Table 1. Thermodynamic data of warfarin and phenylbutazone displacement by FFA

Fatty acid	F	$nK(P)$	$(P)^*$ ($M \times 10^5$)	K ($M^{-1} \times 10^{-3}$)	ΔG (cal/mole)	$\Delta\Delta G$ (cal/mole)
			Warfarin			
None	0·829	4·85	6·70	72·3	−6740	0
Stearic	0·515	1·06	7·99	13·3	5720	1020
Myristic	0·306	0·44	8·85	4·98	−5129	1611
Lauric	0·160	0·19	9·44	2·02	−4584	2156
			Phenylbutazone			
None	0·913	10·49	6·36	165	−7237	0
Stearic	0·677	2·10	7·32	28·6	−6181	1056
Myristic	0·509	1·04	8·01	12·9	−5703	1534
Lauric	0·286	0·40	8·93	4·49	−5065	2172

* Values of (P) were calculated from the reported [10] values of F and the total concentrations of drug and albumin assuming $n = 1$.

and mechanisms for pairs of drugs, such as warfarin and phenylbutazone, are possible via molecular probes such as fatty acids.

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Elevation of central γ-aminobutyric acid levels by isoniazid in mice and convulsant thresholds

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The accumulating evidence for a role of γ-aminobutyric acid (GABA) as a central inhibitory transmitter [1, 2] has prompted several studies about a relationship between the central levels or the metabolism of this amino acid and the sensitivity to convulsions. The results of these studies have been contradictory: some authors did not find any such relationship [3–5], whereas the results of others seem to indicate a role of GABA for central excitability [6] or seizure activity [7–9]. Interpretation of the results was made difficult by the use of drugs with powerful pharmacodynamic properties, as for example thiosemicarbazide or aminooxyacetic acid, as tools for alterations in the central GABA metabolism. Recently, however, Perry and Hansen [10] reported that isoniazid administered in the food over several days in nontoxic doses was able to elevate central GABA levels considerably. Wood, Peesker and Urton [11] and Wood and Peesker [12] showed that treatment with isoniazid indeed protected chicks against seizures elicited by exposure to hyperbaric oxygen or by injection of picrotoxin or pentetrazole, but was ineffective in rodents against hyperbaric oxygen seizures.

Since a daily uptake of 100 mg/kg isoniazid in the drinking water was tolerated by mice without behavioral changes, and since this treatment neither seemed to affect the central metabolism of monoamines, the effect of which on convulsant thresholds had been studied previously [13], we thought it worthwhile to look for a correlation between central GABA concentration and the electro- and chemoconvulsant thresholds in this species.

MATERIAL AND METHODS

The study was done in mice of NMRI-strain (Mollegaard Hansens Avlslaboratorier A/S, Fjby, DK-4632 Fl.

Table 1. Influence of treatment with isoniazid on the central concentrations of GABA and monoamines, on the activity of GAD and GABA-T, and on the thresholds for electroconvulsion and convulsions elicited by pentetrazole

