Biochemical Pharmacology, Vol. 26, pp. 741–747. Pergamon Press, 1977. Printed in Great Britain.

EFFECTS OF DISULFIRAM AND CHLORAL HYDRATE ON THE METABOLISM OF CATECHOLAMINES IN RAT LIVER AND BRAIN*

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(*Received* 5 *April* 1976; *accepted* 17 *September* 1976)

Abstract—Disulfiram and chloral hydrate are known inhibitors of aldehyde dehydrogenase (ALDH), an enzyme involved in the metabolism of dopamine and noradrenaline as well as acetaldehyde. The inhibition *in vivo* of this enzyme by these drugs was investigated in rat liver and brain by measuring the distribution of catabolites obtained from catechol amines. Incubations of brain and liver tissues as well as perfusions of the caudate nucleus of conscious animals were performed with ^{14}C-labeled catechol amines. Data from slice incubations revealed that disulfiram at a dose of 200 mg/kg·day given for at least 3 days inhibits brain ALDH more effectively (48 per cent) than the liver enzyme (16 per cent), as measured by the decreased formation of 3,4-dihydroxyphenylacetic acid. Furthermore, disulfiram increases the rate of deamination of dopamine in liver slices by approximately 25 per cent, but did not alter the rate of dopamine deamination in the brain tissues. Tetrahydropapaveroline (THP) was formed in every experiment utilizing dopamine. Approximately 10 per cent of the deaminated dopamine was recovered as the alkaloid. In addition, a non-identified metabolite of dopamine was isolated, primarily from incubations of brain tissues. Some, but substantially less, of the non-identified metabolite was obtained from incubations of liver slices as well as from brain perfusions. Chloral hydrate at a dose of 100 mg/kg·day given for at least 3 days did not significantly affect the metabolism of dopamine in either organ. The metabolism of noradrenaline was not significantly altered by either drug. Data from the brain perfusions confirmed the conclusions from incubations of slices: disulfiram is a potent inhibitor of 3,4-dihydroxyphenylacetic acid formation, while chloral hydrate is virtually ineffective at inhibiting the acid formation. The major differences found from the perfusion studies compared to the slice incubations were in the increased concentration of homovanillic acid (HVA) and the decreased formation of the non-identified metabolite. The finding that disulfiram only inhibited by 16 per cent the liver metabolism of dopamine while drastically inhibiting the liver metabolism of acetaldehyde suggests that different isozymes of aldehyde dehydrogenase are involved in the oxidation of these two aldehydes. The lack of inhibition of 3,4-dihydroxymandelic acid formation suggests that the aldehyde derived from noradrenaline may be metabolized by still a different isozyme of aldehyde dehydrogenase.

Aldehyde dehydrogenase (aldehyde:NAD oxido reductase, EC 1.2.1.3, ALDH) catalyzes the oxidation of aldehydes to acids. The aldehydes are formed *in vivo* from either alcohols such as ethanol or from biogenic amines such as the catechol amines and serotonin. These amines are converted to aldehydes by an oxidative deamination catalyzed by monoamine oxidase (monoamine:O_2 oxido reductase, EC 1.4.3.4, MAO) while alcohols are oxidized to aldehydes by alcohol dehydrogenase (alcohol:NAD oxido reductase, EC 1.1.1.1, ADH). Though aldehydes such as acetaldehyde are almost exclusively oxidized to acids, biogenic aldehydes can be either oxidized to acids or reduced to alcohol derivatives. Prior to excretion, the catechol amine-derived compounds can be methylated by catechol-*O*-methyl transferase (*S*-adenosylmethionine:catechol-*O*-methyl transferase, EC 2.1.1.6, COMT). Though acetaldehyde is primarily formed and metabolized in the liver [1], ALDH is located in other tissues such as brain and heart. In these tissues, the role of the enzyme is presumed to be to oxidize biogenic aldehydes derived from amines to their acid derivatives. The catabolic pathway for biogenic amines is found to be essentially the same in the liver [2], heart [3] and brain [2]. Thus, a drug which inhibits liver aldehyde dehydrogenase may also inhibit the enzyme located in non-hepatic tissue.

