

## FUNCTIONAL CHANGES IN CEREBRAL 5-HYDROXYTRYPTAMINE METABOLISM IN THE MOUSE INDUCED BY ANTICONVULSANT DRUGS

D. CHADWICK, J.W. GORROD\*, P. JENNER, C.D. MARSDEN & E.H. REYNOLDS

University Department of Neurology, Institute of Psychiatry and King's College Hospital Medical School, Denmark Hill, London, SE5 8AF

\*Department of Pharmacy, Chelsea College, University of London, Manresa Road, London, S.W.3

- 1 Acute administration of clonazepam, diazepam and diphenylhydantoin to mice elevated cerebral 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA); chronic administration had less effect.
- 2 Acute administration of clonazepam and diazepam but not diphenylhydantoin raised cerebral tryptophan levels; chronic administration of clonazepam caused a smaller elevation of cerebral tryptophan but chronic administration of diazepam still caused a large rise in cerebral tryptophan.
- 3 Neither clonazepam nor diazepam caused induction of drug metabolizing enzymes on chronic administration but diphenylhydantoin had a marked effect.
- 4 These data suggest that the altered 5-HT metabolism caused by these compounds is unrelated to a common action on tryptophan levels, and that the reduced effect of clonazepam and diazepam on chronic administration cannot be attributed to increased metabolism of these compounds.
- 5 Clonazepam induced abnormal head movements in mice in a dose-dependent manner. Pretreatment of animals with tranlylcypromine increased the intensity of movement, although pargyline was without effect. Similar effects were observed with diazepam and diphenylhydantoin, suggesting that the increase in cerebral 5-HT caused by these compounds is of functional significance in stimulating 5-HT receptors.

### Introduction

Manipulation of cerebral 5-hydroxytryptamine (5-HT) is associated with an alteration of seizure threshold (Meldrum, Balzamo, Wada & Vuillon-Cacciuttolo, 1972; Wada, Balzamo, Meldrum & Naquet, 1972; Boggan, 1973). Conventional anticonvulsant drugs also elevate brain 5-HT in experimental animals (Bonnycastle, Giarman & Paasonen, 1957; Jenner, Chadwick, Reynolds & Marsden, 1975). Changes in cerebral 5-HT metabolism may occur in anticonvulsant-treated human epileptics and could contribute in part to the therapeutic action of these drugs (Chadwick, Jenner & Reynolds, 1975). On the other hand, the elevation of cerebral 5-HT in animals only occurs at dosage levels well above those causing a marked anticonvulsant activity and the alterations reported in humans are most marked in those subjects showing clinical signs of intoxication. Thus, the influence of these compounds on brain 5-HT may either be related to some of their toxic side effects or be of no therapeutic significance.

The object of the present study was to investigate the action of anticonvulsant drugs on cerebral

5-HT systems. Tryptophan hydroxylase is generally regarded as the rate limiting step in 5-HT synthesis (Moir & Eccleston, 1968); however, since the brain concentration of tryptophan is normally below the enzyme saturating concentration, alterations in the availability of the substrate may be important in the control of 5-HT synthesis (Wurtman & Fernstrom, 1972; Hamon & Glowinski, 1974).

We have investigated the ability of three conventionally used anticonvulsant drugs, clonazepam, diazepam and diphenylhydantoin to alter brain tryptophan, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in mice.

Since the clinical activity of some anti-epileptic drugs, in particular, benzodiazepines, decreases on chronic administration the influence of chronic drug administration on cerebral 5-HT metabolism has also been investigated.

Drugs raising brain 5-HT levels in animals cause a number of behavioural changes, such as head and body jerking in guinea-pigs (Klawans, Goetz & Weiner, 1973), head twitching in mice (Corne, Picker-

ing & Warner, 1963), wet-dog shakes (Bedard & Pycoc, 1977) or hyperactivity in rats (Grahame-Smith, 1971). In order to determine whether the changes in 5-HT metabolism caused by the drugs studied is of functional significance, a behavioural assessment for the production of abnormal head movements has been made.

## Methods

Swiss 'S' or 'P' strain male mice (20–25 g, Animal Suppliers Ltd.) were used in all experiments. Animals were housed under normal conditions using a 12 h light-dark cycle. The diet fed to the animals was Dixons 41B.

