Biochemical Basis of the Sulfonylurea-induced Antabuse Syndrome

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SUMMARY

The antabuse reaction, seen in patients imbibing alcohol while being treated with an antidiabetic sulfonylurea, is thought to be due to either an increase in acetaldehyde levels or an alteration in the metabolism of serotonin. Both can occur through an inhibition of aldehyde dehydrogenase. The oral antidiabetic sulfonylureas, chlorpropamide and tolbutamide, were found to be noncompetitive inhibitors of this enzyme. Acetaldehyde, a metabolite of ethanol, can competitively inhibit aldehyde dehydrogenase. Moreover, the oxidation of ethanol reverses the DPN/DPNH ratio so that the favored pathway of serotonin metabolism is altered, resulting in a decrease of 5-hydroxyindoleacetic acid (5-HIAA) and an increase of 5-hydroxytryptophol. Thus, ethanol augments the action of the sulfonylureas. Whether the syndrome results from an accumulation of acetaldehyde due to a block in ethanol metabolism, or whether it is a result of an alteration in the metabolism of serotonin, the noncompetitive inhibition of aldehyde dehydrogenase by the sulfonylureas, coupled with the actions of ethanol, may be considered as a potential cause. DIABETES *17*:679-82, November, 1968.

In 1948, Hald and Jacobsen demonstrated the causal relationship between ethanol hypersensitivity and the previous ingestion of tetraethylthiuram disulfide (TETD, antabuse).[1] The resulting symptom complex has been called the antabuse syndrome. It has also been reported in patients imbibing ethanol after use of, or exposure to, other pharmacologic agents.[1] In recent years, a number of reports have implicated the oral antidiabetic sulfonylureas in the pathogenesis of the antabuse syndrome.[2-7] These agents are extensively used in the treatment of patients with diabetes mellitus, many of whom use ethanol. Because of the common use of both the sulfonylureas and ethanol in patients with diabetes mellitus, side effects resulting from the combination are important.

The precise etiology of the syndrome has not yet been elucidated. Two theories have been proposed: (1) increased levels of acetaldehyde due to a block in the metabolism of ethanol;[6-8] (2) liberation of increased amounts of serotonin coupled with a block in its metabolism.[4] In this communication, in vitro data are presented which show that 1-propyl-3-p-chlorobenzenesulfonylurea (chlorpropamide) and 1-butyl-3-p-tolylsulfonylurea (tolbutamide) inhibit aldehyde dehydrogenase, a key enzyme in the metabolism of both ethanol and serotonin[6,9-11] (figures 1 and 2). This inhibition would serve to elevate levels of acetaldehyde and alter the metabolism of serotonin.

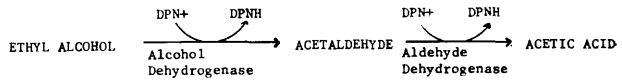

FIG. 1. Metabolism of ethanol.

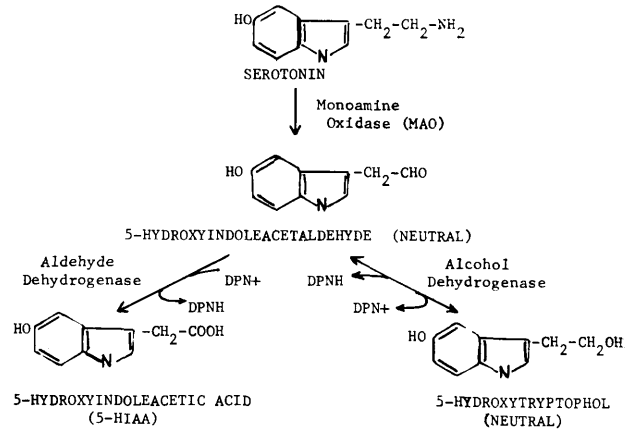

FIG. 2. Metabolism of serotonin.

MATERIAL AND METHODS

Liver aldehyde dehydrogenase, specific activity 83 U./mg. protein, was prepared according to the method of Racker as modified by Dietrich, Hellerman, and Wein[10] up to the treatment of the enzyme preparation with RNA. The oxidation of acetaldehyde was calculated from the rate of DPNH formation by following the absorption change at 340 mμ. in a Beckman DU spectrophotometer coupled to a Gilford recorder.