Disulfiram is a potent inhibitor of ALDH, both *in vivo* [4] and *in vitro* [5]. If alcohol is ingested when disulfiram is present, acetaldehyde levels rise. The increased blood acetaldehyde is presumably partly responsible for the well-known disulfiram–alcohol reaction [6]. For this reason the drug is often given to the alcoholic patient in order to deter drinking. Chloral hydrate is another inhibitor of ALDH, and is sometimes used for the treatment of withdrawal syndrome in conjunction with other drugs [7], and in cases of insomnia of alcohol-addicted individuals [8].

Since ALDH is involved in both ethanol and biogenic amine metabolism, any drug which inhibits the enzyme could also affect biogenic amine metabolism. In this paper, we report on the effects of disulfiram and chloral hydrate on catechol amine metabolism in the liver and brain of rats.

* Journal paper number 6245 of the Purdue University Agricultural Experiment Station.

EXPERIMENTAL

Materials

All chemicals were reagent grade and used, except when stated, without further purification. Soluble carboxymethyl cellulose, disulfiram, 2,4-dinitrophenylhydrazine, catecholamines and other catechol products were purchased from Sigma Chemical Co. (St. Louis, Mo.), and chloral hydrate from Fisher Scientific Co. (Fair Lawn, N.J.). [Ethylamine 1-^{14}C] dopamine hydrochloride and DL[carbinol-^{14}C]noradrenaline bitartrate were obtained from Amersham/Searle Corp. (Arlington Heights, IL). Parafilm M from American Can Co. (Greenwich, CT), phenol reagent (Folin) from Harleco (Gibbstown, NJ) and 3 MM chromatography paper from Whatman W & R Balston Ltd. (Maidstone, England). Tetrahydropapaveroline was a gift of Dr. A. Collins, Inst. for Behavioral Genetics, Univ. of Colorado (Boulder, CO). All other chemicals were purchased from Mallinckrodt Chemical Works (St. Louis, MO). Wistar rats were supplied by the breeding facilities of Purdue's Department of Biochemistry. Double-distilled water was used throughout this work.

Methods

Drugs administration. Disulfiram (recrystallized from water–acetone mixtures [9]) was suspended (200 mg/ml) in 1% carboxymethyl cellulose. Chloral (distillated after treatment of the hydrate crystals with concentrated sulfuric acid [10]) was mixed (100 mg/ml) with 1% carboxymethyl cellulose. Either one of the drugs was intubated in the stomach of 300-g male Wistar rats (1 ml/kg) every 24 hr for at least 3 days, when tissue slices were to be incubated. Reference animals were treated with an equivalent amount of carboxymethyl cellulose. An additional dose of chloral hydrate was given 1 hr before sacrificing the animal. Either a dose (3 ml/kg) of chloral hydrate or disulfiram (0.5 or 16 hr before dopamine injection respectively) was intubated in the stomach of the animal when drug inhibition was to be measured by brain perfusion.

Incubation of tissue slices. Brain and liver were removed from the body within 2 min after decapitation. Slices (50 mg) were prepared with a Stadie Riggs microtome, briefly washed in standard Krebs Ringer phosphate buffer [11] and incubated for 4 hr in 1 ml of the same solution containing 2 mM (0.2 μCi) ^{14}C-labeled dopamine or noradrenaline. The incubation tubes (13 × 100 mm) were flushed with oxygen, sealed with Parafilm and shaken continuously in a thermostatic bath at 37°.

Separation of products. After removing the slice from the incubation mixture, a 25-μl sample was subjected to paper electrophoresis in 50 mM sodium borate buffer, pH 9.5 (3 hr, 10 V/cm, 4°), in order to separate the metabolites of dopamine or noradrenaline [12, 13]. Standard solutions of expected products were run simultaneously. Spots were visualized with 50% aqueous phenol reagent, cut from electrophoregrams and quantitated by scintillation counting as described by Tank *et al.* [13]. Individual components were identified by comparison of electrophoretic mobility with reference standard solutions in either sodium borate or 30 mM sodium phosphate buffer, pH 6.8. The presence of aldehydic function was recognized by spraying with 0.4% of 2,4-dinitrophenylhydrazine in 2 N HCl. Schematic electrophoregrams are presented in Fig. 1.