### Biochemical experiments

In acute experiments, animals received clonazepam base (Rivotril, Roche; 1–8 mg/kg), diazepam base (Valium, Roche; 4–32 mg/kg) or diphenylhydantoin sodium salt (Epanutin, Parke-Davis, 5–40 mg/kg) or 0.9% w/v NaCl solution (saline, 0.1 ml) intraperitoneally 0.5–24 h before they were killed. Experiments were always carried out at the same time of day (i.e. between 08 h 30 min and 18 h 30 min). Animals were killed by cervical dislocation and decapitation, the brain quickly removed and frozen at  $-20^{\circ}\text{C}$ . Whole brain levels of 5-HT and 5-HIAA were determined fluorimetrically according to the method of Curzon & Green (1970) and tryptophan by a modification (Eccleston, 1975) of the method of Denckla & Dewey (1967).

In chronic experiments, animals received clonazepam (4 mg/kg), diazepam (32 mg/kg), diphenylhydantoin (20 mg/kg), or saline intraperitoneally daily for 8 days. On day 9, drug-treated animals received either the drug chronically administered on days 1–8 or saline; saline-treated animals received saline, clonazepam (4 mg/kg), diazepam (32 mg/kg), or diphenylhydantoin (20 mg/kg).

Animals were killed 0.5–24 h after the final administration and whole brain 5-HT, 5-HIAA and tryptophan were determined as outlined above.

Paralysis and sleeping times were determined on day 9 following chronic drug treatment on days 1–8 as outlined below. On day 9 animals received either zoxazolamine (150 mg/kg) or hexobarbitone (100 mg/kg). Paralysis or sleeping time was defined as the time between loss and recovery of the righting reflex. The end point was taken as the time when the animal righted itself twice in quick succession.

Cytochrome  $\text{P}_{450}$  and  $\text{b}_5$  levels were determined in washed hepatic microsomes from chronically treated animals according to the method of Omara & Sato (1964). Protein levels were determined by a

modification (Miller, 1959) of the method of Lowry, Rosebrough, Farr & Randall (1951).

### Behavioural experiments

Animals were housed individually in  $4 \times 4$  inch metal cages with clear perspex lids. Clonazepam (0.5–8.0 mg/kg), diazepam (32 mg/kg), diphenylhydantoin (40 mg/kg) or saline was administered 45 min before behavioural assessment. Some animals were pre-treated with pargyline hydrochloride (50 mg/kg, William Warner Ltd.) or tranlycypromine sulphate (25 mg/kg, SKF Ltd.) 30 min before anticonvulsant administration.

Behavioural assessment was made by counting the number of twitches, head jerks, head weaves, or 'wet-dog shakes' observed in a 2 min period. The response to pinna stimulation was determined by stroking with a bristle paint brush. General observations of activity were also employed.

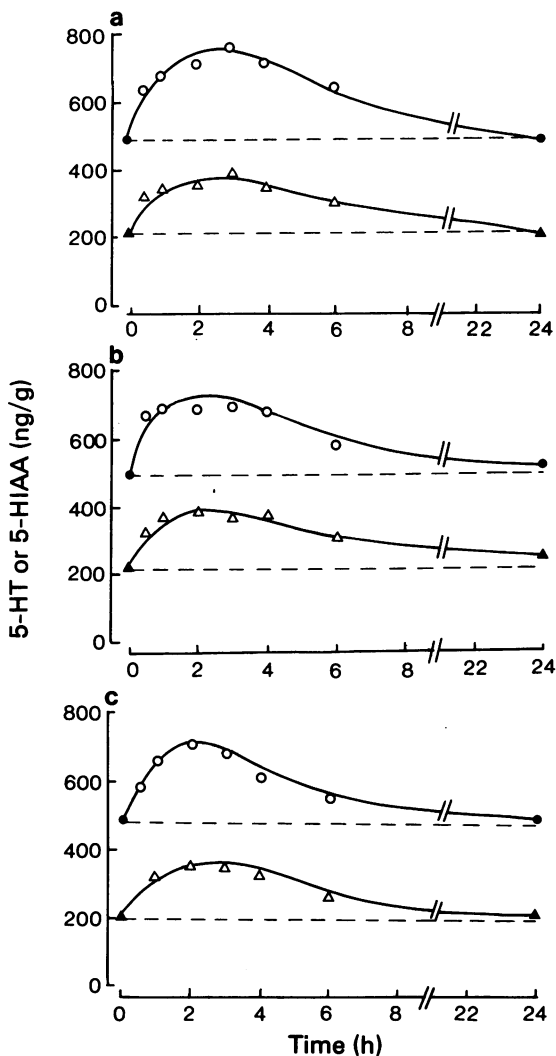
All results were examined using Student's *t* test.

