by acetaldehyde, the primary metabolite of ethanol, enhances the formation *in vitro* of tetrahydropapaveroline (THP), a tetrahydroisoquinoline (THIQ) alkaloid derived from the condensation of dopamine (DA) with 3,4-dihydroxyphenylacetaldehyde. Unlike the DA-derived aldehyde, the aldehyde derivative of norepinephrine (NE) is not appreciably oxidized in brain tissue to the corresponding acid by brain aldehyde dehydrogenase, but is primarily reduced to the glycol, 3,4-dihydroxyphenylglycol (DHPG), by an NADPH-dependent aldehyde reductase. Since it has been demonstrated that this partially purified aldehyde reductase from bovine brain is inhibited by barbiturates, an investigation was conducted to define the effects of barbiturates on the over-all metabolism of ^{14}C-NE and its aldehyde by rat brainstem homogenates. In the absence of exogenous NAD or NADPH, the major metabolite of deaminated NE was found in a fraction that would contain the THIQ alkaloids. A smaller portion of the deaminated NE was isolated as the DHPG and the 3,4-dihydroxymandelic acid (DHMA) metabolites. A substantial amount of the deaminated NE was also accounted for as the free aldehyde. Adding NADPH markedly increased DHPG formation while decreasing alkaloid synthesis. Incorporating barbiturates into incubation mixtures containing NADPH, or a mixture of NAD and NADPH, appreciably inhibited DHPG production, thereby enhancing free glycolaldehyde levels and augmenting alkaloid formation. Thus, barbiturates—like ethanol, as mediated by acetaldehyde—markedly modify neuroamine-derived aldehyde metabolism.

THE DEAMINATION of biogenic amines, mediated by monoamine oxidase (MAO), results in the formation of their respective aldehyde derivatives. These derivatives are subject to oxidation to the corresponding acid metabolites by an NAD-dependent aldehyde dehydrogenase.[1,2] Certain amine-derived aldehydes can also be reduced to the corresponding alcohol or glycol metabolites by the NAD/NADH-dependent alcohol dehydrogenase in liver or by an NADPH-linked aldehyde reductase in brain.[3,4]

The metabolic option in brain appears to be dependent upon the structural characteristics of the amine-derived aldehyde. The aromatic aldehyde intermediates of such phenylethylamines as tyramine and dopamine (DA) are primarily oxidized to the corresponding acids. On the other hand, the β-hydroxylated aldehydes—e.g. those derived from octopamine and norepinephrine (NE)—are largely reduced to glycol derivatives.[5–7]

Interruption of the major degradative pathways of phenylethylamine or β-hydroxylated phenylethylamine-derived aldehydes by inhibition of aldehyde dehydrogenase or aldehyde reductase, respectively, could be expected to alter markedly the intermediary metabolism of these aldehydes in brain tissue. Acetaldehyde, the primary meta-

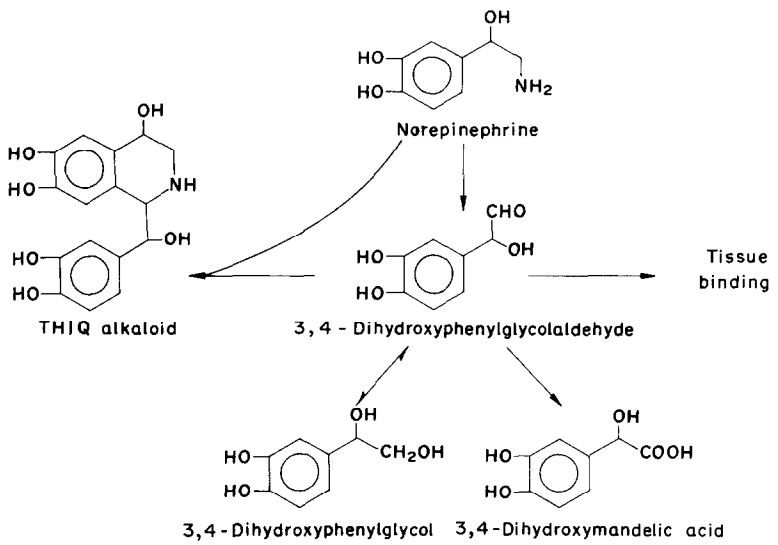

FIG. 1. Schematic representation showing pathways of norepinephrine and 3,4-dihydroxyphenylglycolaldehyde metabolism.

bolite of ethanol, competitively inhibits oxidation of amine-derived aldehydes.[8] Chloral hydrate is also a potent inhibitor of brain aldehyde dehydrogenase.[2] These inhibitors disrupt the major route for disposition of DA's aldehyde derivative in brain tissue—a disruption demonstrated by decreased oxidation of the biogenic aldehyde to the corresponding acid, increased steady state levels of the biogenic aldehyde, and enhanced formation of tetrahydropapaveroline (THP).[9,10] THP is a pharmacologically active tetrahydroisoquinoline (THIQ) alkaloid, which is formed by the condensation of DA and the aldehyde derived from the oxidative deamination of another DA molecule.[11–18]

Barbiturates have been shown to be inhibitors of an NADPH-linked aldehyde reductase partially purified from bovine brain.[3,19] Thus, it is conceivable that barbiturate intervention may, to an important extent, evoke aberrant metabolism of the aromatic aldehydes derived from β-hydroxylated phenylethylamines by brain tissue.