Surgery. A thin-wall stainless steel guide tube was implanted stereotaxically in the brain of the rat under aseptic conditions, as described by Myers [14]. The coordinates of the site to be perfused, the caudate nucleus, were determined using the stereotaxic atlas of Pellegrino and Cushman [15]: $AP = 8.2$, $L = 3.0$ and $V = 4.0$. After surgery, the animals were allowed 5 days for recovery.

Push–pull perfusions. The microinjection and the push–pull perfusion canulae were fashioned and filled with a physiological sterile solution as described by Myers [16]. Approximately 1 μCi (2 μl, 0.6 to 1.0 μmole) [^{14}C]dopamine was injected at 1 μl/min 1 mm below the tip of the implanted guide tube. Five min later, the push–pull perfusion was started and performed for 30 min continuously at 25 μl/min; the perfusate was stored at 0° in a long coil of PE 50 polyethylene tubing, and fractions of *ca.* 50 μl were collected and acidified to pH 4.0 with 0.1 N HCl. The products were analyzed as described above for the incubation experiments. Each animal was submitted to one perfusion daily. Reference metabolism was determined before any drug was given to the rat. Histology of the area of implantation was performed according to Wolf [17].

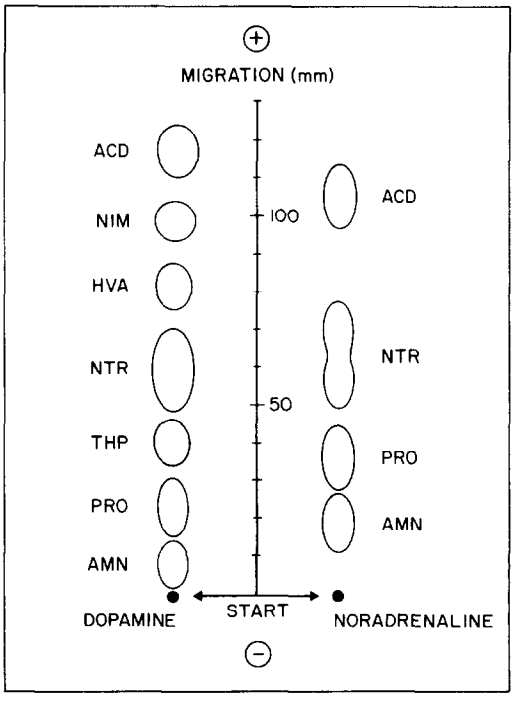

Fig. 1. Schematic electrophoregrams of metabolites from dopamine (left) or noradrenaline (right) incubated with brain or liver tissues. Migration is in borate buffer, as described in the Experimental section. Abbreviations are as follows: AMN: dopamine or noradrenaline; PRO: ^{14}C-labeled compound(s) bound to protein; NTR: neutral metabolites (alcohol and aldehydes); THP: tetrahydropapaveroline; HVA 4-hydroxy-3-methoxyphenylacetic acid; NIM: non-identified metabolite; and ACD: 3,4-dihydroxyphenylacetic acid or 3,4-dihydroxymandelic acid.

Calculations. Except when stated, all values are expressed as mean and standard deviation of duplicate analysis from eight animals. Statistical significance was determined by Student's *t*-test for two means with a programmed Hewlett–Packard 25 calculator.

RESULTS

Dopamine metabolism in tissue slices

Liver metabolism. Approximately 50 per cent of the dopamine was deaminated during the course of the incubation. The major metabolites isolated were acids (48 per cent), either 3,4-dihydroxyphenylacetic acid (DOPAC) or 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA), and neutrals (28 per cent), which were previously shown to be a mixture of both 3,4-dihydroxyphenylethanol (DOPET) and 3,4-dihydroxyphenylacetaldehyde (DOPAL) [13]. Tetrahydropapaveroline (THP), a condensation product of dopamine and DOPAL [18], was also obtained. Two additional radioactive components were found. The first, which migrated between dopamine and THP, contained proteins. It was not ascertained which metabolites were associated with, or how they were bound to, this proteic material. The second was a metabolite which is not yet identified (non-identified metabolite, NIM) migrating close to DOPAC. Since the chemical nature of this component is still unknown, the same specific activity as dopamine (1 ^{14}C atom/molecule) was assumed to calculate the levels of this component (Table 1). The distribution of these products is presented in Fig. 2A. When dopamine was incubated with free intact hepatic cells, the same distribution of metabolites was obtained, within experimental error.