## Results

### Comparison of acute and chronic administration on whole brain 5-hydroxytryptamine and 5-hydroxyindoleacetic acid

Acute administration of clonazepam (4 mg/kg), diazepam (32 mg/kg) and diphenylhydantoin (20 mg/kg) caused a rise in both whole brain 5-HT and 5-HIAA levels (Figure 1). No change in 5-HT or 5-HIAA levels ( $P > 0.05$ ) was observed in saline-treated animals during the time course over which experiments were carried out. The effect of all three drugs was maximal between 1 and 3 h after administration and levels reverted to control values by 6 hours. At the time of maximal effect (2 h), this elevation represented 139%, 147% and 148% of control 5-HT levels and 182%, 165% and 176% of control 5-HIAA levels for clonazepam, diazepam and diphenylhydantoin respectively ( $P < 0.001$  in each case). Administration of clonazepam (1–8 mg/kg), diazepam (4–32 mg/kg) and diphenylhydantoin (5–40 mg/kg) showed the elevation of 5-HT and 5-HIAA to be dose-dependent when animals were killed 2 h after drug administration (Figure 2).

Whole brain 5-HT or 5-HIAA levels 24 h after the last administration of the chronic anticonvulsant schedule were not different from those of saline-treated or normal animals ( $P > 0.05$ ). Pretreatment with saline caused some differences in the percentage rise observed on subsequent anticonvulsant administration (see Figure 4); this was particularly marked in the case of diazepam. To overcome these 'injection effects' the elevation of 5-HT and 5-HIAA following



**Figure 1** The time course of the effect of acute administration of (a) diazepam (32 mg/kg), (b) clonazepam (4 mg/kg) and (c) diphenylhydantoin (20 mg/kg) on whole brain levels of 5-hydroxytryptamine (5-HT, ●, ○) and 5-hydroxyindoleacetic acid (5-HIAA, ▲, △). Open symbols represent values significantly different ( $P < 0.05$ ) from saline-treated control values. Closed symbols are not significant. Each point represents the mean of 12 determinations. Standard errors in no case exceed 12 ng/g for 5-HIAA and 20 ng/g for 5-HT.

chronic drug administration has been compared with the saline-treated controls to assess the drug-induced effects.

Pretreatment of animals for 8 days with saline followed by administration of clonazepam (4 mg/kg) on day 9 again resulted in a rise in both whole brain

5-HT (152%) and 5-HIAA (181%) ( $P < 0.001$ ) 2 h following drug administration comparable to that seen on acute administration of clonazepam to untreated animals (Figures 3 and 4). Pretreatment of animals for 8 days with clonazepam (4 mg/kg) followed by administration of a further dose on day 9, produced a smaller rise in 5-HT (126%) and 5-HIAA (155%) ( $P < 0.001$ ) 2 h after drug administration, compared to those observed after clonazepam administration either to animals given saline or to untreated animals ( $P < 0.001$ ) (Figures 3 & 4).

Following chronic saline treatment, administration of diazepam (32 mg/kg) or diphenylhydantoin (20 mg/kg) on day 9 produced elevation in 5-HT (160%, 135% respectively) ( $P < 0.001$ ) 2 h following drug administration compared to untreated animals. However, pretreatment with either drug followed by a further administration on day 9 again resulted in a smaller rise in 5-HT (137%, 121% respectively) and 5-HIAA (153%, 139% respectively) ( $P < 0.001$ ) 2 h following drug administration compared to those observed following drug administration either to saline-treated or to untreated animals ( $P < 0.001$ ) (Figure 4).

#### *Comparison of acute and chronic administration on whole brain tryptophan levels*

No change in whole brain tryptophan levels was observed in animals receiving saline at the time intervals used for these experiments.