Treatment	Isoniazid daily intake (mg/kg)	GAD (μM/g/hr)	GABA (μg/g)	GABA-T (FU*/2 hr/g)	Noradrenaline (μg/g)	Dopamine (μg/g)	5-HT (μg/g)	5-HIAA (μg/g)	Thresholds (ED_{50}) Electroconvulsion (Volts)	Pentetrazole clonic component (mg/kg)	Pentetrazole tonic component (mg/kg)
Controls		44 ± 3.5† (18)‡	195 ± 12 (12)	91 ± 17	0.45 ± 0.053 (18)	0.95 ± 0.16 (18)	0.54 ± 0.06 (18)	0.23 ± 0.03 (18)	151 (140–164)**	43 ± 2.7	85 ± 17
Isoniazid (0.3 mg/ml)											
4 days	67 ± 12	45 ± 7.7	225 ± 11¶	96 ± 4	0.4 ± 0.031	1.08 ± 0.13	0.57 ± 0.07	0.18 ± 0.02¶	158 (148–170)	38 ± 5.8§	75 ± 17¶
7 days		44 ± 3.1	275 ± 28¶	102 ± 4	0.51 ± 0.036‖	1.1 ± 0.19§	0.56 ± 0.067	0.18 ± 0.029¶	139 (129–150§	52 ± 9.5¶	93 ± 23
Isoniazid (0.5 mg/ml)											
4 days	100 ± 19	40 ± 7.5	280 ± 22¶	63 ± 2¶	0.38 ± 0.0698	0.86 ± 0.076	0.59 ± 0.0268	0.21 ± 0.024	153 (142–166)	43 ± 9.9	80 ± 18
7 days		46 ± 7.1	255 ± 15¶	95 ± 2	0.44 ± 0.047	0.95 ± 0.063	0.55 ± 0.065	0.26 ± 0.0348	162 (153–172)	45 ± 8.6	80 ± 17
Isoniazid (0.3 mg/ml) + Pyridoxine (0.06 mg/ml)											
4 days	69 ± 9.3	45 ± 7.3	230 ± 9.7¶	70 ± 8¶	0.36 ± 0.034¶	1.02 ± 0.1	0.59 ± 0.0748	0.19 ± 0.03¶	152 (143–162)	44 ± 8.1	96 ± 23
7 days		47 ± 6.9	255 ± 22¶	70 ± 2¶	0.36 ± 0.052¶	1.26 ± 0.14¶	0.54 ± 0.036	0.18 ± 0.025¶	131 (120–143)¶	49 ± 13	98 ± 26§
Isoniazid (0.5 mg/ml) + Pyridoxine (0.1 mg/ml)											
4 days	103 ± 12	48 ± 1.8¶	285 ± 27¶	64 ± 2.5¶	0.39 ± 0.035¶	0.93 ± 0.044	0.57 ± 0.04	0.23 ± 0.033	141 (132–151)	44 ± 7.4	86 ± 18
7 days		44 ± 2.0	335 ± 27¶	68 ± 5.3¶	0.46 ± 0.04	1.0 ± 0.11	0.59 ± 0.05	0.16 ± 0.034¶	150 (138–162)	44 ± 5.9	86 ± 16

* Activity given in arbitrary fluorescence units.
† Values are expressed as means ± S. D.
‡ Figures in parentheses = number of determinations, if not indicated: n = 6.
§ $P < 0.05$, ‖ $P < 0.02$, ¶ $P < 0.01$.
** Confidence limits for 95% probability.

Skensved, Denmark) weighing 20–25 g. Equal numbers of male and female mice were used in all experiments, but the sexes were kept apart.

The animals were kept in groups not exceeding ten in Macrolon-cages and had free access to food (Altromin R 10-pellets) and water.

Isoniazid treatment. Isoniazid was dissolved in the drinking water in concentrations of 0.3 or 0.5 mg/ml. In other experiments, 0.06 or 0.1 mg/ml of pyridoxine HCl was added to the concentrations of isoniazid mentioned. The relation of 5:1 between both drugs had been adopted from the work of Perry and Hansen [10]. Fresh solutions were made up every second day. Treatment with isoniazid, both with and without pyridoxine, was given for 4 and 7 days preceding the determination of the convulsant thresholds and the biochemical parameters. By weighing the bottles for the drinking water and the mice every day, the daily intake of isoniazid could be calculated. Controls were used both without treatment and with pyridoxine only.

Determination of convulsant thresholds. Electroshock was applied by eye electrodes using an A-615-B shocker, (Lafayette Co.). Stimulation data were 50 cycles/sec for 0.2 sec with the serial resistance of the apparatus set to 10 kΩ. Endpoint was the tonic extension of the hind limbs. For the determination of the electroconvulsant threshold, the voltage of the shock was changed according to the 'up and down' method of Kimball, Burnett and Doherty [14]. The threshold for clonic and tonic chemoconvulsions was determined in freely moving mice by the method of Hint and Richter [15]. A 1% solution of pentetrazole was infused intravenously at a rate of 0.3 ml/min by means of a Sage syringe pump, model 351. Endpoints were the fully developed clonic seizure with loss of the righting reflexes and the tonic extension of the hind limbs. Twenty mice were used for each threshold determination. Determinations were always done at the same time of the forenoon. The thresholds are given as the voltage or the dose of pentetrazole provoking the respective endpoint in 50 per cent of the mice. Details of the method have been described in a previous paper [13].

