Increased cesium-induced catecholamine release from isolated adrenals by lidocaine, 0.1 mM

Experiment	Total catecholamine µg/min above resting secretion Control	Lidocaine
1	78.2	119.3
2	224	235
3	75.6	165.7
4	87.5	76.6
5	24.5	36
6	28.1	69.3
7	92.7	119.3
8	126.6	213.6
Mean	92.2	129.4

Paired glands from the same animal were used for each experiment. 4 right glands and 4 left glands were used in each group. Release was measured during a 1-min-exposure to 56 mM Cs^+. Lidocaine significantly enhanced release by comparison of paired differences ($p<0.05$). Lidocaine alone has no effect on arenal catecholamine release.

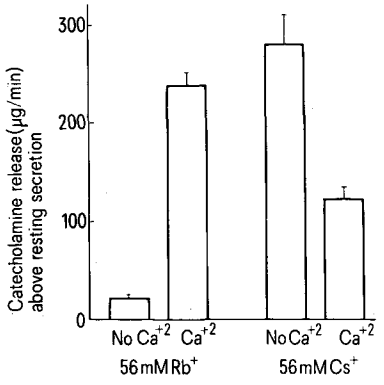

Fig. 2. Effect of a calcium-free medium on the response of bovine adrenal medulla to Rb^+ and Cs^+. Glands were infused for 20 min with calcium-free Lockes solution and then stimulated with 10 ml (1 min) of Rb^+ or Cs^+ containing solution (sodium content was decreased to maintain isotonicity) in the absence of calcium. 2 min after the initial stimulation, calcium was added back to the medium and the glands were restimulated with the same ion 18 min later. 4 glands were used for each ion.

small response is seen when calcium is absent from the medium (figure 2). The response to Cs^+, by contrast is greater without calcium in the medium (figure 2).

Thus, mechanism of adrenal catecholamine release by alkali metal varies with atomic weight. In the absence of extracellular calcium, the lightest ion K^+ is ineffective, Rb^+ is slightly active, and the heaviest ion Cs^+ shows greater effectiveness than in the presence of calcium. Potassium probably acts on adrenal medulla in the classical manner to depolarize the plasma membrane and admit extracellular calcium into the cell. Cesium as well as Ba^{+2} may act intracellularly since these ions are more potent under conditions of increased membrane permeability (calcium-free media). Lidocaine may increase membrane 'fluidity' or induce 'prelytic' changes, which allow increased penetration of Ba^{+2} and Cs^+ (see ref.[8]). Rubidium's effect appears to be a mixture of Cs^+-like and K^+-like actions. Many small metallic cations are essentially ineffective as agonists in adrenal medulla, e.g. Mg^{+2}, Co^{+2}, Ni^{+2}, Mn^{+2}, Zn^{+2}, Li^+ [3,6,8], whereas large metallic cations like Hg^{+2} are very good releasers of adrenal catecholamines[7]. Some metals may fail to release because they cannot activate the secretory apparatus[9]. Other ions e.g. Ba^{+2}, Cs^+ and possibly Sr^{+2} [9], may fail to release or release relatively small amounts because they cannot penetrate well into adrenomedullary cells. Plasma membrane surfaces of adrenomedullary cells may carry weak fixed negative charges which would favour penetration of ions of low charge density[10].

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Possible role of GABA in the development of tolerance to alcohol[1]

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Summary. Rat cerebellar GABA levels were reduced following 2 weeks alcohol administration. Animals also exhibited alcohol tolerance with an air righting reflex. This tolerance was mimiced by picrotoxin administration in control animals and was reduced in animals chronically administered alcohol by aminooxyacetic acid.

Acute exposure to ethanol appears to have a variable effect on central nervous system GABA (gamma amino butyric acid) levels. Both increases[2,3] and decreases[4,5] in the level of this putative transmitter have been reported. Chronic alcohol administration more generally causes a reduction in GABA levels[3], and such a reduction is greatest at that time when the signs of alcohol withdrawal are most severe[6]. Drugs that increase brain GABA levels reduce alcohol withdrawal convulsions[7]. The present communication deals with the question of whether or not GABA is involved in alcohol tolerance. The air righting reflex was used as the test procedure as it involves a cerebellar component, which includes GABAergic neurones[8].

Materials and methods. Male Sprague-Dawley rats, 200 g (CD outbred, Charles Rivers Breeding Labs., Inc., Wilmington, MA.) were divided into 3 groups. The control group was fed unsupplemented Purina lab chow meal and water ad lib. The alcohol group was fed one half the mean weight of food consumed by the control animals, and 10% ethanol was their only source of drinking water. The glucose group was fed the same amount of meal as the alcohol group, was given a glucose solution isocaloric with the mean daily alcohol consumption of the alcohol

Rat cerebellum GABA levels following 2 and 4 weeks on the experimental protocol

	GABA (μg/mg protein) Control	Glucose	Alcohol
2 weeks	1.58 ± 0.18	1.24 ± 0.17	0.99 ± 0.04*
4 weeks	1.42 ± 0.11	1.36 ± 0.07	1.00 ± 0.11*,**

