Effect of diazepam on γ-aminobutyric acid (GABA) content of mouse brain

Diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one), has been reported to have anticonvulsant and antiepileptic effects (Banziger, 1965; Eidelberg, Harry & Miller, 1965; Galambos, 1965; Gastaut, Naquet & others, 1965). Highly significant correlation of γ-aminobutyric acid (GABA) content in brain and excitability was suggested by Woodbury & Esplin (1959). The GABA content in the brains of epileptics was shown to be low (Elliott, Roberts & Baxter, 1959) and its formation decreased in brains of idiopathic epileptics and of rabbits sensitized to convulsions (Kokudo, 1959; Kuroda; 1959). For these reasons, the effect of diazepam on the normal cerebral GABA levels in the hemisphere and in hemispheres in which the GABA levels were reduced by the administration of isoniazid, was studied.

GABA was quantitatively determined using the chromatographic method of Saad (1970). Both cerebral hemispheres, including white matter, from the brains of 3 decapitated animals were quickly frozen (−4 to −6°) and pooled for each analysis. The elution of GABA spots was as described by Sytinskii & Priyatkina (1966) and the colour was read at 512 nm. Diazepam (Valium 10, amp., Hoffmann-La Roche) was administered intraperitoneally in doses of 5 or 10 mg/kg to adult male mice, 20–25 g, in 4 groups of 9 animals for each dose. Each group was subdivided into 3 subgroups from which the cerebral hemispheres were pooled and the GABA content determined 1, 2, 3 or 4 h after injection of diazepam. The mean GABA content for each group was compared with that from cerebral hemispheres of 9 untreated male mice in pooled samples each from 3 animals. The difference was considered significant if by Student's *t*-test $P < 0.05$.

The GABA content of cerebral hemispheres of 9 mice was depleted by isoniazid (Sugawara, 1958), administered (50 mg/kg, i.p.) and the GABA content determined for each subgroup of 3 animals 1 h later; this corresponds to the maximal fall in cerebral hemisphere GABA content (Saad, El Masry & Scott, 1972). Two groups of 9 mice were given isoniazid (50 mg/kg, i.p.) and diazepam (5 or 10 mg/kg, i.p.) separately, 1 h before decapitation and then the procedure was as described above. The mean GABA content for each group was compared with that of untreated animals, and that of isoniazid-treated animals. The state of activity of mice was observed before decapitation.

The mean control GABA content in the cerebral hemispheres was 415 ± 22 μg/g wet tissue. The effect of diazepam on the normal cerebral GABA levels in the hemispheres is summarized in Table 1.

Table 1. *GABA content in the cerebral hemispheres of adult male mice at different times after i.p. administration of diazepam.*

Diazepam (mg/kg)	Mean *GABA content (μg/g wet tissue) at times (h) 1	2	3	4
5	490 ± 16†	520 ± 17†	465 ± 20	430 ± 22
10	512 ± 20†	503 ± 12†	482 ± 32	470 ± 24

* Mean ± s.e. of 3 pooled samples, each sample from 3 cerebral hemispheres.
† Significant difference from the control ($P < 0.05$).

Diazepam in doses of 5 and 10 mg/kg increased the normal cerebral GABA levels in the hemispheres 1 h after injection by about 18 and 23% respectively. At 2 h, the

level increased by about 25 and 21% respectively, while no significant effect was seen at 3 and 4 h. Diazepam was more effective in increasing the levels of GABA in hemispheres previously depleted of it by isoniazid. Thus in doses of 5 and 10 mg/kg and 1 h after injection, it increased the isoniazid—reduced GABA levels significantly higher by about 88 and 80% respectively, and this was accompanied by protection of mice from the convulsions. Control GABA content: 415 ± 22 μg/g wet tissue; isoniazid treated 205 ± 9 μg/g; diazepam, 5 mg/kg, 490 ± 16 μg/g and 10 mg/kg, 512 ± 20 μg/g; isoniazid + diazepam (5 mg/kg) 385 ± 15 μg/g; isoniazid + diazepam (10 mg/kg) 370 ± 18 μg/g wet tissue.

Thus diazepam produces a much higher increase in GABA in hemispheres of mice in which GABA had been reduced by isoniazid than in normal mice. This indicates that the diazepam has a higher activity in brain disorders leading to decreased GABA content. The decreased GABA content is associated with excitability and lower electroshock-seizure threshold (Woodbury & Esplin, 1959) and probably correlates with McIlwain's conclusion (1962) that the characteristic action of anticonvulsants is not on the normal brain, but on that subjected to abnormal excitation of an epileptic seizure. My results are also similar qualitatively to those seen after certain anticonvulsant barbiturates and hydantoins (Saad & others 1972). Many workers e.g., Hawkins & Sarett, 1957; Trifaro, Mikulio & others, 1964 have found that GABA, or drugs that increase its content in brain, antagonize convulsions. The effect of diazepam in increasing the normal and depleted GABA levels in the hemispheres may be related to its anticonvulsant and antiepileptic actions.

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