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The effect of chlorpropamide on the metabolism of serotonin was also studied. White male guinea pigs weighing 500-600 gm. were used. The animals were killed by a blow to the head. Their livers were quickly excised and homogenized (100 mg./ml.) in 0.25 M sucrose. 5-Hydroxytryptamine-3-1-C-14-creatinine sulfate (serotonin-C-14), 39.6 mc/mM, was obtained from Nuclear Chicago. Assays which followed the metabolism of serotonin as modified by the addition of chlorpropamide and/or ethanol were performed using the method of Feldstein and Wong[11] for extraction and separation of 5-HIAA and the neutral products. The assays were done as follows: incubations were carried out as shown in tables 2 and 3. The reactions were terminated by pouring the contents of the flasks into 40 ml. screw-top centrifuge tubes containing 3 ml. of 0.3 N HCl. Four grams of NaCl were added followed by 25 ml. of ether. The tubes were shaken mechanically for five minutes, centrifuged for five minutes and 20 ml. aliquots of the ether layers placed in additional 40 ml. centrifuge tubes. Three milliliters of salt saturated pH 10, 0.5 M borate buffer were added to each aliquot. They were again shaken for five minutes and centrifuged for five minutes. One milliliter of the ether layer was removed and counted. This was the neutral fraction. The remaining ether was removed and borate solution twice washed with 15 ml. of ether. Finally 1 ml. of 2 N HCl was added. The tubes were shaken and 0.2 ml. was removed for counting of the 5-HIAA fraction. Controls with added radioactive C-14-5-HIAA were run and 90 per cent recoveries obtained. Samples were placed in a total volume of 15 milliliters of toluene containing 2,5 diphenyloxazole (4 gm./liter) and 1,4-bis-[2-(4-methyl-5-phenyloxazolyl)]-benzene (100 mg./liter). Radioactivity was determined with a Packard Tri-Carb liquid scintillation spectrometer.

RESULTS

The effects of chlorpropamide and tolbutamide on aldehyde dehydrogenase. The effect of the sulfonylureas on the oxidation of acetaldehyde by liver aldehyde dehydrogenase is shown in table 1. Both chlorpropamide and tolbutamide inhibited the enzyme. The data of one experiment of table 1 are shown plotted in figure 3 to demonstrate the noncompetitive nature of the inhibition. All yielded similar changes. The nonparametric sign test was used to test the significance of the changes. The decreased DPNH production by both tolbutamide and chlorpropamide was significant at the level of $p < 0.01$. Chlorpropamide appeared to be the more potent inhibitor of the enzyme.

The effect of chlorpropamide and ethanol on serotonin metabolism. The in vivo metabolism of serotonin yields 5-HIAA as the major product, whereas 5-hydroxy-

TABLE 1

Effect of tolbutamide and chlorpropamide on the oxidation of acetaldehyde*

Acetaldehyde (mμmoles)	DPNH Production (mμmoles/ml./minute)				
	Control	Tolbutamide		Chlorpropamide	
2.5	4.85	3.15		0.67	
	4.67	2.58		1.25	
	3.55	2.67	(40 per cent)	0.94	(68 per cent)
	3.60	2.15		1.23	
	4.04	3.05		2.00	
	3.78	1.95		0.75	
5.0	4.82	2.74		1.13	
	3.81	2.10		1.05	
	2.95	1.47	(47 per cent)	0.63	(61 per cent)
	5.10	3.05		1.10	
	4.65	1.90		0.85	
	3.37	1.86		2.04	
25.0	6.59	3.22		1.85	
	5.08	2.59		2.13	
	7.16	5.03	(37 per cent)	0.92	(71 per cent)
	8.23	4.85		2.20	
	6.18	4.16		2.28	
	5.58	4.57		1.84	

*Numbers in parentheses represent the average per cent decrease in DPNH production due to the presence of tolbutamide or chlorpropamide. The values were obtained by averaging six controls and six tolbutamide values (or chlorpropamide values) at each acetaldehyde concentration. The per cent decrease was derived from these average figures.