Brain metabolism. The same metabolites were isolated from incubations of brain and liver tissues. However, the rate of deamination of dopamine was 50 per cent lower than in incubations of liver slices (Table 1). The major difference in product distribution between results from these two organs was in the level of the non-identified metabolite. After incubations of brain tissues, this component was a major metabolic product, reaching a level comparable to that of DOPAC, as shown in Fig. 2B. In addition, more radioactivity was recovered in the protein fraction.

Effect of disulfiram and chloral hydrate. Assays *in vitro* [19, 20] using *p*-nitrobenzaldehyde or acetaldehyde revealed that disulfiram is a potent inhibitor of aldehyde oxidation; less than 10 per cent of ALDH activity remained when 25 μM disulfiram was present in the assay. However, only a modest 16 per cent decrease in DOPAC formation was observed in the incubation of liver slices. A higher inhibition (48 per cent) was found in incubations of brain tissues. For both organs, the level of HVA was unaffected by the drug. Concomitant with the decrease of DOPAC concentration was a proportional increase of the neutral accumulation. The overall effect of disulfiram on the metabolism is presented in Fig. 2 and Table 1.

Chloral hydrate, though a potent inhibitor *in vitro* of ALDH, is relatively ineffective in producing alterations in DOPAC or neutral levels as shown in Fig. 2. The response *in vivo* of ALDH to the drug, as measured by the alteration of DOPAC formation in incubation of tissues, is summarized in Table 2, where only the concentrations of major metabolites were considered. Chloral hydrate produced a small but statistically significant increase (*ca.* 12 per cent) in brain neutral levels. However, the *ca.* 7 per cent decrease in brain DOPAC levels that was observed did not reach a statistical significance due to broader individual variation.

Disulfiram-treated animals, but not chloral hydrate-treated ones, exhibited an enhancement of the overall deamination of dopamine in the liver slices. Neither drug affected this deamination in the brain tissues.

Noradrenaline metabolism in tissue slices. In contrast with the deamination of dopamine, disulfiram did not affect the rate of noradrenaline disappearance. After incubations of liver and brain slices from any control or drug-treated animals, two major fractions were isolated. The neutral one comprised 3,4-dihydroxyphenylglycol (DOPEG) and 3,4-dihydroxyphenylglycolaldehyde; the acid one contained only 3,4-dihydroxymandelic acid (DOMA). A slightly higher level of acid was found as a result of liver metabolism (19 per cent), as compared to brain metabolism (14 per cent). Some radioactivity was also found in a fraction containing proteins; 17 and 22 per cent of the recovered labeled components were associated with this fraction in the incubation mixtures of liver and brain tissues respectively. The drugs did not alter these values (data not presented).

Brain perfusions

Basal metabolism. The metabolism of dopamine in the brain was also measured *in situ* by perfusion of

Table 1. Radioactivity (cpm) found in compounds isolated from incubations of [^{14}C]dopamine with liver or brain tissue in control and drug-treated animals

Drug	Activity* (cpm) AMN	PRO	THP	NTR	NIM	HVA	DOPAC	Total count recovered†
Liver								
Reference ± S. D.	2375 ± 981	184 ± 21	467 ± 106	599 ± 132	66 ± 20	174 ± 73	927 ± 457	4763 ± 564
Disulfiram ± S. D.	1814 ± 824	218 ± 29	482 ± 210	968 ± 154	123 ± 68	259 ± 98	1033 ± 582	4899 ± 347
Chloral hydrate ± S. D.	2507 ± 986	180 ± 26	418 ± 145	604 ± 150	75 ± 27	192 ± 93	877 ± 587	4855 ± 440
Brain								
Reference ± S. D.	3063 ± 406	130 ± 26	219 ± 51	236 ± 77	232 ± 91	61 ± 12	271 ± 73	4211 ± 460
Disulfiram ± S. D.	2982 ± 256	143 ± 98	227 ± 92	355 ± 127	265 ± 111	60 ± 14	159 ± 70	4192 ± 339
Chloral hydrate ± S. D.	2994 ± 339	165 ± 108	211 ± 62	260 ± 91	219 ± 80	62 ± 22	242 ± 79	4154 ± 484

* For abbreviations of compounds see Fig. 1.
† *Ca.* 5000 cpm was placed in the paper.

