The effect of ethanol on GABA content of rat brain

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IN A RECENT communication Ferrari and Arnold[1] reported that ethanol administered orally to rats at a dose level of 4·3 g/kg as an aqueous 33 per cent solution produced a decrease of γ-aminobutyric acid (GABA) of 16 per cent in 1 hr in brain of Sprague-Dawley rats, while in the Wistar strain of rats the decrease amounted to only 1·6 per cent. Häkkinen and Kulonen,[2] on the other hand, using Wistar rats, found an increase of 34 per cent under the same conditions, and the GABA level returned to normal in about 2 hr. A neurohumoral function for GABA was questioned, and it was suggested that its main role is probably via its participation in the metabolic pathway for entry of glutamate into the citric acid cycle at the succinate level or as a by-pass of the supposed rate-limiting α-keto glutarate dehydrogenase step. Results reported in the present communication have a bearing on the interpretation of these findings and support the view that strain differences exist in regard to levels of GABA in brain and the related influence of ethanol.

Weanling Sprague-Dawley rats were divided into two groups. The first, or B_6-deficient, group was fed *ad libitum* a diet composed of 24 per cent casein, 65 per cent glucose monohydrate, 4 per cent hydrogenated vegetable oil, 4 per cent Wesson salt mixture, 2 per cent cottonseed oil, and 1 per cent cod liver oil. To this were added 100 mg choline, 2·5 mg nicotinamide, 1 mg calcium pantothenate, 0·4 mg riboflavin, and 0·4 mg thiamine per 100 g of diet. The second, or control, group was fed a similar diet which contained in addition 0·4 mg pyridoxine per 100 g. After 1 month on the diets (average weight of controls, 261 g; B_6-deficient, 99 g) one-half of each group was injected intraperitoneally with 3 g ethanol per kg body weight (10 per cent ethanol in 0·9 per cent NaCl). The remainder of each group was injected with saline only. After 4 hr the rats were decapitated and the brains were removed, weighed, and homogenized immediately in ice-cold 75 per cent ethanol. At least 5 replicate samples from each brain were assayed for GABA.[3] Although results in Table 1 represent GABA

TABLE 1. EFFECT OF ETHANOL ON LEVELS OF GABA IN BRAIN OF SPRAGUE-DAWLEY RATS

		GABA content (μg/g brain)*	
	No. of rats	Saline only	3 g ethanol/kg
Controls	5	257 ± 16	230 ± 27
B_6-deficient	6	206 ± 12	207 ± 10

* Animals were decapitated 4 hr after receiving either saline or 3 g ethanol/kg intraperitoneally. Brains were extracted and assayed by chromatography[3] of at least 5 replicate samples of each tissue. Results are expressed as means ± standard error.

values 4 hr after ethanol or saline, values for the control Sprague-Dawley rats are in close agreement with those reported by Ferrari and Arnold[1] 1 hr after treatment with a somewhat higher, orally administered, ethanol dose (4·3 g/kg). However, in Wistar rats under these conditions as employed by Häkkinen and Kulonen,[2] ethanol *increased* rather than *depressed* the GABA content of brain. Moreover, the normal content of GABA in the brain of Wistar rats, reported by the latter authors, was much higher than that of the Sprague-Dawley rats presented here (440 μg vs 257 μg per g fresh tissue). Finally, the effect of ethanol on GABA in brain was still pronounced after 4 hr in the Sprague-Dawley rats, whereas it disappeared after about 2 hr in the Wistar strain. It is concluded from these data that a strain difference exists between Sprague-Dawley and Wistar rats regarding the influence of ethanol on levels of brain-GABA. Since GABA levels were depressed in the Sprague-Dawley rats after ethanol, it is questionable whether GABA may be assigned a general role as a depressant of the central nervous system. At any rate, in view of this observed strain difference, perhaps caution should attend the consideration of other influences on metabolism of GABA among animals of different strains and species.

Pyridoxine deficiency produces a marked depression in GABA levels (Table 1, "Saline only" groups). This finding is in accord with earlier reports in which pyridoxal binding agents or B_6 anti vitamins caused lowering of GABA in brain, and in which glutamate decarboxylase activity was shown to be substantially depressed in B_6-deficient animals.[4] The lowered level in the B_6-deficient group was not further depressed by the administration of ethanol, however. Thus the "GABA shunt" appears to have little quantitative significance under conditions of B_6-deficiency and therefore the depressant effect of ethanol on this pathway is not manifest. In this regard it is noteworthy that both glutamate decarboxylase and GABA transaminase require pyridoxal phosphate as coenzyme.

Department of Biochemistry,
Medical College of Virginia, and
Division of Alcohol Studies and Rehabilitation,
Commonwealth of Virginia, Richmond, Va., U.S.A.

EDWIN S. HIGGINS

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Inhibition of monoamine oxidase activity in sympathetic ganglia of the cat

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IN CATS, the administration of certain drugs that inhibit monoamine oxidase (MAO) is followed by a transient blockade of transmission of electrical impulses through the superior cervical ganglion (1–4). In order to determine whether there is any relationship between ganglionic blockade and inhibition of MAO, the present experiments were designed to characterize this inhibition in the sympathetic ganglia after treatment with two drugs: iproniazid* and N-benzyl-N-methyl-2 propynylamine hydrochloride (MO-911).*

From cats anesthetized with α-chloralose the superior cervical and stellate ganglia were removed before and after intravenous injections of MO-911 and iproniazid, and assayed for MAO activity by a method described previously.[5] The MAO-activity of ganglia removed prior to the administration of a MAO-inhibiting drug ranged from 2·8 to 12·4 (expressed as μmoles indoleacetic acid formed from tryptamine/g tissue per hr of incubation). In each of 10 individual animals, however, the MAO activities of the four ganglia differed by no greater than 15%, and no significant difference between stellate and superior cervical ganglia was detected. It was therefore possible to study inhibition of MAO by using the enzymic activity of one ganglion as a control to compare with that of other ganglia from the same cat. The control ganglion was removed prior to the injection of an MAO-inhibiting drug and was designated as having 100% MAO activity. The levels of MAO in ganglia removed later from the same cat were expressed as the percentage of activity with respect to the control ganglion.

The results of these experiments are presented in Table 1. MO-911, in doses of 10 mg/kg or greater, produced significant inhibition of MAO activity within 10 min and maximal inhibition within 30–60

* MO-911 (pargyline) was kindly supplied by Abbott Laboratories and iproniazid by Hoffmann-LaRoche, Inc.