Mean ± SEM values (N = 5 or 6). *Significantly different from control, $p < 0.02$. **Significantly different from glucose, $p < 0.02$. Statistical evaluation performed using the Student t-test.

group, and was given water ad lib. Animals were tested for their ability to right themselves when dropped from an inverted position. In reflex testing experiments ethanol was given by stomach tube, while all other drugs were administered s.c. Blood alcohol levels were measured using an alcohol dehydrogenase kit method (California Biochemical Corp., La Jolla, CA.) Cerebellum GABA levels were also measured in animals from these 3 groups, but without additional alcohol administration. Animals were decapitated in mid-morning and their heads immediately immersed in liquid nitrogen. The cerebellum was dissected frozen and homogenized in 9 volumes ice cold water, following which $^1/_4$ volume of 2 M perchloric acid was added and samples were cleared by centrifugation (50,000 × g for 30 min). Each supernatant was applied to 1 ml of Dowex 50 (H^+) (6 mm diameter column) followed by 8 ml water and 1 ml 1 M NH_4OH. The GABA, eluted in the next 5 ml of 1 M NH_4OH, was lyophilized and redissolved in water and assayed by the Gabase cell-free system[9] (Worthington Biochemical Corp., Freehold, N. J.). GABA values were normalized to mg protein[10] and corrected for loss of recovery using trace amounts of [^{14}C] GABA added to the initial homogenates. Of several methods of extraction tried this provided greatest reproducibility.

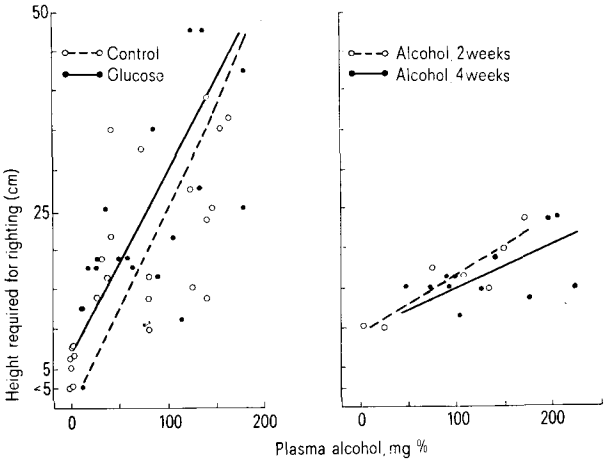

Fig. 1. Height required for animals to accomplish a complete air righting reflex, plotted as a function of the plasma alcohol concentration at the time of testing. Regression lines for control and glucose group animals represent data pooled from both 2- and 4-week treated animals. Alcohol group animals had a mean daily alcohol consumption of 8.5 and 9.0 g/kg at 2 and 4 weeks respectively.

Results. Chronic alcohol administration resulted in the development of tolerance as measured by the air righting reflex (figure 1), and caused a significant reduction in cerebellum GABA levels (table). At 2 weeks the difference between glucose and alcohol group GABA levels was not statistically significant. We tested the air righting reflex of animals that had never been exposed to alcohol, before and after receiving ethanol via stomach tube. When picrotoxin, an inhibitor of GABA activity, was administered 30 min after the alcohol, the animals showed a marked improvement in their righting ability (figure 2A). 2 sub-

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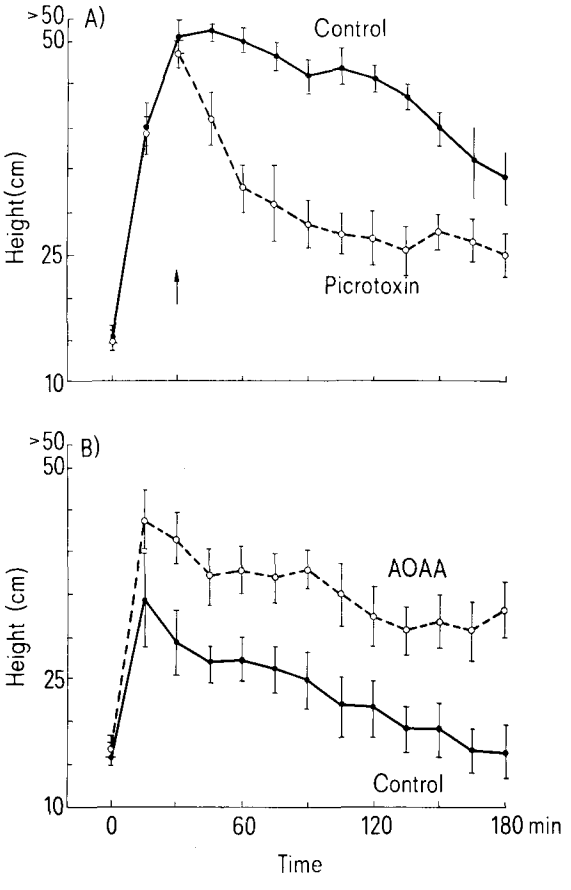