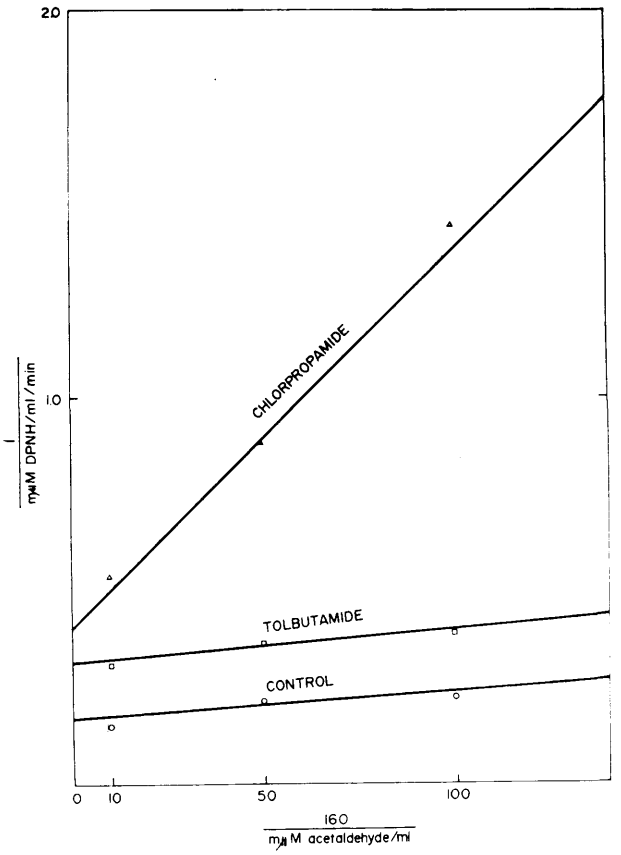

FIG. 3. Noncompetitive inhibition of acetaldehyde oxidation. For conditions of reactions see table 1.

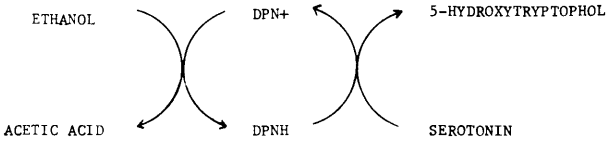

FIG. 4. DPN link of ethanol and serotonin metabolism.

indoleacetaldehyde and 5-hydroxytryptophol (neutrals) are minor products (cf. figure 2).[12-14] Inhibition of aldehyde dehydrogenase would result in a decreased formation of 5-HIAA and an increased formation of neutrals. The effect of chlorpropamide on serotonin metabolism by guinea pig liver homogenates is shown in table 2. Chlorpropamide resulted in a decrease in the production of 5-HIAA and an increase in the neutrals. This experiment represents one of eight which were done. All of these experiments showed a decrease in the production of 5-HIAA (ranging 12-19 per cent) and an increased production of neutrals (ranging 21-41 per cent). By the sign test, the changes were highly

TABLE 2

Effect of chlorpropamide on serotonin metabolism

Chlorpropamide (μmoles)	Neutrals (cpm/60 min.)	5-HIAA
—	109,375	337,525
0.5	114,550	326,975
2.5	125,100	317,950
5.0	150,800	294,425

Each reaction mixtures contained the indicated amounts of chlorpropamide, 150 mg. liver homogenate in 1.5 ml. 0.25 M sucrose, 50 μg. serotonin-C-14, (7×10^5 cpm) and 0.5 ml. 0.5 M phosphate buffer, pH 7.4. Final volume was 3 ml. Incubations were for 15 min. prior to the addition of the serotonin-C-14, and 60 min. after it, at 37° C. with constant shaking. The reactions were terminated by the addition of 3 ml. 0.3 N hydrochloric acid.

TABLE 3

Effects of ethanol and/or chlorpropamide on serotonin metabolism

Ethanol (μmoles)	Chlor-propamide (μmoles)	Neutrals (cpm/60 min.)	5-HIAA
—	—	39,425	178,650
—	5.0	93,950	144,125
17.3	—	145,100	106,175
17.3	5.0	158,640	93,375

Each reaction mixtures contained the indicated amounts of ethanol and/or chlorpropamide, 150 mg. liver homogenate in 1.5 ml. 0.25 M sucrose, 50 μg. serotonin-C-14, (5.4×10^5 cpm) and 0.5 ml. 0.5 M phosphate buffer, pH 7.4. Final volume was 3 ml. Incubations were for 60 min. at 37° C. with constant shaking. The reactions were terminated of the addition of 3 ml. 0.3 N hydrochloric acid.

significant ($p < 0.01$). In these experiments 60 per cent (ranging 45-70) of the added serotonin was metabolized.

The addition of ethanol to the reaction mixture simulated the effect of chlorpropamide on serotonin metabolism (table 3). The addition of ethanol plus chlorpropamide had a greater effect on serotonin metabolism than either one alone. This experiment represents one of four which were done. All of the experiments showed similar changes. Ethanol produced a more marked effect on serotonin metabolism.