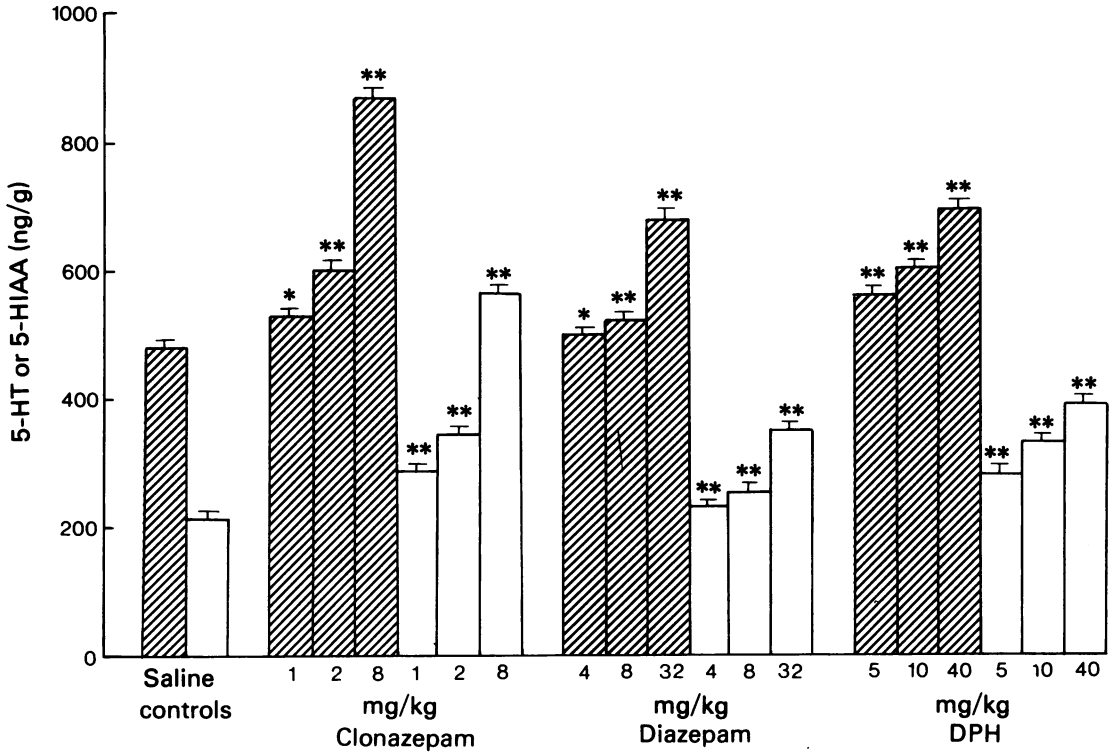
Administration of clonazepam (4 mg/kg) produced a rise in whole brain tryptophan levels. The effect was maximal between 2 and 3 h after drug administration, but declined rapidly such that by 4 h, levels were the same as those found in control animals (Figure 5).

Adopting a response time of 2.5 h, the effects of clonazepam (4 mg/kg), diazepam (32 mg/kg) and diphenylhydantoin (20 mg/kg) on whole brain tryptophan were compared (Table 1). Both clonazepam and diazepam produced a marked elevation of whole brain tryptophan ( $P < 0.001$ ). Diphenylhydantoin was without effect.

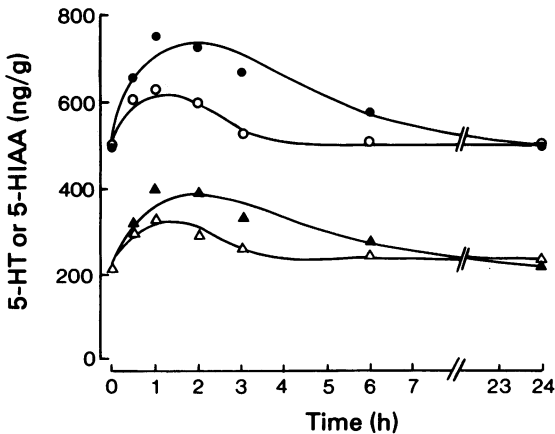
Following chronic administration of the drugs according to the schedule described above (Table 2) clonazepam no longer produced an elevation of whole brain tryptophan. Diazepam still produced an effect compared to acute administration, and diphenylhydantoin remained without effect. In animals pretreated with saline, identical changes to those observed on acute administration of the drugs were observed.

#### *Comparison of effects on sleeping times and cytochrome $P_{450}$ levels*

Chronic administration of clonazepam and diazepam (as detailed above) produced no change in hepatic