The present investigation was conducted to study *in vitro* the effects of barbiturates on NE metabolism (Fig. 1). The study sought to determine if NE metabolism was subject to a concatenation similar to that created by modifying the dominant route of DA metabolism by aldehyde dehydrogenase inhibitors. A preliminary account of this investigation has been presented.[20]

METHODS

Incubation procedure. Brains or livers were rapidly removed from male rats, Sprague–Dawley descendants, weighing 175–250 g (Texas Inbred Mice Co., Houston, Texas), which had been killed by decapitation. The cerebellum and cerebral hemispheres were discarded, and 10% brainstem or 5% liver homogenates were prepared with ground glass homogenizers in 0·067 M phosphate buffer (pH 7·4) containing 0·5 mg ascorbic acid/ml.

The reaction mixtures consisted of 1-ml portions of the homogenates, equivalent to 100 mg wet wt of brainstem or 50 mg wet wt of liver, 1 ml NE substrate (0·5 μCi DL-NE-7-^{14}C, Amersham–Searle Corp., Arlington Heights, Ill., sp. act. 54 mCi/mmole, plus 12 μmoles NE hydrochloride), and 2 ml of 0·067 M phosphate buffer (pH 7·4) containing 0·5 mg ascorbic acid/ml. Additions of pyridine nucleotide coenzymes or barbiturates were made in 1 vol. of 1 ml at the expense of the phosphate buffer, and the total vol. of the incubation mixtures was maintained at 4·0 ml. Reactions were initiated by adding the NE substrate, the barbiturates having been introduced 2 min earlier. All components were prepared in the phosphate buffer immediately before use. Incubations were carried out for 60 or 90 min in 25-ml Erlenmeyer flasks exposed to air in a shaking water bath at 37·5°.

The reaction mixtures were deproteinized by adding 0·25 ml of 45% perchloric acid. Control samples, to which the perchloric acid was added before the NE substrate, were included in each experiment. After deproteinization, the mixtures were centrifuged at 4°, the supernatants were adjusted to pH 5 with 5 N potassium carbonate, and the perchlorate salts were removed by centrifugation. The supernatants thus obtained were then adjusted to pH 8·4 with sodium hydroxide and diluted to 15 ml.

Isolation of radioactive NE metabolites. The amounts of NE and the THIQ alkaloid(s) formed by condensation of NE with 3,4-dihydroxyphenylglycolaldehyde (DHPGA), as well as the deaminated NE metabolites—i.e. 3,4-dihydroxyphenylglycol (DHPG), 3,4-dihydroxymandelic acid (DHMA) and DHPGA—were determined by the procedure described for assay of labeled DA and its corresponding metabolites.[9] The determinations involved isolation of the catechol compounds on alumina; separation of the bases, NE and THIQ derivative of NE, on the cation exchange resin, Dowex AG-50W; and separation of the deaminated NE catechol metabolites with the anion exchange resin, Amberlite CG-4B. The only modification of the procedure consisted in the differential elution of NE and the THIQ derivative of NE from the cation exchange resin. Complete elution of NE was achieved with 50 ml of 0·5 N HCl, which contained 10 μg EDTA and 1 μg ascorbic acid/ml. The THIQ alkaloid derivative of NE was then eluted with 10 ml of 3 N HCl in 50% ethanol.

Radioactivity in the effluents and eluates from the different columns was measured by liquid scintillation spectrometry. The composition of the scintillation fluid was 0·2 g 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP), 4 g 2,5-diphenyloxazole (PPO), 333 ml Triton X-100 (Rohm & Haas, Co., Philadelphia, Pa.) and 667 ml toluene. The specific activity of each NE substrate preparation was established for each experiment.

Thin-layer chromatography. Glass plates, 50 by 200 mm, were coated with 250 μm of Silica gel G. The layer was applied as a slurry consisting of 30 g Silica gel G suspended in 60 ml of 0·067 M sodium phosphate buffer (pH 6·8) containing 1% sodium bisulfite. The sodium bisulfite was incorporated to prevent possible oxidation of NE and the alkaloid metabolites. The plates were air dried for 20 min and then heated in an oven at 110° for 30 min. Aliquots of both the NE and alkaloid fractions were evaporated *in vacuo*. The residue was dissolved in a small volume of 0·1 N HCl in 95% methanol and applied to the chromatographic plates. The chromatograms were developed in a butanol–acetic acid–water (4:1:1) system under N_2 for 15 cm in glass chambers lined with solvent-saturated filter paper. Ferric chloride–potassium ferri-

cyanide (10% aq. ferric chloride–5% aq. potassium ferricyanide–water, 2:1:8) was used for visualization.

Gas chromatography. Analyses were performed with a Barber–Coleman (series 5000) gas chromatograph equipped with a flame ionization detector. Gas chromatographic conditions were: 6-ft U-shaped glass column (i.d. 4 mm) packed with 3% OV-1 on Gas Chrom Q, 100–120 mesh, detector temperature 290° and nitrogen flow rate 60 ml/min. The basic metabolites of NE were analyzed under isothermal conditions at 250°.