DISCUSSION

No correlation has been ascertained between blood levels of acetaldehyde and the appearance of toxic symptoms in diabetic patients on sulfonylureas in whom the antabuse syndrome is elicited by ethanol ingestion.[3]

Much of the data concerning the pathogenesis of the

antabuse syndrome points to a disturbance in the metabolism of serotonin. Normally, the majority of serotonin in the body is metabolized by monoamine oxidase (MAO) to 4-hydroxyindoleacetaldehyde, which is in turn converted to 5-HIAA by aldehyde dehydrogenase. A small amount of the aldehyde is converted to 5-hydroxytryptophol via alcohol dehydrogenase.[12-14] The latter two reactions are dependent on the relative availability of DPN and DPNH (figure 2).[13] Alterations of this pathway suggest an effect on serotonin metabolism by both ethanol and the sulfonylureas as a possible cause of the antabuse syndrome.

5-HIAA and 5-hydroxytryptophol (5-HT) are the main products of serotonin metabolism with 5-HIAA the major product.[14] Ethanol ingestion causes a decrease in 5-HIAA and an increase in 5 HT.[15-19] This metabolic alteration might be due to (1) competitive inhibition of aldehyde dehydrogenase by acetaldehyde generated from ethanol[20]; (2) increased production of DPNH from ethanol metabolism (cf. figure 1). Each of these factors would favor the formation of more 5-HT (cf. figure 4). DPNH is itself capable of competition with DPN for its site on aldehyde dehydrogenase.[21] Ethanol has been found to increase hepatic DPNH in vivo.[22-24]

Ethanol ingestion produces a toxic reaction in patients with the serotonin-producing malignant carcinoid tumor, which is very similar, if not identical, to the antabuse syndrome.[25] It has been reported that ethanol (or acetaldehyde) can release stores of serotonin in vivo and in vitro.[26,27] This could also augment the production of neutrals. Although the neutral products of serotonin metabolism can cross the blood-brain barrier,[19] it is not known that they are toxic.

The sulfonylureas would accentuate the effects of ethanol by noncompetitively inhibiting aldehyde dehydrogenase. Moreover, the metabolism of tolbutamide results in an aldehyde metabolite whose conversion to carboxytolbutamide is catalyzed by aldehyde dehydrogenase.[28] The aldehyde derivative thus competitively inhibits the enzyme by acting as a substrate for it.

ACKNOWLEDGMENT

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Anorexia Nervosa

Patients with anorexia nervosa frighten nutritionists. In this land of plenty it is most distressing to encounter individuals who refuse food and will go to great lengths to circumvent the attempts of others to feed them. They do not appear angry or defiant; there is no ready explanation for their behavior (at least to a nonpsychiatrist); they often appear as placid, passive individuals who slowly waste away despite all that the mechanics of modern nutrition can offer.

These patients also confound the psychophysiologist and the neurophysiologist. They may complain of hunger, but they do not eat; some will take pleasure in preparing and serving food to others; the usual signals thought to be of some importance in appetite control, such as loss of body fat, mild hypoglycemia, gastric peristalsis, et cetera, seem to have no effect. They may resort to vomiting or purgation in response to force feeding. One can only conclude that the disturbance is far above the hypothalamus, that some superior integrative and perceptive mechanisms are involved.

The psychiatrist is concerned about the psychodynamics of anorexia nervosa; the nutritionist is worried that the patient will starve herself (about 75 per cent are young females) to death. A recent paper points out, however, the prognosis for life is somewhat better than had previously been supposed. C. H. Browning and S. I. Miller (Am. J. Psychiat. *124*:1128, 1968) review the course of thirty-six patients seen at their hospital during the past twenty-four years. Follow-up inquiry revealed that eighteen of the patients were definitely improved, nine showed questionable improvement, six were no better, and three were dead. Of the latter, one died of chronic asthma, a condition which antedated the onset of anorexia, while the other two died of complications of treatment. One had been given hypertonic gastric tube feedings, developed azotemia, and died following tracheal aspiration; the other had been given prolonged intravenous feedings without supplemental potassium. Another series of 115 patients revealed a death rate of 10 per cent (J. A. Sours, N.Y. State J. Med. *68*:1363, 1968), but no details are given as to the cause of death.

The degree of metabolic adaptability shown by these patients is truly remarkable. Most are hypometabolic, exhibiting hypothermia, bradycardia, low blood pressure and basal metabolic rate. Amenorrhea is common, and some show a diminution in pituitary and adrenocortical activity which has led some observers to postulate a state of "functional hypopituitarism." These phenomena all can serve to prolong life under conditions of restricted food intake. Overt vitamin deficiencies are rare, and plasma protein levels are usually normal. These findings suggest that dietary imbalance is somehow avoided.

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