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Altered 5-HT metabolism with clonazepam, diazepam and diphenylhydantoin

Brain 5-hydroxytryptamine (5-HT) has been implicated in the control of both seizure threshold and action myoclonus, a disorder of movement usually caused by prolonged cerebral anoxia. Thus, in animals with experimental epilepsy, such as the photosensitive baboon or rodents with chemically or electrically induced fits, raising brain 5-HT elevates seizure threshold, while lowering brain 5-HT increases seizure susceptibility (Azzaro, Wenger & others, 1971; Killan & Frey, 1973; Wada, Balzamo & others, 1972). Furthermore, several conventional anticonvulsants, including diphenylhydantoin(DPH) have been shown to raise brain 5-HT (Bonnycastle, Giarman & Paasonen, 1957). In human epileptics "therapeutic" concentrations of phenobarbitone and DPH are associated with a rise in cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid (5-HIAA) which is particularly striking in clinically intoxicated patients (Chadwick, Jenner & Reynolds, 1975). In subjects with action myoclonus we have found CSF 5-HIAA to be low and treatment with 5-hydroxytryptophan dramatically relieves the abnormal movements (Chadwick, Harris, Reynolds & Marsden, unpublished results), as has been demonstrated by Lhermitte, Marteau & Degos (1972) and Van Woert & Sethy (1975).

Clonazepam, a benzodiazepine derivative with more potent anticonvulsant properties than diazepam in experimental models of epilepsy (Swinyard & Castillion, 1966; Killam, Matsuzaki & Killam, 1972) has recently been introduced into clinical practice for the treatment of epilepsy. In addition to its anticonvulsant action it has been found to be effective in the treatment of post-anoxic action myoclonus (Bourdourques, Roger & others, 1971). We have found it to be markedly more potent than diazepam or other anticonvulsants in this condition although its benefits appear to be transient. We have therefore examined the effect of clonazepam on brain 5-HT metabolism in the mouse and compared it with the effects of diazepam and DPH.

Male Swiss Albino mice (20–25 g; Animal Suppliers Ltd) were treated intraperitoneally with clonazepam (0·5–8·0 mg kg^{-1}; Rivotril, Roche Products Ltd), diazepam (2·0–32·0 mg kg^{-1}; Valium, Roche Products Ltd), diphenylhydantoin (2·5–40·0 mg kg^{-1}; Epanutin, Parke-Davis Ltd) or normal saline (0·1 ml). Animals were housed under conditions of standard laboratory lighting and temperature and were allowed free access to food and water. After 3 h animals were decapitated and the

brain rapidly removed and cooled to −20°. All animals were killed at the same time of day. Parallel experiments had shown all drugs to exhibit a maximal effect on brain 5-HT and 5-HIAA concentrations at 3 h after administration. Whole brain levels of 5-HT and 5-HIAA were subsequently determined fluorimetrically by the method of Curzon & Green (1970). The changes in whole brain 5-HT and 5-HIAA concentrations resulting from the above treatments are shown Fig. 1.

Clonazepam caused a dose-dependant rise in brain 5-HT and 5-HIAA concentrations in the mouse at much lower doses than either diazepam or diphenylhydantoin, which had smaller absolute effects in the dose range administered. Fennessy & Lee (1972), have also reported that clonazepam in a single dose of 2·5 mg kg^{-1} (oral) increased whole brain 5-HT concentration in the mouse although they did not find any significant change in brain 5-HIAA; clonazepam also increased whole brain dopamine and noradrenaline concentrations. The same authors were unable to show any effect of diazepam (7·3 mg kg^{-1}, oral) or other benzodiazepine derivatives on brain 5-HT concentrations in agreement with the findings of Lidbrink, Corrodi & Fuxe (1974), who gave diazepam (25 mg kg^{-1}, i.p.) to rats. However, Fernstrom, Shabshelowitz & Faller (1974) showed dose related increases in rat brain and spinal cord 5-HT and 5-HIAA concentrations which were maximal between 1–2 h following the intraperitoneal injection of diazepam (5–20 mg kg^{-1}). With regard to diphenylhydantoin, Bonnycastle & others (1957), demonstrated that repeated doses of 100 mg kg^{-1} daily for 16 days elevated whole brain 5-HT concentrations in rats. Green & Grahame-Smith (1975) found that while a single dose of 75 mg kg^{-1} of diphenylhydantoin had no effect on the rate of synthesis or on absolute brain concentrations of 5-HT in the rat, the same dose administered twice daily for two days did increase the rate of 5-HT synthesis.

In our clinical studies, the effect of clonazepam on action myoclonus was transient in two patients, the excellent initial response disappearing after some six to eight weeks continuous therapy. A similar loss of efficiency in epilepsy has been noted by (Aarli, 1973). We therefore examined the effect of clonazepam on brain 5-HT and 5-HIAA after a period of prolonged administration. Mice were treated daily for 8 days with either clonazepam (4 mg kg^{-1}, i.p.) or saline (0·1 ml). On the ninth day, the chronically treated animals received a further dose of clonazepam (4 mg kg^{-1}) at