Aliquots of the alkaloid fraction obtained from the incubation mixtures were dried *in vacuo*. Each residue was reconstituted in 1 ml of 0·1 N HCl in 95% methanol and transferred to a reaction vial. The samples were again dried *in vacuo*. Trimethylsilyl derivatives were formed by allowing the alkaloid metabolites to react with hexamethyldisilazane (90 μl) and trimethylchlorosilane (10 μl) at 65° for 6 hr. A 20 μl aliquot of an authentic THP standard (1 μg/μl), prepared in the acid-methanol, was similarly dried and reacted. The retention times of the alkaloid metabolites of NE and THP were then determined.

RESULTS

Effect of pyridine nucleotide coenzymes on NE metabolism by rat liver homogenates. Incubation of rat liver homogenates with NE, in the absence of exogenous pyridine nucleotide coenzymes, resulted in the accumulation of large quantities of DHPGA, representing 55 per cent of the total metabolites. The oxidation product of the glycolaldehyde, DHMA, comprised 26 per cent of the metabolites, and the THIQ alkaloid formed by condensation of NE and DHPGA, 16 per cent. The formation of DHPG was minimal at only 2·5 per cent of the total catabolites (Table 1).

Incorporation of NAD, NADH or NADPH—the cofactors for the oxidation of the glycolaldehyde metabolite of NE by aldehyde dehydrogenase or for the reduction of the glycolaldehyde by alcohol dehydrogenase or aldehyde reductase—produced a marked alteration in the pattern of NE catabolites in incubation mixtures of rat

TABLE 1. EFFECT OF VARIOUS PYRIDINE NUCLEOTIDE COENZYMES ON NE METABOLISM BY RAT LIVER HOMOGENATES*

Condition	% Total metabolites determined†			
	THIQ	DHPG	DHMA	DHPGA
Control	16·3 ± 1·4	2·6 ± 0·3	25·9 ± 0·7	55·3 ± 0·6
NAD	4·7 ± 0·8‡	7·1 ± 0·5‡	51·7 ± 0·5§	36·5 ± 0·9§
NADH	10·1 ± 1·3	10·5 ± 0·6§	41·3 ± 1·2§	38·1 ± 1·2§
NADPH	13·9 ± 0·8	26·8 ± 1·2§	25·5 ± 0·4	33·8 ± 0·5§

* Control incubation mixtures consisted of 1 ml rat liver homogenate (equiv. 50 mg liver), 1 ml NE substrate (0·5 μCi NE-^{14}C, sp. act. 54 mCi/m-mole, plus 12 μmoles NE hydrochloride) and 2 ml of 0·067 M phosphate buffer containing 0·5 mg ascorbic acid/ml, pH 7·4. Additions of indicated pyridine nucleotide coenzymes (2 μmoles) were made at expense of buffer. All components were prepared in buffer immediately before use. Incubation was for 60 min at 37·5°.

† Each value represents mean ± S. E. M. of five determinations. The total metabolites, expressed as nmoles for each incubation condition, were as follows: control, 545·6 ± 9·7; NAD, 426·3 ± 7·0; NADH, 434·4 ± 15·5; NADPH, 438·6 ± 18·2.

‡ $P < 0.01$.

§ $P < 0.001$.

TABLE 2. EFFECTS OF VARIOUS PYRIDINE NUCLEOTIDE COENZYMES ON NE METABOLISM BY RAT BRAINSTEM HOMOGENATES*

Condition	% Total metabolites determined†			
	THIQ	DHPG	DHMA	DHPGA
Control (35)	25·3 ± 0·8	4·7 ± 0·5	17·8 ± 0·6	52·3 ± 1·4
Semicarbazide (5)	13·4 ± 0·4‡	4·1 ± 0·8	17·4 ± 1·0	65·2 ± 1·0‡
NAD (10)	24·1 ± 1·2	6·1 ± 1·3	23·0 ± 0·7‡	46·9 ± 1·2
NADH (5)	21·2 ± 0·5	3·7 ± 0·3	22·8 ± 0·3§	52·1 ± 1·0
NADPH (20)	11·7 ± 0·8‖	48·7 ± 1·5‖	10·6 ± 0·6‖	28·9 ± 1·4‖
NAD + NADPH (20)	12·9 ± 0·9‖	52·5 ± 1·1‖	9·7 ± 0·6‖	24·9 ± 1·7‖

* Control incubation mixtures consisted of 1 ml rat brainstem homogenate (equivalent to 100 mg tissue), 1 ml NE substrate (0·5 μCi NE-^{14}C, sp. act, 54 mCi/m-mole, plus 12 μmoles NE hydrochloride) and 2 ml of 0·067 M phosphate buffer containing 0·5 mg ascorbic acid/ml, pH 7·4. Additions of the pyridine nucleotide coenzymes (2 μmoles) and semicarbazide (10 μmoles) were made at expense of buffer. All components were prepared in buffer immediately before use. Incubation was for 90 min at 37·5°.