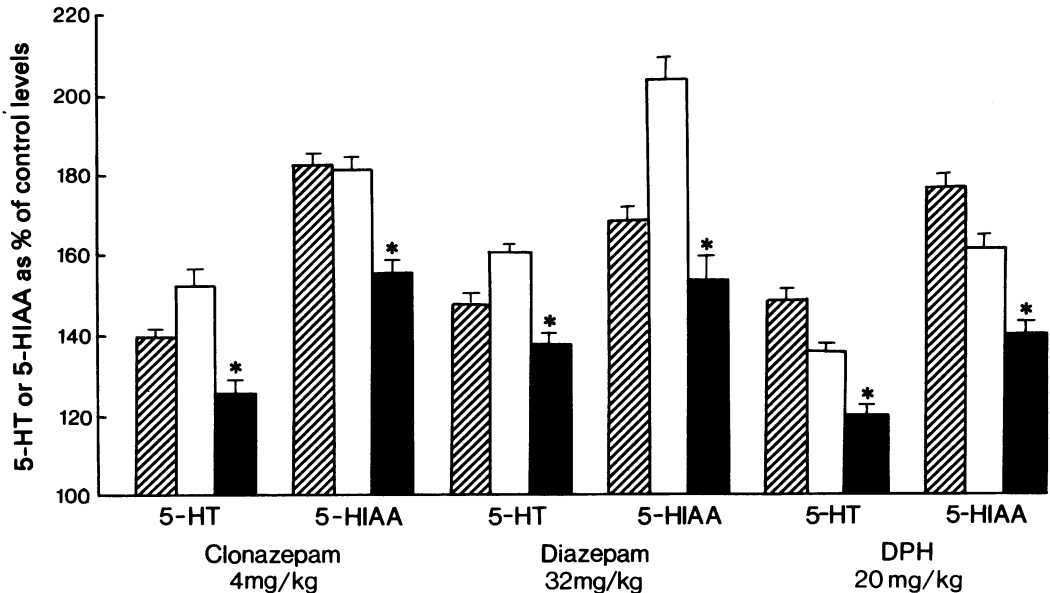
**Figure 2** The effect of dosage of clonazepam, diazepam and diphenylhydantoin (DPH) on whole brain levels of 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) 2 h following drug administration. Open columns: 5-HIAA; hatched columns: 5-HT. Each column represents the mean of at least 12 determinations. Vertical lines show s.e. mean. \*  $P < 0.005$ ; \*\*  $P < 0.001$ .



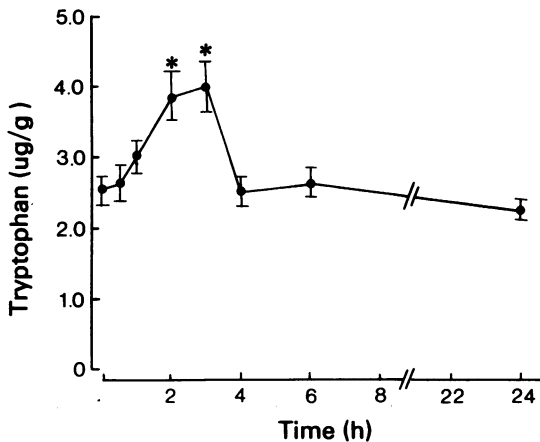
**Figure 3** The time course of the effect on whole brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) of chronic administration (8 days) of clonazepam (4 mg/kg) and of a subsequent administration of clonazepam (4 mg/kg) on day 9 (5-HT ○; 5-HIAA △) in comparison to saline-treated animals (8 days) receiving clonazepam (4 mg/kg) on day 9 (5-HT ●; 5-HIAA ▲). Each point represents the mean of 12 determinations. Standard errors in no case exceed 12 ng/g for 5-HIAA and 18 ng/g for 5-HT.

microsomal protein, cytochrome  $b_5$  and cytochrome  $P_{450}$  levels compared to those found in control animals or those chronically treated with saline (Table 3). Similarly, these drugs induced no change in hexobarbitone sleeping times or zoxazolamine paralysis

times. In contrast, chronic administration of diphenylhydantoin increased hepatic cytochrome  $P_{450}$  and cytochrome  $b_5$  levels and reduced hexobarbitone sleeping time and zoxazolamine paralysis time (Table 3).



**Figure 4** The effect of chronic administration of clonazepam (4 mg/kg), diazepam (32 mg/kg) or diphenylhydantoin (DPH; 20 mg/kg) for 8 days on the elevation of whole brain 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) caused 2 h following a subsequent administration of the same regime on day 9 in comparison to the effect of these drugs in untreated and saline-treated (8 days) animals. Hatched columns: untreated plus drugs; open columns: saline-treated plus drugs; solid columns: drug-treated plus drugs. Each column represents the mean value as a percentage of basal levels for at least 12 determinations. Vertical lines show s.e. mean. \* Represents a significant difference ( $P < 0.05$ ) between the saline-treated animals and drug pre-treated groups.



**Figure 5** The time course of the effect of clonazepam (4 mg/kg) on whole brain tryptophan levels. Each point is the mean of at least 12 determinations. Vertical lines show s.e. mean. \*  $P < 0.005$ .