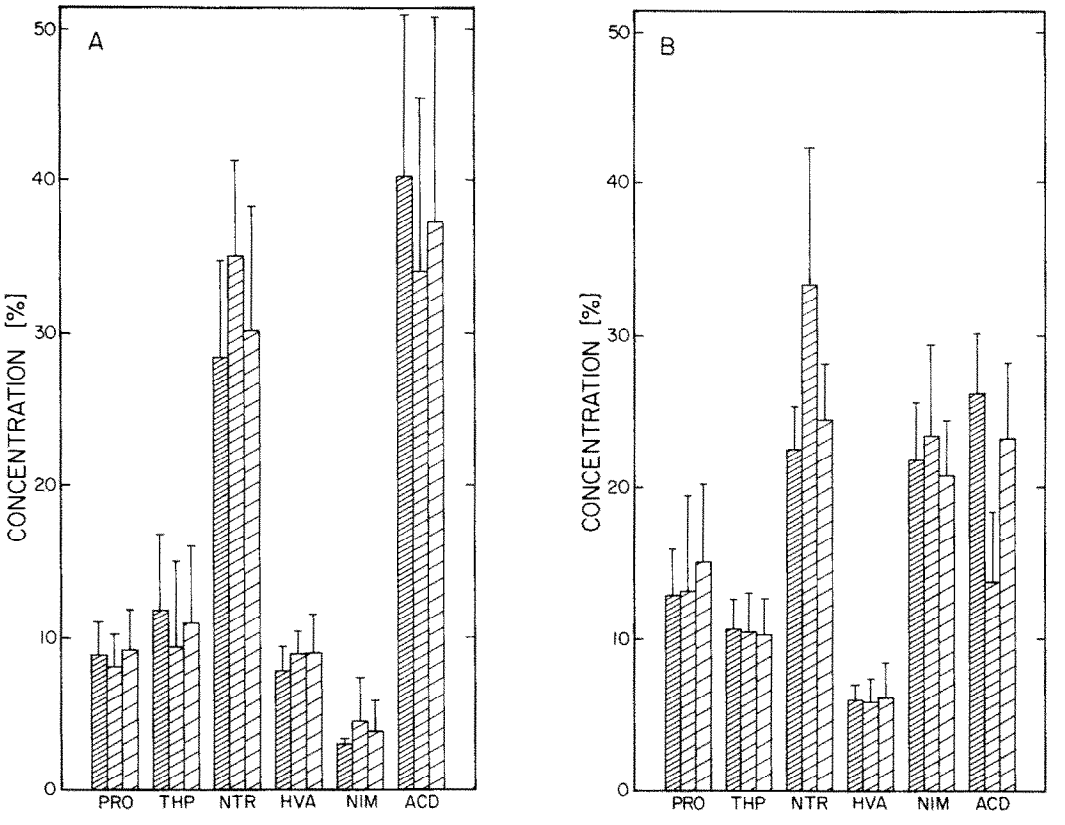

Fig. 2. Relative concentration of the deaminated products isolated from incubation of liver (A) or brain (B) slices with dopamine. Abbreviations are as follows: PRO: ^{14}C-labeled compound(s) bound to proteins; THP: tetrahydropapaveroline; NTR: neutral metabolites (3,4-dihydroxyphenylacetaldehyde (DOPAL) + 3,4-dihydroxyphenylethanol DOPET); HVA: 4-hydroxy-3-methoxyphenylacetic acid; NIM: non-identified metabolite; and ACD: 3,4-dihydroxyphenylacetic acid (DOPAC). Comparison of control animals (////, left), animals treated with disulfiram (▨, center) or chloral hydrate (▤, right). Conditions are as described in the Experimental section.

the caudate nucleus of conscious rats. In Table 3 are presented representative results obtained at selective time points during perfusion. In contrast to results from incubation of brain slices, where the ratio of HVA to DOPAC was 0.2, the perfusion data showed a ratio from 1.2 to 4.0. Also, the level of the non-identified metabolite was three to five times lower.