† Number of determinations are given in parentheses. Each value represents mean ± S. E. M. The total metabolites, expressed as nmoles for each incubation condition, were as follows: control, 409·3 ± 14·7; semicarbazide, 257·4 ± 9·9; NAD, 278·9 ± 11·5; NADH, 366·2 ± 8·3; NADPH, 423·6 ± 22·7; NAD + NADPH, 450·0 ± 21·8.

§ $P < 0.05$.

‡ $P < 0.01$.

‖ $P < 0.001$.

liver homogenates. Adding NAD, the cofactor for aldehyde dehydrogenase, significantly enhanced DHMA formation to 52 per cent ($P < 0.001$) and modestly increased the level of DHPG to 7 per cent ($P < 0.01$). It simultaneously diminished the THIQ alkaloid production to only 5 per cent ($P < 0.01$) and the free DHPGA level to 37 per cent ($P < 0.001$) of the metabolites. Adding NADH, the cofactor for the reduction of the glycolaldehyde by alcohol dehydrogenase, resulted in increased DHPG formation to about 11 per cent ($P < 0.001$) of the NE catabolites and DHMA formation to 41 per cent ($P < 0.001$). The DHPGA level decreased to 38 per cent ($P < 0.001$) and THIQ alkaloid formation diminished by a modest degree. Supplementing the incubation mixtures with NADPH, the cofactor for aldehyde reductase, diverted glycolaldehyde metabolism to the reductive path, thus raising DHPG levels to almost 27 per cent ($P < 0.001$) of the metabolites. Consequently, the amounts of metabolites present as DHPGA were attenuated to almost 34 per cent ($P < 0.001$), whereas THIQ alkaloid production decreased slightly.

Effect of pyridine nucleotide coenzymes on NE metabolism by rat brainstem homogenates. The metabolism of NE by unsupplemented incubation mixtures of rat brainstem homogenates (Table 2) differed from the pattern observed with rat liver homogenates. DHMA comprised approx. 18 per cent of the NE catabolites obtained with the former, but about 26 per cent of the metabolites produced by the latter. Although DHPG and DHPGA levels were similar with both tissue preparations, comparison of the two tissues revealed decreased DHMA formation and increased THIQ alkaloid generation by brainstem, accounting for 25 per cent of the NE metabolites in comparison with approximately 16 per cent generated by liver.

Unlike the marked effect of NAD, NADH and NADPH on the distribution of NE metabolites by rat liver homogenates, only NADPH strongly influenced the metabolic disposition of NE by rat brainstem homogenates. Adding NAD or NADH to

brainstem incubation mixtures moderately increased DHMA formation from about 18 per cent in unsupplemented mixtures to 23 per cent ($P < 0.01$ for NAD and $P < 0.05$ for NADH) of the NE catabolites. No significant change in aldehyde, glycol or THIQ alkaloid metabolite production was effected with these cofactors.

In the presence of exogenous NADPH, the activity of brainstem aldehyde reductase was unmasked; DHPG became the dominant metabolite, comprising approximately 48 per cent ($P < 0.001$) of the NE catabolites. Because glycolaldehyde metabolism was diverted to the reductive route, the level of the free DHPGA was decreased to about 29 per cent ($P < 0.001$), DHMA accounted for only about 11 per cent, and the diminished glycolaldehyde levels depressed THIQ alkaloid formation to only 12 per cent ($P < 0.001$) of the NE catabolites. Adding both NAD and NADPH to the brainstem incubation mixtures produced the same metabolite distribution pattern that was observed when NADPH alone was added.

THIQ alkaloid formation depended upon the availability of glycolaldehyde for condensation with NE. In the presence of exogenous NADPH, therefore, the glycol-