#### *Behavioural effects of acute and chronic drug administration*

Administration of clonazepam (0.5–4.0 mg/kg) induced a dose-dependent incidence of abnormal head

movement (Table 4). A higher dose of clonazepam (8 mg/kg) caused ataxia and sedation. These effects lasted several hours. Clonazepam also induced a pinna response manifested by a 'wet dog shake' that was not observed in normal animals. Both the abnormal head movements and the 'wet-dog shake' were maximal at around 2.0 mg/kg but declined at higher dosage levels when animals were increasingly sedated. In the presence of pargyline (50 mg/kg) this effect was maximal at around 1.0 mg/kg clonazepam and again declined at higher dosage levels. Animals pretreated with pargyline generally appeared less active than animals treated with clonazepam alone and were less easily aroused.

Pretreatment of animals with tranlycypromine (25 mg/kg) alone caused a well coordinated increase in motor activity compared to animals receiving saline, and an increase in abnormal head movement was observed but no pinna response was seen. On administration of clonazepam, this behavioural effect was transformed into the type of hyperactivity syndrome seen on administration of tryptophan plus a monoamine oxidase (MAO) inhibitor to rats described by Grahame-Smith (1971) although reciprocal forepaw treading was not evident. Clonazepam, in the presence of tranlycypromine, also produced a marked

**Table 1** The effect of clonazepam, diazepam or diphenylhydantoin on whole brain tryptophan levels in mice

<i>Drug treatment*</i>	<i>Tryptophan** (µg/g)</i>	<i>% of control values</i>	<i>P</i>
Saline	2.31 ± 0.13 (16)	—	—
Clonazepam 4 mg/kg	3.95 ± 0.24 (16)	171%	< 0.001
Diazepam 32 mg/kg	4.71 ± 0.29 (8)	203%	< 0.001
Diphenylhydantoin 20 mg/kg	2.69 ± 0.19 (8)	116%	> 0.05

\* Animals were pre-treated with drugs 2.5 h before they were killed.

\*\* All values are quoted as the mean of all determinations ± s.e. mean; numbers in parentheses are the number of animals used for each group.

**Table 2** The effect of chronic pre-treatment with clonazepam (4 mg/kg), diazepam (32 mg/kg) or diphenylhydantoin (20 mg/kg) on mouse whole brain tryptophan levels

<i>Pre-treatment Day 1–8</i>	<i>Treatment Day 9</i>	<i>Tryptophan ** (µg/g)</i>	<i>% of *** saline treated controls</i>
Saline	Saline	1.70 ± 0.24 (8)	—
Clonazepam	Saline	1.41 ± 0.11 (8)	—
Diazepam	Saline	1.55 ± 0.18 (8)	—
Diphenylhydantoin	Saline	1.83 ± 0.19 (8)	—
Clonazepam	Clonazepam	1.68 ± 0.18 (8)	119% <sup>NS</sup>
Diazepam	Diazepam	3.76 ± 0.33 (8)	243% <sup>†</sup>
Diphenylhydantoin	Diphenylhydantoin	2.24 ± 0.19 (8)	122% <sup>NS</sup>
Saline	Clonazepam	2.88 ± 0.14 (8)	169% <sup>†</sup>
Saline	Diazepam	5.56 ± 0.37 (8)	327% <sup>†</sup>
Saline	Diphenylhydantoin	2.21 ± 0.24 (8)	130% <sup>NS</sup>

\* Animals were killed 2.5 h following drug administration on day 9.

\*\* All values are quoted as the mean of all determinations ± s.e. mean; numbers in parentheses are the number of animals used for each group.

\*\*\* Values are expressed as a percentage of the respective pretreatment group treated with saline on day 9. NS: not significant ( $P > 0.05$ );  $† P < 0.001$ .

**Table 3** The effect of chronic pretreatment with clonazepam (4 mg/kg), diazepam (32 mg/kg) or diphenylhydantoin (20 mg/kg) on drug metabolizing ability as judged by hexobarbitone sleeping time, zoxazolamine paralysis time and the level of cytochrome  $b_5$  and  $P_{450}$  in washed hepatic microsomes