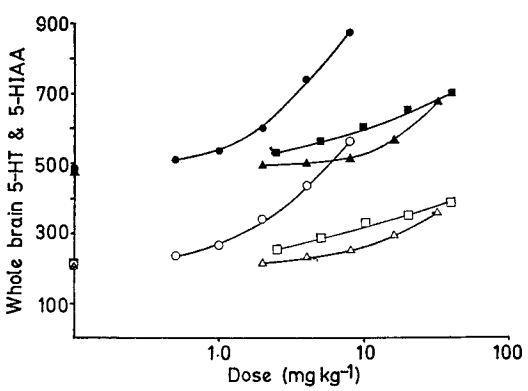

FIG. 1. The effect of dosage of clonazepam (—●—, —○—), diazepam (—▲—, —△—) and diphenylhydantoin (—■—, —□—) on mouse whole brain 5-HT (closed symbols) and 5-HIAA (open symbols) (ng g^{-1}) 3 h after i.p. administration. Each point represents the mean for 16 observations. One s.e.m. in no case exceeds 16 ng g^{-1} for 5-HT and 12 ng g^{-1} for 5-HIAA. The increase in brain 5-HT and 5-HIAA concentrations produced by all three drugs was significantly different from values in saline treated animals (P <0·005–0·001; Student's t-test) for all but the lowest doses administered.

least 24 h after the last administration of the chronic clonazepam treatment. Animals were killed 3 h after drug administration and whole brain 5-HT and 5-HIAA concentrations measured (Table 1). Control groups of saline-pretreated and nontreated animals were similarly examined.

The results showed that the increase in 5-HT and 5-HIAA observed after acute administration of clonazepam was not evident following chronic treatment with a high dose for eight days. Such findings could be explained in a number of ways. Chronic treatment with clonazepam could result in hepatic enzyme induction as shown for other benzodiazepine derivatives in rats (Vallerino, Vessel & others, 1973) and in man (Heubel, 1969), thus leading to a decreased effect on brain 5-HT and 5-HIAA concentrations due to decreased plasma concentrations. Alternatively, these results may represent a true tolerance to the effects of clonazepam on 5-HT neurons. This would explain the transient response to clonazepam we have observed clinically.

There are a number of possible explanations for the observed effect of clonazepam on whole brain 5-HT and 5-HIAA concentrations. Clonazepam may increase the rate of synthesis of 5-HT and, to a lesser extent, its release. Such an effect would explain the rise in concentration of both the amine itself and its metabolite. Alternatively 5-HT release might be decreased leading to a rise in the amine concentration but egress of its metabolite might be impeded causing a rise in the concentration of 5-HIAA, or there may be a combination of these actions. Further data on clonazepam to distinguish between these possibilities are required. With diazepam, (20 mg kg^{-1}, i.p. in rats) Chase, Katz & Kopin (1970) have demonstrated increases in the absolute concentrations of labelled brain 5-HT and 5-HIAA 3 h after a single intraventricular injection of ^{14}C-5-HT. These authors have interpreted these data as suggesting that diazepam interferes with the metabolism of the parent amine. They also showed a reduced efflux of labelled ^{14}C-5-HIAA from brain after intraventricular injection, suggesting that diazepam decreases the transport of 5-HIAA out of brain. Chase, Katz & Kopin (1969) found that diphenylhydantoin (200 mg kg^{-1}, i.p.) had the same effects as diazepam in similar experiments. The suggestion that diazepam reduces 5-HT turnover and serotoninergic neuronal activity is further supported by the findings of Lidbrink & others (1974) that this drug protects against 5-HT depletion following the administration of α-propyldopacetamide, an inhibitor of 5-HT synthesis. However, Green & Grahame-Smith (1975) were able to demonstrate that repeated treatment with diphenylhydantoin caused an increase in the rate of synthesis of brain 5-HT, as judged by the rate of accumulation of 5-HT after blockade of monoamine oxidase with tranylcypromine.

Table 1. *The effect of chronic treatment (8 days) with clonazepam (4 mg kg^{-1}) on the acute response of mouse whole brain 5-HT and 5-HIAA to clonazepam (4 mg kg^{-1})*

Pretreatment	Drug administered	Whole brain (ng g^{-1}) 5-HT	5-HIAA
None	Saline	503·2 ± 11·8 (12)	223·9 ± 5·7 (12)
None	Clonazepam (4 mg kg^{-1})	694·4 ± 5·5 (12)*	389·2 ± 6·3 (12)*
Saline 8 days	Saline	485·5 ± 6·2 (12)	223·4 ± 3·0 (12)
Saline 8 days	Clonazepam (4 mg kg^{-1})	667·8 ± 7·0 (6)*	364·5 ± 7·1 (6)*
Clonazepam (4 mg kg^{-1}) 8 days	Clonazepam (4 mg kg^{-1})	523·3 ± 17·2 (12)	252·9 ± 12·9 (12)

* Significantly different ($P < 0{\cdot}001$) from respective values for whole brain 5-HT and 5-HIAA in saline-treated animals which had not undergone pretreatment.

Clearly, more work is required to establish the effect of benzodiazepines and other anticonvulsants on the functional activity and impulse traffic in brain 5-HT neurons. Such studies are of relevance to the understanding of the mode of action of these drugs and the role of cerebral 5-HT in both epilepsy and action myoclonus. Others have suggested that anticonvulsant drugs may owe some of their clinical efficacy to their ability to alter cerebral 5-HT metabolism (Anderson, Markowitz & Bonnycastle, 1962; Meyer & Frey, 1973), although this notion has been disputed (Green & Grahame-Smith, 1975). In the case of action myoclonus the evidence for the involvement of 5-HT in its genesis and the therapeutic effects of clinically beneficial drugs is even more compelling.

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