On the mechanism of the convulsivant effect of hydrazides in mice

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METHODS

1. *Manometric estimation of glutamic acid decarboxylase activity of the brain.* Two ml homogenate 1 : 4, 0·067 M phosphate buffer, pH 6·5; 40 mg Na-glutamate in 0·3 ml distilled water, 37 °C, N_2 atmosphere.

2. *Amino acid estimation.* γ-Aminobutyric acid (GABA), glutamic acid, aspartic acid. For each experiment three mice brains were homogenized with 5 ml 70% ethanol. Separation of the amino acids of the alcoholic extracts by high voltage electrophoresis; direct quantitative estimation on the paper with the "Spinco Analytrol" (Beckman).

RESULTS

It is known that after the injection of hydrazines[1, 2] in convulsive doses the GABA content of the brain is decreased.[3] The cause is an inhibition of the glutamic acid decarboxylase. The hydrazides form hydrazones with the coenzyme pyridoxal-5-phosphate.

(1) Pyridoxine which inhibits the convulsions does not prevent the decrease of the GABA-content of the brain. On the contrary, after simultaneous injections of pyridoxine and hydrazides the fall in GABA content was even more pronounced (Table 1), in spite of a normal activity of the glutamic acid decarboxylase (Table 2, thiosemicarbazide). The explanation probably is that pyridoxine catalyses the

TABLE 2. THE EFFECT OF PYRIDOXINE ON THE *in vivo* INHIBITION OF GLUTAMIC ACID DECARBOXYLASE ACTIVITY OF THE BRAIN CAUSED BY HYDRAZIDES

	μl. CO_2 90 min	% Activity
Controls	55	100
Pyridoxine 50–300 mg/kg i.m.	71	129
Thiosemicarbazide 16 mg/kg i.m. (CD100 + 25%)	34	62 (−38)
Thiosemicarbzide 16 mg/kg i.m. + pyridoxine 50 mg/kg	55	100
Isonicotinic hydrazide 200 mg/kg i.m. (CD100)	28	51 (−49)
Isonicotinic hydrazide 200 mg/kg i.m. + pyridoxine 300 mg/kg (simultaneously)	38	69 (−31)
Isonicotinic hydrazide 200 mg/kg i.m. + Pyridoxine 300 mg/kg (30 min before INH)	19	35 (−65)

The mice were sacrificed 60 min after the injection of hydrazides (45 min after injection of INH).

transamination between GABA and α-ketoglutaric acid more strongly than the decarboxylation of glutamic acid.

It is concluded that the seizures are caused not by the actual decrease in brain GABA, but rather by the reduced turnover in the glutamic acid-GABA-α-ketoglutaric acid metabolism.

(2) Seizures caused by isonicotinic acid hydrazide (INH) were not prevented by pyridoxine (Py). Simultaneous administration of pyridoxine reduced the inhibition of decarboxylase by INH; if Py was injected 30 min before INH, it enhanced the inhibition (Table 2). After high doses of Py-5-phosphate the INH-seizures occurred earlier than after INH alone.

TABLE 1. THE EFFECT OF HYDRAZIDES AND PYRIDOXINE ON THE GABA GLUTAMIC ACID AND ASPARTIC ACID CONTENT OF THE BRAIN

	No. of mice	No. of exp.	γ-Aminobutyric acid			Glutamic acid			Aspartic acid
			μmole/g	decrease	% significance	μmole/g	decrease	% significance	μmole/g
Controls	180	60	3·49 ± 0·14			17·5 ± 0·38			4·38 ± 0·11
Thiocarbohydrazide 4 mg/kg i.m.	21	7	2·07 ± 0·19	−40	$t = 3·46$ $p = 0·001$	14·5 ± 1·27	−17	$t = 5·22$ $p = 0·001$	3·62 ± 0·31
Thiosemicarbazide 13 mg/kg i.m.	57	19	3·56 ± 0·21	0		17·7 ± 0·84	0		4·20 ± 0·25
Isonicotinic hydrazide 200 mg/kg i.m.	27	9	1·84 ± 0·22	−47	$t = 4·25$ $p = 0·001$	13·5 ± 1·09	−23	$t = 3·15$ $p = 0·01$	4·66 ± 0·42
Thiocarbohydrazide 4 mg/kg i.m. +pyridoxine 50 mg/kg	21	7	1·32 ± 0·13	−62	$t = 5·3$ $p = 0·001$	14·1 ± 0·96	−19	$t = 2·9$ $p = 0·01$	3·98 ± 0·17
Thiosemicarbazide 13 mg/kg i.m. +pyridoxine 50 mg/kg	24	8	2·50 ± 0·24	−28	$t = 2·5$ $p = 0·02$	13·5 ± 1·06	−23	$t = 2·4$ $p = 0·01$	3·51 ± 0·42
Isonicotinic hydrazide 200 mg/kg i.m. +pyridoxine600mg/kg	12	4	1·64 ± 0·23	−53	$t = 3·36$ $p = 0·01$	16·7 ± 1·32	− 5		3·77 ± 0·32

Pyridoxine and convulsive doses of the hydrazides simultaneously were injected i.m. to groups of three mice; 60 min later the animals were sacrificed.

It is concluded that Py and Py-5-phosphate are not merely biochemical antagonists of INH, but can potentiate the action of INH, perhaps by facilitating the penetration of INH into the brain across the blood–brain barrier.

Nicotinamide (400–500 mg/kg) abolished the seizures caused by INH (200–250 mg/kg) in 50 per cent of the experimental animals.

TABLE 3. THE EFFECT OF RESERPINE ON THE GABA-CONTENT OF THE BRAIN AND THE THRESHOLD OF ELECTROSEIZURES IN MICE

	Numbers of mice (n)	Numbers of exp.	γ-Aminobutyric acid			Glutamic acid (μmole/g)	Aspartic acid (μmole/g)	threshold for electro-seizures (100 Hz, 5 msec, 5 sec)
			μmole/g	% de-crease	signifi-ance			
Controls	$n = 180$	60	$3{\cdot}49 \pm 0{\cdot}14$			$17{\cdot}5 \pm 0{\cdot}38$	$4{\cdot}38 \pm 0{\cdot}11$	5 mA ($n = 10$)
Reserpine 5 mg/kg s.c. 3 hr before injection	$n = 30$	10	$2{\cdot}42 \pm 0{\cdot}51$	-30	$t = 2{\cdot}67$ $p = 0{\cdot}01$	$17{\cdot}7 \pm 1{\cdot}71$	$3{\cdot}96 \pm 0{\cdot}38$	1·0–2·5 mA ($n = 10$)

(3) The *tonic* component of hydrazide induced seizures was abolished when the dopamine content of the brain was raised by the injection of iproniazid (100 mg/kg i.m.) and L-DOPA (100 mg/kg i.p., 2 hr before). This may explain why low doses of pyridoxine (18 mg/kg) antagonized the convulsive action of *iso*propylhydrazine (400 mg/kg), although they had only a weak effect on the inhibition of glutamic acid decarboxylation: *iso*propylhydrazine is a potent inhibitor of monoamine oxydase.

(4) Doses of reserpine which decreased the threshold for electroshock, diminished the content of GABA in the brain, but not that of glutamic and aspartic acid (Table 3).

(The results of our experiments will be published *in extenso* in *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmak.*)

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