<i>Pre-treatment Day 1–9*</i>	<i>Hexobarbitone sleeping time (min)</i>	<i>Zoxazolamine paralysis time (min)</i>	<i>Cytochrome <math>P_{450}</math> (OD 450–490 nm)</i>	<i>Cytochrome <math>b_5</math> (OD 424–470 nm)</i>	<i>Microsomal protein (mg/g)</i>
Saline	46.6 ± 1.8 (59)	68.9 ± 2.7 (58)	0.12 ± 0.02 (7)	0.16 ± 0.05 (7)	31.4 ± 2.3 (7)
Clonazepam	45.6 ± 3.2 (19)	67.9 ± 4.7 (20)	0.10 ± 0.01 (3)	0.15 ± 0.03 (3)	37.1 ± 0.9 (3)
Diazepam	49.8 ± 2.8 (19)	59.7 ± 4.1 (19)	0.19 0.05	0.16 0.07	30.0 ± 5.2
Diphenylhydantoin	19.0 ± 1.2** (20)	23.8 ± 2.4** (20)	0.29*** 0.32	0.16 0.26	38.0 ± 2.2

\* Animals were killed 2 h following the final dosing on day 9.

The number of observations made for hexobarbitone sleeping time and zoxazolamine paralysis time are shown in parentheses.

The biochemical parameters represent at least 2 observations on tissue pooled from groups of 6 mice.

\*\*  $P < 0.001$ ; \*\*\*  $P < 0.005$ .

increase in head movements compared with clonazepam alone, and induced a pinna response.

Administration of diazepam (32 mg/kg) also produced abnormal head movements, and a behavioural profile similar to that seen following clonazepam (4 mg/kg). Pretreatment of animals with pargyline (50 mg/kg) or tranylcypromine (25 mg/kg) again increased the intensity of abnormal head movements. In the presence of tranylcypromine, diazepam produced the hyperactivity syndrome (Grahame-Smith, 1971) as observed with clonazepam.

Diphenylhydantoin (40 mg/kg) produced some abnormal head movements either alone, or in the presence of pargyline. These movements were not enhanced by tranylcypromine and pinna stimulation was not effective in diphenylhydantoin-treated animals. Sedation was not observed following diphenylhydantoin administration, although animals were slightly ataxic. Diphenylhydantoin in the presence of tranylcypromine did not produce a hyperactivity syndrome, in contrast to diazepam and clonazepam.

Following chronic administration of clonazepam (4 mg/kg), diazepam (32 mg/kg) or diphenylhydantoin (20 mg/kg) for 8 days, the behavioural response to these drugs was less marked and of shorter duration. Animals receiving saline over the same period showed behavioural changes consistent with acute administration when given any of the drugs on day 9.

## Discussion

The ability of the anticonvulsant drugs to induce head shaking in mice would suggest that the alterations in 5-HT turnover observed are of functional significance since such movements have previously been associated with altered function of this neuronal pathway. Similarly, the enhancement of these effects by tranylcypromine provides further evidence for the involvement of a monoamine system, although the lack of effect of pargyline is difficult to explain. However, the two MAO inhibitors did induce a different behavioural pattern when administered alone to mice; pargyline, in general, produced slight sedation, whereas tranylcypromine enhanced activity. The involvement of 5-HT is also suggested by the ability of clonazepam and diazepam to transform the tranylcypromine-induced increase in motor activity into the 5-HT-related hyperactivity syndrome previously described by Grahame-Smith (1971). The failure of tranylcypromine to enhance diphenylhydantoin-induced abnormal head movements cannot be explained, although other behavioural changes were apparent, for example, tremor and ataxia. It may well be that this drug alters 5-HT function in a different manner from benzodiazepine derivatives. The facilitation of a pinna evoked head response by the benzodiazepines might also suggest these compounds act by inhibition of an

**Table 4** The production of abnormal head movements in mice by clonazepam, diazepam or diphenylhydantoin

Drug*	Alone	Number of head movements**	
		Plus pargyline (50 mg/kg)	Plus tranylcypromine (25 mg/kg)
Saline	0.6 ± 0.3 (1/12)	0.3 ± 0.2 (1/12)	2.2 ± 0.8 (0/12)
Clonazepam 0.5 mg/kg	0.6 ± 0.3 (9/12)	1.1 ± 0.3*** (10/12)	3.6 ± 0.9 (9/12)
Clonazepam 1.0 mg/kg	1.3 ± 0.5*** (11/12)	1.7 ± 0.5*** (9/12)	10.4 ± 2.0*** (11/12)
Clonazepam 2.0 mg/kg	2.1 ± 0.8*** (10/12)	1.2 ± 0.4*** (6/12)	5.2 ± 1.2*** (11/12)
Clonazepam 4.0 mg/kg	2.5 ± 0.5*** (5/12)	0.8 ± 0.3 (0/12)	18.0 ± 2.2*** (8/12)
Clonazepam 8.0 mg/kg	0 (0/12)	0 (0/12)	3.2 ± 1.0 (1/12)
Diazepam 32 mg/kg	3.2 ± 0.9*** (6/12)	0.8 ± 0.6 (3/12)	9.8 ± 1.3*** (9/12)
Diphenylhydantoin 40 mg/kg	1.3 ± 0.3 (0/12)	2.7 ± 0.6*** (0/12)	2.8 ± 0.6 (1/12)

\* Anticonvulsants were administered 45 min before behavioural assessment. Pargyline and tranylcypromine were administered 30 min prior to anticonvulsant treatment.

