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The effect of isoniazid and some anticonvulsant drugs on the γ-aminobutyric acid content of mouse brain in insulin hypoglycaemia

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Phenobarbitone, prominal and primidone protect mice from insulin convulsions and raise their lowered cerebral hemisphere GABA content. Phenobarbitone and primidone are superior to prominal and produce a significant increase in the insulin depleted GABA content. Isoniazid potentiates insulin convulsions and significantly lowers the insulin depleted GABA content. It is probable that insulin depletion of cerebral hemisphere GABA content is a rationale of hypolgycaemic convulsions.

The mechanism by which a high dose of insulin produces convulsions is vague. According to Sollmann (1957) it is not due to hypoglycaemia directly but to some secondary process. Insulin convulsions may be suppressed by narcotics, such as barbiturates, even with very low blood sugar.

Insulin produces a decrease in brain γ-aminobutyric acid (GABA) content (Cravioto, Massieu & Izquierdo, 1951; Okumura, Otsuki & Nasu, 1959; De Ropp & Snedeker, 1961; Maynert & Kaji, 1962). Saad (1969) found that the lowest level of GABA in the cerebral hemispheres of mice occurred 1 h after insulin administration. The hyperexcitability and convulsions in insulin-treated mice correlates with the significantly lowered levels of GABA. Woodbury & Esplin (1959) suggested that there is a striking and highly significant correlation of GABA content in brain and the electroshock seizure threshold. Decrease in GABA content was accompanied by an increase in brain excitability.

Isoniazid produces a decrease in brain GABA content (Sugawara, 1958; Bukin, 1959). Saad, El Masry & Scott (1969) found that its intraperitoneal administration to mice produces a sharp lowering of the cerebral hemispheric GABA content with a maximal effect after 1 h. The degree of convulsive activity increased as the GABA content fell.

Certain barbiturates which are effective against grand mal epilepsy increase the GABA content in the cerebral hemispheres of mice. Saad & others (1969) found that the GABA content increased to a peak 1 h after the intraperitoneal administration of the sodium salts of prominal or primidone, and 2 h after administration of phenobarbitone sodium. The barbiturates returned to normal the isoniazid depleted GABA contents and protected animals from convulsions.

The synergism and antagonism of the effects of isoniazid and certain barbiturates on insulin convulsions in mice has been examined. The effects have been related to the levels of GABA in their cerebral hemispheres.

EXPERIMENTAL

Determination of GABA

GABA was quantitatively determined using a chromatographic and colorimetric method previously described by Saad (1970) using the cerebral hemispheres from the brains of 3 animals for each analysis.

Experimental design

Adult male mice, 20 to 30 g, were kept on a bread diet several days before the experiment as suggested by Rowlinson & Lesford (1948) to give a more accurate response to insulin. The mice were fasted 1·5 h before the experiment. They were divided into 7 groups, each was subdivided into 3 sub-groups of 3 mice from which the cerebral hemispheres were pooled. The mice were killed at the time corresponding to maximal effect of the drugs on their cerebral hemispheric GABA content.

One group of animals was used as control. A second group was killed 1 h after the subcutaneous injection of insulin (2 u/kg). A third group was injected with phenobarbitone sodium (50 mg/kg i.p.) 2 h, and with insulin (2 u/kg, s.c.) 1 h before death. Prominal sodium, primidone sodium and isoniazid (50 mg/kg) were administered intraperitoneally separately into groups of mice. Insulin (2 u/kg) was administered subcutaneously at the same time to each group. The animals were killed 1 h later. The seventh group of mice was killed 1 h after the intraperitoneal administration of isoniazid alone (50 mg/kg).

Each group of mice was left after the treatment at 29° before being killed for the determination of their cerebral hemisphere GABA content. The state of activity of all the mice just before death was noted.

RESULTS

The mice injected with phenobarbitone and insulin were markedly depressed before death and 78% were hypnotized. Phenobarbitone sodium completely protected the animals from insulin convulsions; primidone sodium protected 78% and prominal sodium 56% of the mice. The other animals showed mild convulsions just before death. The mice given isoniazid and insulin showed severe clonic convulsions, three died.

The GABA content in the cerebral hemispheres of mice was determined for each group and the results are in Table 1.

Table 1. *GABA content in the cerebral hemispheres of adult male mice after the treatment with insulin, phenobarbitone, prominal, primidone or isoniazid.* Insulin was administered subcutaneously in a dose of 2 u/kg 1 h before death. Drugs were administered intraperitoneally in a dose of 50 mg/kg 1 h before death except phenobarbitone (2 h).

	GABA content (mg/100 g wet tissue)						
	Controls	Insulin	Phenobarbitone + insulin	Prominal + insulin	Primidone + insulin	Isoniazid	Isoniazid + insulin
1	32·00	18·30	26·30	18·10	23·30	14·20	10·20
2	34·40	18·90	28·30	20·50	24·30	15·10	13·50
3	37·90	19·90	29·90	22·40	26·70	17·20	17·10
x	37·76	19·03	28·16	20·30	24·76	15·50	13·60
s.e.	1·7132	0·4667	1·0413	1·2443	1·0088	0·8892	1·9925
*P**			<0·05		<0·05		<0·05
P†		<0·05	<0·05	<0·05	<0·05	<0·05	<0·05

* The difference between the mean GABA content and that after insulin treatment.
† The difference between the mean GABA content and the controls.

DISCUSSION

The protection of mice from insulin convulsions by barbiturates was accompanied by an increase in the cerebral hemisphere GABA level reduced by insulin administration. The effect was significant after the administration of phenobarbitone and primidone. Both the degree of protection of the mice from insulin convulsions and effect on the level of cerebral hemisphere GABA was greatest with phenobarbitone, then primidone, finally prominal. However none of these barbiturates returned the insulin lowered level of GABA to normal.

The potentiation of insulin convulsions by isoniazid was accompanied by a small but significant decrease in GABA content from the already lowered level of GABA induced by insulin. However, the difference in terms of convulsive activity was marked.

Since there is a striking and highly significant correlation of GABA content in brain and excitability (Woodbury & Esplin, 1959), and since there is a tendency for a change in GABA content in protection from or potentiation of insulin convulsions it is possible that insulin depletion of cerebral hemisphere GABA content is a rationale of hypoglycaemic convulsions.

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