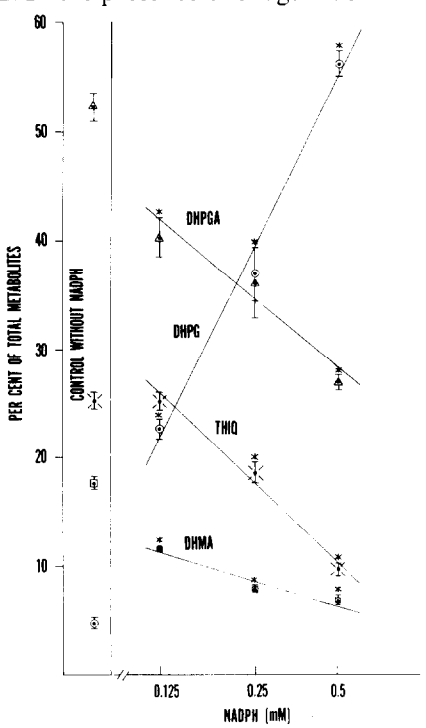

FIG. 2. Effect of NADPH (abscissa, log scale) on the metabolism of norepinephrine (NE) by rat brainstem homogenates. The 4-ml reaction mixtures consisted of 1 ml of 10 per cent rat brainstem homogenate, 1 ml NE substrate consisting of 0·5 μCi/NE-^{14}C (sp. act. 54 mCi/m-mole) plus 12 μmoles unlabeled NE hydrochloride, the indicated amounts of NADPH cofactor, and 0·067 M phosphate buffer, pH 7·4. All components were prepared in the phosphate buffer. The systems were incubated for 90 min at 37·5° with shaking. Each point represents mean per cent of total NE metabolites present as indicated compounds; the vertical bars, S. E. M. Number of determinations: control, 35; each of various cofactor concentrations, 5. The asterisk (*) denotes a significant difference from the control mean. THIQ, tetrahydroisoquinoline alkaloid formed by condensation of NE and the aldehyde metabolite of NE; DHPG, dihydroxyphenylglycol; DHMA, dihydroxymandelic acid; DHPGA, dihydroxyphenylglycolaldehyde. The total metabolites, expressed as nmoles for each concentration of NADPH, were as follows: control, 409·3 ± 14·7; 0·125 mM NADPH, 463·2 ± 12·5; 0·25 mM NADPH, 479·9 ± 4·4; 0·5 mM NADPH, 560·7 ± 14·2.

aldehyde was rapidly converted to DHPG, and synthesis of the aberrant alkaloid was consequently depressed. THIQ alkaloid production was also restricted when the free aldehyde was trapped as a semicarbazone (Table 2). These findings are in accord with the effects of cofactors of the major (i.e. oxidative) pathway for the disposition of the aldehyde derivative of DA.[9] In the absence of exogenous NAD, THP was the predominant catabolite of DA with brainstem homogenates, representing 70 per cent of the DA deaminated while the aldehyde derivative and dihydroxyphenylacetic acid each represented 3 per cent. Even when NAD was added, THP constituted the major metabolite of DA by brainstem homogenates (51 per cent) and the formation of the acid derivative increased to 10 per cent. These results contrast to liver where THP was only a minor metabolite (2 per cent) when exogenous NAD was present, and formation of dihydroxyphenylacetic acid represented almost 66 per cent of the DA

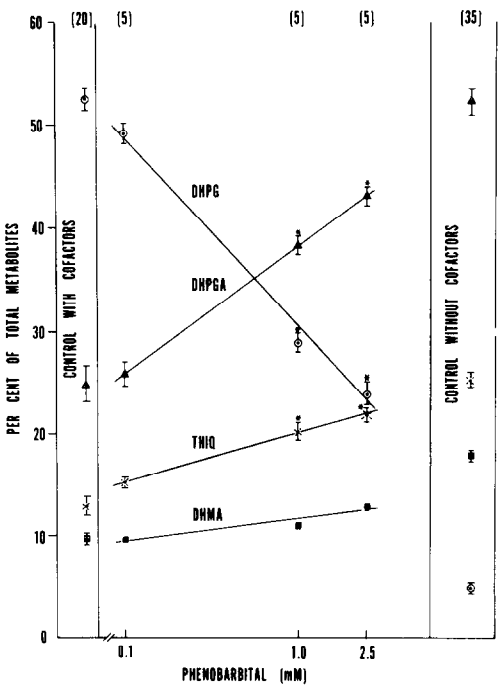

FIG. 3. Effect of phenobarbital (abscissa, log scale) on NE metabolism by rat brainstem homogenates. The reaction mixtures consisted of 1 ml of 10 per cent rat brainstem homogenate, 1 ml of a mixture containing 2 μmoles NAD and 2 μmoles NADPH, 1 ml NE substrate consisting of 0·5 μCi NE-^{14}C (sp. act. 54 mCi/m-mole) plus 12 μmoles unlabeled NE hydrochloride, the indicated amounts of sodium phenobarbital and 0·067 M phosphate buffer, pH 7·4, to a total vol. of 4·0 ml. All components were prepared in the phosphate buffer. Phenobarbital was added 2 min before initiation of the reaction by addition of substrate. The systems were incubated for 90 min at 37·5° with shaking. Each point represents the mean per cent of the total NE metabolites present as the indicated compounds; vertical bars, S. E. M. The number of determinations is indicated in parentheses at the top of the figure above corresponding points. The asterisk (*) denotes a significant difference ($P < 0·05$, Student's t-test) from control mean. THIQ, tetrahydroisoquinoline alkaloid formed by condensation of norepinephrine and the aldehyde derivative of the parent amine; DHPG, dihydroxyphenylglycol; DHMA, dihydroxymandelic acid; DHPGA, dihydroxyphenylglycolaldehyde. The total metabolites, expressed as nmoles for each concentration of phenobarbital, were as follows: control with cofactors, 450·0 ± 21·8; 0·1 mM phenobarbital, 490·2 ± 16·9; 1·0 mM phenobarbital, 474·3 ± 4·9; 2·5 mM phenobarbital, 453·3 ± 15·5; control without cofactors, 409·3 ± 14·7.

deaminated. These differences are probably attributable to the relatively low oxidizing capacity of brain tissue in comparison with liver.[1]

Effects of varying NADPH concentrations. DHPGA oxidation by the NAD-linked aldehyde dehydrogenase of brainstem was of minimal consequence in the metabolic removal of this amine-derived aldehyde. However, the metabolic disposition of the NE-derived glycolaldehyde by brainstem homogenates was markedly influenced by incorporating NADPH into the reaction mixtures. The influence of varying NADPH concentrations on the metabolism of NE by rat brainstem homogenates is shown in Fig. 2.