The relative levels of acid and neutral metabolites at different times after micro injection of dopamine are presented in Fig. 3. Each time point of the curves is analogous to the single entry of Table 2. The levels of non-identified metabolite were omitted from Fig. 3 for clarity of presentation.

Effect of disulfiram and chloral hydrate. The total radioactivity isolated at any time point differed prior to and after drug treatment (Table 3). It cannot be concluded from this difference that the drug altered the uptake of dopamine or MAO activity, for it is often observed that there is a high degree of variability in the levels of radioactivity in the effluent. This variability does not prevent the measurement of the inhibition of aldehyde dehydrogenase activity. As

Table 2. Distribution of the major metabolites isolated from incubation of brain or liver tissues with dopamine (normalized to equal 100 per cent)

Organ	Drug	Neutrals* (%) Mean ± S.D.	P	Acids† (%) Mean ± S.D.	P	NIM‡ (%) Mean ± S.D.	P
Brain		29.4 ± 2.9		42.2 ± 5.4		28.4 ± 3.9	
	Disulfiram	43.6 ± 10.7	< 0.001	25.8 ± 4.7	< 0.001	30.5 ± 7.3	NS§
	Chloral hydrate	32.9 ± 4.4	< 0.02	39.3 ± 6.0	NS	27.8 ± 4.2	NS
Liver		36.4 ± 10.0		59.8 ± 10.3		3.8 ± 0.6	
	Disulfiram	43.2 ± 10.0	NS	51.3 ± 10.8	< 0.05	5.5 ± 3.6	NS
	Chloral hydrate	38.2 ± 11.6	NS	57.0 ± 13.1	NS	4.8 ± 2.5	NS

* Neutrals: 3,4-dihydroxyphenylethanol (DOPET) + 3,4-dihydroxyphenylacetaldehyde (DOPAL).
† Acids: 3,4-dihydroxyphenylacetic acid (DOPAC) + 4-hydroxy-3-methoxyphenylacetic acid (HVA).
‡ NIM: non-identified metabolite.
§ NS = not significant.

Table 3. Radioactivity (cpm) found in compounds derived from dopamine isolated at various times for perfusion of the caudate nucleus in the presence and absence of drugs

Time* (min)	Activity† (cpm) AMN	PRO	THP	NTR	NIM	HVA	DOPAC	Total counts recovered
Reference (animal 1)								
12.5	4,091	126	257	199	31	75	102	4,881
15.0	1,263	31	67	85	15	41	124	1,627
15.7	865	22	53	82	12	45	112	1,190
Disulfiram-treated								
12.5	216	46	324	627	122	225	139	1,700
15.0	161	31	213	399	115	165	111	1,196
17.5	107	18	116	227	53	89	67	677
Reference (animal 2)								
12.5	8,786	314	954	1,707	274	301	221	12,555
15.0	2,945	162	377	664	136	281	138	4,703
17.5	1,984	97	210	511	107	168	120	3,197
Chloral hydrate-treated								
12.5	3,449	166	949	1,445	173	473	160	6,815
15.0	1,116	91	403	622	102	284	142	2,761
17.5	536	43	222	325	54	239	109	1,528

* Time after injection of dopamine.
† For abbreviations of compound see Figs. 1 and 2.

expected from the incubation of brain slices, disulfiram drastically inhibited the formation of acids as shown in Fig. 3A. The inhibition is observed *ca.* 10 min after the onset of perfusion. Similar effects can be noted if either HVA or DOPAC was plotted separately, since both of their respective levels were similarly decreased by this drug. The expected increase in the level of neutral metabolites was observed as well as the unaltered amounts of both THP and non-identified metabolite.

No alteration of the metabolism was observed with the chloral hydrate-treated animals. Though the representative results presented in Table 3 and Fig. 3B indicate a slight difference between the metabolic levels, it was established that they were within the range of metabolic variation observed for a given animal, as opposed to experimental errors.