Biochemical determinations. Groups of six mice were used for the determination of the central levels of the catecholamines, 5-hydroxytryptamine (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and of GABA. Noradrenaline and dopamine were determined by the method of Chang [16], 5-HT and 5-HIAA by that of Curzon and Green [17]. GABA was extracted from whole brains that had been homogenized in chilled ethanol. After separation by paper chromatography, GABA was coloured by ninhydrin, eluted from the paper, and the colour was measured at 575 nm [18]. The concentrations determined in this way in control animals amounted to about 200 μg/g and are considerably lower than those determined later by the fluorometric method of Sutton and Simmonds [19] which gave control values of about 400 μg/g in the same strain of mice. The activity of glutamic acid decarboxylase (GAD) was determined in homogenates of mouse brain by the method of Lowe, Robins and Eyerman [20], that of GABA-γ-oxoglutarate aminotransferase (GABA-T) by the method of Salvador and Albers [21]. The results of the GABA-T determinations are given in fluorescence units (FU), since samples of succinic semialdehyde which had been kindly prepared by Dr. Smalla polymerized too rapidly to permit the construction of a quantitatively reliable standard curve. In the determinations of GABA, GAD and GABA-T, the brains of the mice were homogenized in ice-cold media within 45 sec after decapitation. Determinations were always done at the same time of the forenoon.

RESULTS AND DISCUSSION

The results of the study are summarized in Table 1. Treatment with isoniazid increased the central level of GABA as expected from the work of Perry and Hansen [10], and this increase was fairly well correlated to the total amount of drug consumed ($r = 0.8$, $P < 0.01$). However, these changes in the concentration of GABA were not related to the thresholds for either maximal electroconvulsion or the clonic or tonic components of the pentetrazole seizure. Neither was there any relation between isoniazid intake and central monoamine levels. In some instances, the monoamine levels or the convulsant thresholds showed significant deviations from the control values, but these were in no way related to either drug intake or GABA levels.

GAD activity was hardly altered by isoniazid treatment, but the GABA-T activity was reduced in most of the treated groups. The latter change is probably not the cause of the rise in central GABA since the mitochondrial GABA-T is regarded not as playing a major role for the levels of GABA [22]. Sutton and Simmonds [23] have also reported comparable increases in the GABA concentration after treatment with ethanol for 3 weeks without changes in the activity of both enzymes. Readily reversible changes in enzyme activity induced by isoniazid might have escaped detection with the method used, but we were also unable to show an increase in GAD activity after the addition of the drug *in vitro* (Löscher and Frey, unpublished).

The addition of pyridoxine to the drinking water had no effect on the GABA elevation induced by isoniazid which is in agreement with results of Perry and Hansen [10]. The biochemical data of mice treated with 0.1 mg/ml pyrodoxine HCl for 7 days did not differ from those of untreated controls.

Our results have thus shown that the convulsant thresholds are not elevated by the moderate rises in the central levels of GABA that can be induced by the intake of well-tolerated doses of isoniazid for some days. This lack of correlation between elevated GABA levels and seizure sensitivity is in agreement with results of other authors using less indifferent drugs and regimens of treatment [3–5]. An apparent exception is the protective effect of elevated GABA levels in avian seizures [11, 12, 24]. On the other hand, there is some rather convincing evidence that decreases in central GABA levels lead to increased seizure activity [6, 9, 25, 26]. This would be consistent with the assumption of an optimal 'protective' effect of physiological levels of GABA that cannot be improved by an increase in these levels, and with the hypothesis of disinhibition as the organizing principle in the nervous system [22]. The exact localization of the surplus GABA is also of interest in this regard: Andén [27] has recently suspected an accumulation of GABA in dopaminergic neurons of animals treated with aminoxyacetic acid, which, though considerably increasing the central level of GABA by inhibition of GABA-T, is devoid of a true anticonvulsant effect (Löscher and Frey, in preparation).

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