Activating the reductive metabolism of the glycolaldehyde by aldehyde reductase in the presence of NADPH results in a pronounced increase in DHPG production, accompanied by a decrease in THIQ alkaloid and DHMA formation. Thus, when the major pathway for removing DHPGA is maximally activated, the aldehyde's level is markedly diminished. The decreased availability of DHPGA, with which NE condenses to form the THIQ alkaloid, suppresses alkaloid production. These results suggest that modifications in the activity level of aldehyde reductase in brain preparations, whether effected by cofactor availability or by inhibition of this reductase, should be of primary significance in the disposition of the NE-derived glycolaldehyde and, consequently, in the diversion of NE metabolism to alkaloid formation.

Effect of barbiturates. The effects of different concentrations of phenobarbital on NE metabolism by rat brainstem homogenates were examined. Figure 3 shows that there was a close relationship between the degree of aldehyde reductase inhibition and the extent NE was metabolized to the free aldehyde and to the THIQ alkaloid.

Phenobarbital in low concentrations (0·1 mM) did not significantly alter the metabolic pattern from control values. With 1·0 mM phenobarbital, however, there was a significant decrease in the amount of DHPG formed. The decreased conversion of DHPGA to DHPG was reflected in significant increases in the proportion of DHPGA and THIQ alkaloid. The increase in DHMA levels was not statistically significant. When the highest concentration of phenobarbital (2·5 mM) was tested, an additional decline in the DHPG fraction was observed while the proportions of the free aldehyde and THIQ alkaloid correspondingly increased.

As shown in Fig. 4, phenobarbital, pentobarbital and barbital (1·0 mM) were equipotent in inhibiting the reduction of DHPGA to DHPG by aldehyde reductase. Each barbiturate decreased the proportion of the glycol from 52 to only about 30 per cent of the metabolites. Barbiturate intervention in the primary metabolic pathway of the NE-derived aldehyde consequently raised free glycolaldehyde levels and augmented incorporation of the aldehyde into the THIQ alkaloid.

Characterization of the alkaloid metabolites of NE. The authentic reference standards of the 1-benzyltetrahydroisoquinoline alkaloid derivatives of NE are not presently available. Thus, the structure of NE alkaloid metabolites, shown in Fig. 1, is tentatively assigned based on the physio-chemical characteristics of the isolated material, which provide a strong analogy with prior experiments with DA[9] where the corresponding alkaloid metabolite was THP, a known compound which was characterized by direct comparison with authentic standard. The presence of the catechol moiety is verified by adsorption of the metabolites on alumina. The basic nature of the metabolites is demonstrated by their exchange on the cation exchange resin. In addition, the alkaloid metabolites are distinct from the primary amine, NE,

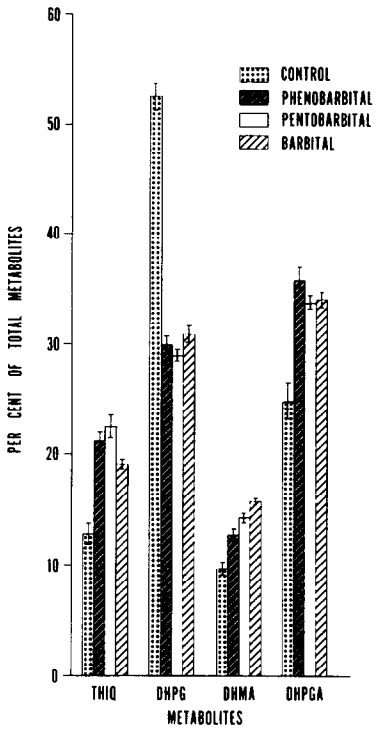

FIG. 4. Effects of various barbiturates on NE metabolism by rat brainstem homogenates. Reaction mixtures consisted of 1 ml of 10 per cent rat brainstem homogenate, 1 ml of a mixture containing 2 μmoles NAD and 2 μmoles NADPH, 1 ml NE substrate consisting of 0·5 μCi NE-^{14}C (sp. act. 54 mCi/m-mole) plus 12 μmoles unlabeled NE hydrochloride, 4 μmoles (1 mM final concentration) of the indicated barbiturates, and 0·067 M phosphate buffer, pH 7·4, in a total vol. of 4·0 ml. All components were prepared in the phosphate buffer. The barbiturates were added 2 min before initiation of the reaction by addition of substrate. The systems were incubated for 90 min at 37·5° with shaking. Each bar denotes the mean per cent $\pm$ S. E. M. of the total NE metabolites present as the indicated compounds. The number of determinations were: control with cofactors, 20; phenobarbital, 15; pentobarbital, 5; and barbital, 5. All three barbiturates effected a significant difference ($P < 0{\cdot}05$) from the control mean in the formation of each NE metabolite. The total metabolites, expressed as nmoles for each incubation condition were as follows: control with cofactors, 450·0 $\pm$ 21·8; 1 mM phenobarbital, 384·4 $\pm$ 24·9; 1 mM pentobarbital, 451·6 $\pm$ 4·9; 1 mM barbital, 434·1 $\pm$ 2·5.

because the two classes of compounds are completely separated by differential elution from the cation exchange resin.