Fig. 2. Height required for air righting reflex completion, plotted as a function of the time after ethanol administration. *A* 500 g rats, not previously exposed to alcohol, were given 1 ml 20% ethanol/100 g by intubation at 0 time, and saline or 3.3 μmoles/kg picrotoxin s.c. at 30 min. *B* Animals previously given alcohol for 2 weeks were given saline or 0.2 or 0.4 mmoles AOAA/kg 3 h prior to administration of 1 ml 12% ethanol/100 g. Mean ± SEM values (N = 5–8).

convulsive doses of strychnine were also employed. With this drug generalized motor excitability increased, but unlike with picrotoxin the righting coordination remained poor. Another group of animals was kept on the alcohol regime for 2 weeks to develop tolerance. Animals were not allowed access to alcohol for 4 h prior to testing, but were given ethanol by intubation immediately prior to testing. Some of these animals were given the GABA transaminase (E.C. 4.1.1.15) inhibitor AOAA (amino-oxyacetic acid) 3 h before testing. Prior to being given ethanol, treated animals were selected so that a group was obtained that was able to right themselves as well as the non-treated control animals. As illustrated in figure 2B the AOAA-treated animals were more adversely affected by the administered alcohol as judged by this motor test.

Discussion. By 2 weeks the mean cerebellum GABA level in the alcohol group was significantly different from that of the control group, and by 4 weeks this statistically significant difference was also observed between the alcohol group and glucose group values. The lack of a statistically significant difference between the 2-week alcohol and glucose values was probably because the glucose group GABA level values were consistently, but non-significantly, lower than the control group values at the same time period. This may suggest a nutritional effect on brain GABA levels. Such a nutritional effect could have been related to the shift of the diet towards a greater proportion of carbohydrate, and an associated change in precursor metabolic pools fed by glucose and amino acids[11]. By 2 weeks the alcohol group animals had also developed tolerance, as demonstrated with the righting reflex. There was a significant difference in the height required for righting between the 2-week alcohol and glucose animals, even though the difference between their mean cerebellum GABA levels was not significant at this time period. With this exception it can be said that a depression in cerebellum GABA levels accompanied the development of alcohol tolerance by this reflex. However, no cause and effect relationship can be concluded from these data. A reduction in neuronal GABA levels would contribute to the development of tolerance by reducing central inhibition, thereby compensating for the depressant effect of the alcohol. Attempts to correlate brain GABA levels with excitability generally have variable success[12], perhaps because GABA levels per se do not distinguish between transmitter synthesis, release and restorage, or degradation, or whether the origin is neuronal or glial. We found that a drug that interferes with GABA neurone activity, picrotoxin[13], mimiced tolerance, while a drug known to increase brain GABA levels, AOAA[14], reduced tolerance that had already developed. These data could be interpreted as being manifestations of the generalized depressant qualities of endogenous GABA, or they could be interpreted as indirectly supporting the view that alcohol tolerance development involves a reduction in brain GABA levels, with an attendant reduction in inhibition or disfacilitation. The apparent specificity of the picrotoxin effect compared to the action of strychnine favours the latter explanation.

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Hypertrophy of pulmonary arteries and arterioles with cor pulmonale in rats induced by seneciphylline, a pyrrolizidine alkaloid

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Summary. Seneciphylline, one of the hepatotoxic pyrolizidine alkaloids, induced, as do also monoclotaline, etc., a marked arterial and arteriolar hypertrophy of the lung of young Wistar rats a month after a single s. c. injection of 50–80 mg/kg. Cor pulmonale with leftward shift of the ventricular septum was also noted.

Recently seneciphylline, m.p. 216 °C $[\alpha]_D^{21°}$ −134 °C ($CHCl_3$), has been isolated from the roots of Japanese Senecio cannabifolius Less (Japanese name: Hangonso) in large content using silica gel column chromatography[1]. Seneciphylline has been known as one of the hepatotoxic pyrrolizidine alkaloids[2] and found in a wide variety of plants of Senecio and Crotalaria species[3]. Some pyrrolizidine derivatives, such as monocrotaline and retrorsine, have been known to cause, besides the liver injury, cor pulmonale, occasionally accompanied by pulmonary arteritis and arteriolitis when fed per os[4,5] as well as single[6] or successive[5] injections. The present preliminary experiment showed that seneciphylline can also induce pulmonary changes with cor pulmonale in rats, similar to those caused by monocrotaline. Although the number of animals used was small, pulmonary and cardiac lesions were convincing.

Materials and methods. 16 male Wistar rats (Nihon Rat Co., Tokyo) of 4 weeks old (40–50 g) were used. They were caged in pairs in a screen-bottom cage and fed commercial pellet food (CE-II, Clea Japan Co.) and water ad libitum. Seneciphylline solution was prepared by dissolving it in 1 N HCl, diluting and then neutralizing with 0.5 N NaOH just before use; it was injected s.c. into the interscapular region (table). The animals were autopsied as soon as they were sacrificed or found dead. After gross examination, the heart, lungs and liver were fixed in 10% neutral buffered formalin, embedded in paraffin, cut and stained with hematoxylin and eosin. Special stainings were used when required.

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