DISCUSSION

Incubations of liver slices or free intact hepatocytes yielded similar patterns for the dopamine metabolism. Comparable results were also obtained with incubations of brain tissues and from perfusions of the caudate nucleus. However, an exception is the low level of HVA obtained; this *O*-methylated acid is not formed in high amounts in the incubations, presumably due to the non-regeneration of endogenous

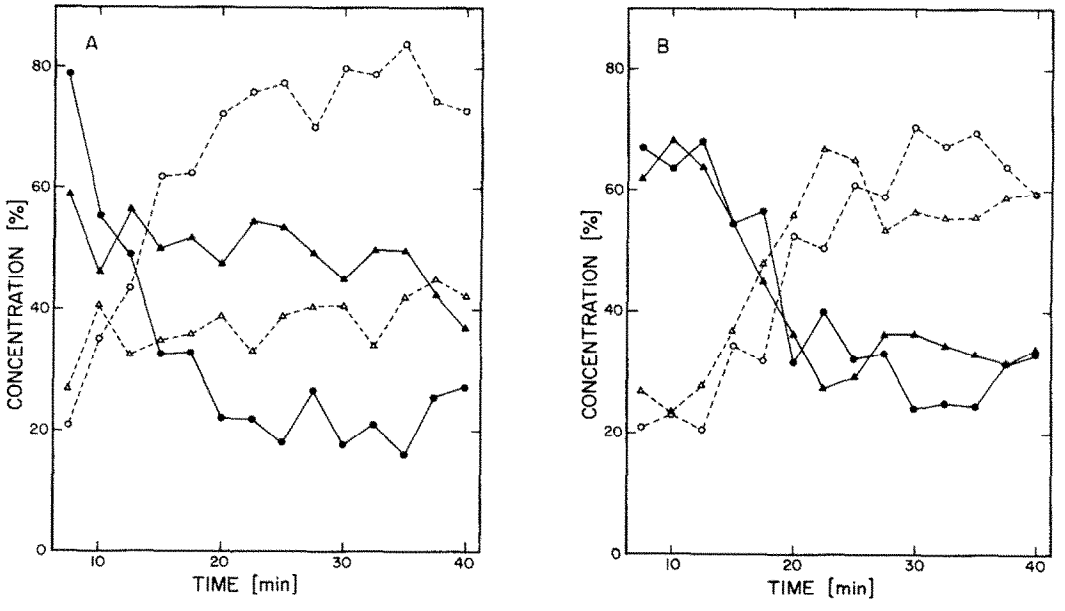

Fig. 3. Representative curves of brain perfusion of animals before (● or ○) or after (▲ or △) treatment with disulfiram (A) or chloral hydrate (B). For simplification, only relative concentrations of neutral [— —, ▲, ●; 3,4-dihydroxyphenylacetaldehyde (DOPAL) + 3,4-dihydroxyphenylethanol (DOPET)] and acid [– – –, △, ○; 3,4-dihydroxyphenylacetic acid (DOPAC) + 4-hydroxy-3-methoxyphenylacetic acid (HVA)] metabolites are shown as a function of the time elaspsed after injection of dopamine. Conditions are as described in the experimental section.

S-adenosylmethionine (SAM) in the cell. In brain perfusates from live animals, where regeneration of SAM is possible, a large fraction of the recovered acids was HVA. Since *O*-methyl amines were not detected in any incubation or perfusion, COMT must have transferred methyl groups to DOPAC and/or DOPAL.

No one has reported the presence of aldehydes derived from the catecholamines in urine. They were identified in incubations of liver [13] and brain tissues and perfusions. This suggests that the aldehyde is further metabolized or oxidized before excretion.

Though not normally found in urine, THP has been detected from subjects treated with 3,4-dihydroxyphenylalanine (L-DOPA) [21]. The isolation of THP in every experiment in this study shows, as originally published by Davis and Walsh [18], that this compound can indeed be formed in the animal. No attempt was made to determine where THP forms in the organs and if it is further metabolized. The relationships between dopamine, DOPAL and THP concentrations will be presented elsewhere.

A presumably new but still not identified metabolite from dopamine was isolated. This compound was found in large amounts from incubation of brain slices but in low concentrations from liver tissues. Finding the compound primarily from incubations of brain slices shows that the compound was not one of the typical catechol degradation products that are formed when incubating catechols in the presence of oxygen. Perfusions of the caudate nucleus yielded a level more similar to that obtained with liver slices, suggesting that the main site of synthesis is located in another region of the brain. Preliminary attempts to characterize this compound by mass spectrometry remained unsuccessful. Its high electrophoretic mobility in borate buffer could be due to either the presence of acidic residues or of additional hydroxyl groups being complexed by the borate anion.