Thin-layer chromatography. The R_f values of the radioactive compounds isolated in both the NE and alkaloid fractions were compared with the standard compounds NE, DA and THP. The primary amines, NE and DA, are readily separated from the benzyltetrahydroisoquinoline alkaloid derivative of DA, THP, by thin-layer chromatography. The R_f values for these reference compounds in the butanol–acetic acid–water (4:1:1) system were 0·26 for NE, 0·34 for DA, and 0·49 for THP. The radioactivity isolated from the incubation mixtures in the NE fraction had an R_f of 0·26, identical to that of the authentic NE standard. Two radioactive metabolites, with respective R_f values of 0·58 and 0·65, were found in the isolated alkaloid fraction, both of which were absent from the control incubation mixtures. Furthermore, no radioactive NE was detected in this alkaloid fraction.

Gas–liquid chromatography. Additional characterization of the basic compounds isolated in the alkaloid fraction was obtained by gas–liquid chromatography. The primary amines are easily separated from the THIQ alkaloids by this technique.[21] Under the conditions described, NE emerges with the solvent front, whereas the products isolated in the alkaloid fraction display the retention times and methylene unit values characteristic of more complex, higher molecular weight compounds. The authentic THP standard has a retention time of 15·4 min and a methylene unit value of 29·3. Two NE metabolites with retention times of 19·3 and 20·6 min and methylene unit values of 30·2 and 30·4 were separated from the alkaloid fraction of the incubation mixtures. The greater retention time of the NE metabolites isolated in the alkaloid fraction, compared with that of THP on the non-polar column employed, is compatible with the structure proposed for the NE alkaloid derivatives (Fig. 1). These compounds were absent in the same fraction obtained from the control incubation mixtures.

The detection of more than one alkaloid reaction product of NE is not unexpected. The identification of *cis*- and *trans*-isomers of the THIQ alkaloid formed by condensation of NE with glyoxylic acid, which were separable by thin-layer chromatography, has been reported earlier.[22] Condensation of NE with acetaldehyde has also been shown by thin-layer chromatography to result in the formation of several reaction products.[23] Both NE and DHPGA possess asymmetric centers. The Pictet–Spengler type of condensation[24] of NE with its aldehyde derivative may thus result in the formation of *cis*- and *trans*-isomers, as well as diastereoisomers of the benzyltetrahydroisoquinoline alkaloid. Studies are now in progress to characterize more definitively these NE metabolites, which are tentatively identified as benzyltetrahydroisoquinoline alkaloids.

DISCUSSION

The formation of predominantly acidic metabolites or of neutral (i.e. alcohol or glycol) metabolites of the neuroamines by brain tissue has been shown to be dependent upon the structure of the aldehyde. In brain, the primary route of metabolism of phenylethylamine derivatives, such as the aldehyde intermediate of DA, is oxidation to the acidic metabolites. On the other hand, the β-hydroxylated phenylethyl analogs of NE, normetanephrine and octopamine, are primarily reduced to the corresponding glycol metabolites by brain slices or *in vivo* after intracisternal injection of the amine.[5–7] The oxidative conversion by brain tissue of amine-derived aldehydes, as well as a broad range of aliphatic aldehydes (including acetaldehyde) to the corresponding acids appears to be mediated by an NAD-linked aldehyde dehydrogenase.[1,2] In contrast to hepatic tissue, which utilizes both the NAD/NADH- and the NADP/NADPH-linked enzymes, the reduction of certain aldehydes derived by oxidative deamination of the biogenic amines in brain appears to be primarily effected by an NADPH-dependent aldehyde reductase.[3,25] The enzyme is believed to have low affinity for most aliphatic aldehydes. The preferential reduction of aldehydes derived from β-hydroxylated phenylethylamines in brain is presumably a reflection of this enzyme's substrate specificity requirements.[3,4]

Previous reports defining the differential route of metabolism of phenylethylamine or β-hydroxyphenylethylamine-derived aldehydes by brain tissue have utilized partially purified enzymes,[2–4] brain slices,[5,7] or the intracerebral administration of

labeled amines.[6] All of these systems have some limitation for investigating the role of cofactors and effects of inhibitors on the over-all metabolism of biogenic amines. Homogenate preparations with broken cells permit the utilization of specific exogenous cofactors, while essentially maintaining all the diverse enzymatic activities required for the total metabolism of the biogenic amines, and thus lend themselves to the simultanous determination of the effects of cofactors and specific inhibitors on the total metabolism of the biogenic amines.

