The effect of hypoxia on isonicotinic acid hydrazide-induced seizures in chicks during ontogenesis

The concentration of γ-aminobutyric acid (GABA) in the brain of various species is elevated by hypobaric hypoxia, or by hypoxia caused by the breathing at ambient pressure of a gas mixture of low oxygen content (Wood, Watson & Ducker, 1968). Hypobaric hypoxia also delays the onset of semi-carbazide- and methionine sulphoximine-induced seizures (Baumel, Schatz & others, 1969) and it has been suggested by Baumel & others that the elevation in brain GABA level may be responsible for the anticonvulsant action. Although the above studies clearly indicate that both an elevation in brain GABA concentration and an anticonvulsant action are associated with hypoxic conditions, the results do not necessarily indicate a causal relation between the first two phenomena. The present investigation was therefore initiated to obtain additional information about this.

GABA metabolism increases during ontogenesis in the chick (Sisken, Sano & Roberts, 1961). If there is a concomitant change in the sensitivity of the brain GABA levels to hypoxia, this would allow a comparison to be made between the elevation of GABA and the anticonvulsant action of hypoxia in chicks of different ages. We have found that the sensitivity of brain GABA concentrations to hypoxia changes with age and that the elevation in brain GABA concentration is related to the delay in onset of the seizures.

White Leghorn cockerels aged 2 and 22-days were used. Isonicotinic acid hydrazide (INH) (2·18 mmol/kg) was injected intramuscularly in a volume of 0·154 M NaCl equivalent to 1% of body weight. The chick was then kept in a normal air environment or exposed immediately at ambient pressure to a hypoxic gas mixture containing 7·5% oxygen in nitrogen at 3–4 litre/min in a small chamber previously flushed with the mixture. The birds were observed continuously and the time to onset of generalized seizures recorded. The CT50 value was used as a quantitative measure of sensitivity. This value was the time required (min) after the administration of INH for seizures to occur in 50% of the birds. It was determined by plotting the percent convulsions on logarithmic probability paper (Miller & Tainter, 1944). Brain GABA levels were determined in control chicks (air) and in birds that had been exposed to hypoxic conditions for 20 min. No hydrazide was administered in these experiments. Brain extracts were prepared and the GABA assayed (Wood & Abrahams, 1971).

The CT50 values for the 2-day old chicks were 21·4 and 27·1 min for air and hypoxia respectively. For the 22-day old birds the values were 22·6 and 31·3 min. Hypoxic conditions delayed the onset of seizures in birds of both age groups, but the effect was more pronounced in the older birds, the changes in CT50 values being 26 and 38% in 2-day and 22-day old birds, respectively (Table 1).

Table 1. *Effect of hypoxia on brain GABA concentrations and on INH-induced seizures.*

Age (days)	Condition	GABA (μmol/g)	CT50† (min)	Change due to hypoxia (%) GABA	Change due to hypoxia (%) CT50	ΔCT50‡ / ΔGABA
2	Normal	2·35 ± 0·03* (10)	21·4			
2	Hypoxic	2·56 ± 0·01 (5)	27·1	+ 9·0	+26·6	2·96
22	Normal	2·80 ± 0·05 (5)	22·6			
22	Hypoxic	3·17 ± 0·03 (5)	31·3	+13·2	+38·5	2·92

* Mean ±s.e.; values in parenthesis indicate number of samples per group.

‡ ration = (% change in CT50 due to hypoxia)/(% change in GABA concentration due to hypoxia).

† Time required after administration of INH for 50% of the chicks to convulse.

In agreement with Sisken & others (1961), the brain GABA concentrations increased during the first 3 weeks of life (Table 1). Moreover the change produced by a standard hypoxic condition was greater in the 22-day old birds than in the 2-day old chicks. The constant ratio (Table 1) indicates that the elevation in GABA is proportional to the anticonvulsant effect (i.e. change in CT50) thereby lending support to the hypothesis of Baumel & others that the elevation in brain GABA concentration is responsible for the anticonvulsant action of hypoxia. However, the evidence is still not unequivocal.

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Triglyceride and precursor concentrations in the fatty liver of rats after chronic administration of ethanol

The most widely held explanation of the induction of fatty liver by ethanol is that an increase in the $NADH_2$:NAD ratio, secondary to the oxidation of ethanol, favours the formation of α-glycerophosphate and impairs the oxidation of fatty acyl-CoA derivatives (Lieber, Rubin & de Carli, 1969). The resulting increase in the concentrations of both precursors should then favour esterification to form triglyceride. In apparent support of this view, a single dose of ethanol has been found to increase the hepatic concentration of α-glycerophosphate and of triglycerides (Nikkilä & Ojala, 1963; Zakim, 1965). We have examined the concentrations of both precursors and product after chronic administration of ethanol in doses which consistently give rise to fatty liver in the rat.

Male Wistar rats, 200–300 g, were given homogenized liquid diets (Khanna, Kalant & Bustos, 1967) for 14 and 21 days. One diet, freely available, provided 35% of the total calories as ethanol, 19% as protein hydrolysate, 5% as sucrose and 41% as fat, and was nutritionally adequate in all other respects. The daily intake of ethanol averaged 10–12 g/kg. In a second diet the pair-fed controls had ethanol replaced by a calorically equivalent concentration of sucrose.

At the end of the treatment the animals were decapitated, the abdomen opened, and a portion of liver frozen instantly *in situ* by Wollenberger tongs precooled in liquid nitrogen. The frozen tissue was ground, deproteinized in 6% (w/v) perchloric acid, and centrifuged at 20 000 *g* for 15 min. The precipitate contained the long-chain fatty acyl-CoA derivatives, which were hydrolysed (Bortz & Lynen, 1963) and assayed for CoA content (Stadtman, 1955). α-Glycerophosphate was measured enzymatically in the neutralized supernatant (Hohorst, 1963). Other portions of the same livers were homogenized in phosphate buffer, and the triglycerides were extracted and measured (Butler, Maling & others, 1961).