Chloral hydrate is metabolized rapidly in the liver [22]. Assays *in vitro* performed under V_{max} conditions using crude rat liver homogenates showed that 10 mM chloral hydrate was necessary to totally inhibit ALDH activity. It was not possible to administer the drug at such a concentration in the animal, so the lack of inhibition in the liver or brain slice experiments could be due to either the fact that the drug was completely metabolized or that there was not enough drug present to inhibit the enzyme in the presence of the substrate. However, Huff *et al.* [23] found that chloral hydrate given to rats greatly affected the serotonin metabolism. It was found in this study that even if an additional dose (0.6 LD_{50}) of chloral hydrate was given intragastrically only 30 min prior to brain perfusion, no alteration in the metabolism of dopamine was observed. It is possible that the enzymes located in the caudate were not as inhibited by chloral hydrate as were the enzymes located in the serotonergic regions of the brain. Thus, the finding of essentially no inhibition by the drug cannot be used as evidence to prove that the drug is not an inhibitor *in vivo* of aldehyde dehydrogenase.

In contrast to chloral hydrate, disulfiram not only inhibited ALDH in brain and liver but stimulated the deamination of dopamine in the liver. It did not affect the deamination of noradrenaline. The cause of this stimulation, presumably of liver MAO activity, was not investigated. There are multimolecular forms of MAO [24] and different isozymes are involved in the oxidative deamination of noradrenaline and dopamine [25]. It appears, then, that disulfiram is affecting these isozymes differently.

As can be seen from the data in Table 2, inhibition of DOPAC formation in disulfiram-treated animals is three times more effective in the brain than in the liver. This small extent of inhibition (16 per cent) of the liver enzyme was unexpected, since it is known that acetaldehyde is barely oxidized in the presence of the drug and that the same mitochondrial ALDH [26] isozyme is preferentially responsible for both acetaldehyde and DOPAL oxidation (to be published). Deitrich and Erwin [4] have shown that all rat liver ALDH isozymes are not equally inhibited by disulfiram. Such an uninhibited isozyme could thus account for the DOPAL oxidation in the drug-treated animals. This would be valid only if the uninhibited isozyme had higher specific activity toward DOPAL than toward acetaldehyde. Preliminary work on substrate specificity supports this hypothesis.

From brain perfusions of control rats, 55–80 per cent of the acid product obtained was HVA. DOPAC as well as HVA levels were decreased in perfusions of disulfiram-treated animals. This suggests that the unaltered levels of HVA found after incubation of brain slices were due not to lack of ALDH inhibition, but more likely to the rapid depletion of SAM as discussed above.

The large standard deviations of the presented results are due to differences between individual animals and not to simple experimental errors. It could be caused by differences in the rates of drug absorption and interactions, as illustrated by the broader variation in incubations of brain slices from disulfiram-treated animals (see Table 2).

Among the multiple effects on the organism, disulfiram affects brain and liver dopamine metabolism mainly by inhibition of ALDH. In addition it inhibits dopamine-β-hydroxylase (3,4-dihydroxyphenylethylamine, ascorbate:oxygen oxido reductase, EC 1.14.2.1, DBH), thus lowering the concentrations of noradrenaline in the organism [6, 27]. Since the drug inhibits DBH and ALDH, the physiological balances of every metabolite from dopamine and noradrenaline are altered. The extrapolations from animal models to humans are always difficult. Rats neither exhibit a "knock-out" effect when chloral hydrate and ethanol are given simultaneously, nor the well-known ethanol–disulfiram syndrome. When disulfiram is prescribed to patients in order to deter drinking, the treatment is usually for a long period of time. If some similarities exist between the metabolism of rats and humans, disulfiram-treated patients would encounter a serious change in their overall catecholamine metabolism. What the long-term effects of such an alteration would be is not predictable.

Acknowledgements The authors wish to thank Dr. R. D. Myers, who permitted free access to his laboratories and instrumentation for brain perfusion experiments, and C. L. Melchior and M. McCaleb for their helpful technical advice. They are also grateful to Dr. T. K. Li, who supplied the hepatocytes. This work was supported by a Postdoctoral Fellowship from the Swiss National Science Founda-

tion and grants from NIH (AA01395) and NSF (BMS 75 03926).

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