Suppressing the major metabolic routes in brain tissue of phenylethylamine or β-hydroxylated phenylethylamine-derived aldehydes whether by decreased availability of the cofactors or by specific inhibitors of aldehyde dehydrogenase or aldehyde reductase, respectively, could reasonably increase the aldehydes' steady state levels and subsequently alter their normal disposition. Indeed, our previous investigations have demonstrated that acetaldehyde, derived metabolically from ethanol, and also the sedative-hypnotic, chloral hydrate, block the normal oxidative disposition of the DA-derived aldehyde and enhance the formation *in vitro* of THP, the benzyltetrahydroisoquinoline alkaloid formed by the condensation of DA with its initial metabolite, 3,4-dihydroxyphenylacetaldehyde.[9,10] This pharmacologically active aberrant alkaloid derivative of dopamine, THP, is of interest because it functions as a β-adrenergic agonist.[11–17] In plants, THP (norlaudanosoline) plays an essential role in the biosynthesis of a broad spectrum of even more complex alkaloids, including the papaverine, hydrophenanthrene, aporphine and tetrahydroprotoberberine (THPB) types, to cite a few examples.[26–30] By way of a corollary, the specific incorporation by plants of radiolabeled NE into the berberine alkaloid, berberastine, is also presumed to proceed through the intermediacy of an NE-derived THIQ alkaloid, 4-hydroxynorlaudanosoline (4-hydroxytetrahydropapaveroline).[31]

In the present study, the effects of various pyridine nucleotide coenzymes on NE metabolism by rat liver and brainstem homogenates further substantiate the cardinal role played by an NADPH-linked aldehyde reductase in the metabolism of NE's aldehyde derivative, particularly by brain tissue. Furthermore, this investigation demonstrates that disruption of the primary (i.e. reductive) metabolic pathway of the NE-derived aldehyde in brain tissue by barbiturates leads to elevated aldehyde levels and to a metabolic diversion resulting in the formation of an aberrant THIQ alkaloid derivative of NE. Although barbiturate concentrations of 10^{-3} M were required to produce significant effects on the over-all metabolism of NE under the conditions employed in the present study, Erwin *et al.*[19] found that the rate of oxidation of NADPH by a partially purified aldehyde reductase from bovine brain was inhibited approximately 50 per cent by 10^{-4} and 73 per cent by 10^{-3} M sodium phenobarbital. These investigators reported that the inhibitor constants for various physiologically active barbiturates are low (i.e. K_i 10–100 μM) and indicated that these concentrations correspond with brain levels of sodium phenobarbital achieved with doses of the barbiturate that produce behavioral changes in rats.[19] Thus, the modification of the metabolism of NE's aldehyde described in the present study is consistent with the previously demonstrated inhibitor effects of barbiturates on a partially purified NADPH-linked aldehyde reductase. The only discrepancy would appear to be in the concentration of the barbiturates required. However, one must remember that, because of the diluted and disrupted enzymatic architecture operant in a closed system, direct quantitative comparison between homogenates and other prep-

arations *in vitro* or the situation *in vivo* is not possible, and the results obtained with homogenates provide only an indication of potential endogenous events.

These data offer further evidence of the metabolic inter-relationships effected by interruption of the major route for disposition of the catecholamine-derived aldehydes. It is possible that enhanced endogenous formation of catecholamine-derived THIQ alkaloids may be mediated by certain pharmacological agents which interfere with the normal disposition of amine-derived aldehydes. Furthermore, although the enzymes directing plant biosynthesis of THIQ-derived alkaloids are undefined, the additional possibility exists that these more complex alkaloids may also be elaborated from benzyltetrahydroisoquinoline alkaloids in mammalian systems. These considerations are strengthened by several recent findings.

The unequivocal evidence[32] that the DA-derived THIQ alkaloids, salsolinol and THP, are formed *in vivo* in man lends considerable credence to these possibilites. Additionally, our own experimentation has demonstrated the capability of mammalian systems to metabolize THP further. Combined GC–MS data revealed that incubating the soluble fraction from rat liver or brain with *S*-adenosylmethionine-methyl-^{14}C and THP results in the formation of two classes of radioactive metabolites. One consists of the readily expected *O*-methylated THIQ derivatives of THP; the other of THPB alkaloid metabolites of THP.[33] Additionally, these same THPB alkaloids have also been detected as THP metabolites in the urine of rats.[34] Most importantly, however, we have recently demonstrated the endogenous formation of these complex THPB alkaloids *in vivo* in man.

At present the only recognized biosynthetic pathway for THPB alkaloid formation involves the intermediacy of benzyltetrahydroisoquinolines such as THP. Thus, the THIQ derivatives of catecholamines should not be considered metabolic end products because mammalian systems, like plants, are now known to be capable of mediating not only the endogenous formation but also biotransformations of the catecholamine-derived THIQ alkaloids to even more complex alkaloids.

Although indirect, the evidence revealed by the present investigation suggests that alteration of neuroamine metabolism resulting in an increase in the steady state levels of certain highly reactive neuroamine-derived aldehydes or augmented formation of aberrant alkaloid metabolites may provide a common biochemical mechanism underlying some of the effects of certain CNS depressant drugs considered pharmacologically equivalent. Direct translation of biochemical effects to pharmacological actions of drugs is not always readily attainable. We may, therefore, still be far from understanding the primary mechanisms subserving the pharmacological equivalency of barbiturates, ethanol, and the large number of related sedative-hypnotic drugs. However, the spectrum of aberrations in neuroamine metabolism evoked by these drugs supports the contention that a whole series of metabolites capable of eliciting potentially important pharmacological consequences exists and awaits discovery.

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