\*\* Results are expressed as the number of abnormal head movements observed in a 2 min period 45 min following anticonvulsant administration. The figures in parentheses represent the number of animals exhibiting a wet dog shake in response to pinna stimulation over the total number of animals used.

\*\*\*  $P < 0.05$  compared to saline-treated controls.

afferent inhibitory brainstem pathway (see Corne *et al.*, 1963). Only at high doses is the pinna response diminished as a general depression of cerebral activity occurs and at these doses, spontaneous abnormal head movements are also reduced.

During the course of this work, other workers (Nakamura & Fukushima, 1976) have also shown clonazepam to induce head twitches in mice following oral administration, and have attributed this phenomenon to an action on 5-HT neuronal pathways. However, they were unable to demonstrate an effect following oral diazepam administration. The dose-ratio (clonazepam:diazepam) used was higher in this work than in the present study, and may explain this discrepancy.

The elevation of cerebral 5-HT and 5-HIAA levels by acute administration of clonazepam, diazepam and diphenylhydantoin provides further support for an action of anticonvulsant drugs on central 5-HT neuronal systems. Similar actions of these and other anticonvulsant drugs on cerebral 5-HT have previously been demonstrated (see Bonnycastle *et al.*, 1957; Chase, Katz & Kopin, 1969; Lidbrink, Corrodi & Fuxe, 1974; Fernstrom, Shabshelowitz & Faller, 1974; Dominic, Jinha & Barchas, 1975; Green & Grahame-Smith, 1975). The time course of action of all three drugs appears similar, but in each case the effect is only seen in the first few hours following administration even though such compounds have prolonged biological half-lives in man at least. Of the three drugs studied, clonazepam was most potent in elevating 5-HT and 5-HIAA. Interpretation of the elevations observed in terms of a basic mode of action of these drugs is difficult, since a number of possibilities exist which cannot be distinguished by the present data alone. Previous studies (Chase *et al.*, 1969; Chase, Katz & Kopin, 1970; Lidbrink *et al.*, 1974) have suggested that anticonvulsants decrease 5-HT turnover (leading to an accumulation of the transmitter) and block the egress of 5-HIAA from the CNS. However, other studies with diphenylhydantoin (Green & Grahame-Smith, 1975) have suggested an increased turnover of 5-HT.

In man and animals the cerebral concentration of tryptophan may be influenced by the availability of free tryptophan in plasma (Curzon & Knott, 1974; Gessa & Tagliamonte, 1974; Young, Lal, Feldmuller, Sourkes, Ford, Kiely & Martin, 1976). Since the bulk of the plasma tryptophan is bound to albumin (McMenamy & Oncley, 1958) drugs which compete for the same binding site may increase the plasma concentrations of free tryptophan, hence the cerebral levels, and consequently lead to increased 5-HT synthesis (Tagliamonte, Biggio, Vargiu & Gessa, 1973; Iwata, Okamoto & Koh, 1975). In particular, benzodiazepines have such actions and this may in part explain their ability to alter cerebral 5-HT function

(Sjöholm & Sjödin, 1972; Muller & Wollert, 1975). Indeed, in the present study, both clonazepam and diazepam increased cerebral tryptophan levels on acute administration, suggesting one mechanism by which an increase in 5-HT and 5-HIAA might be brought about. However, as pointed out by Muller & Wollert (1975) the relevance of such an effect to the activity of these drugs in man is probably small, in view of the low therapeutic plasma levels attained. Further, there is evidence for reduced, rather than increased, incorporation of [<sup>3</sup>H]-tryptophan in cerebral 5-HT synthesis following pretreatment of mice with diazepam, flurazepam and chloridiazepoxide (Dominic *et al.*, 1975).

We have recently reported increased cerebrospinal fluid (CSF) levels of 5-HIAA and tryptophan in treated, compared to untreated, epileptic patients (Chadwick, Jenner & Reynolds, 1977). This suggests that changes in 5-HT metabolism similar to those we have reported here may occur in man. These differences were most marked in patients showing clinical signs of anticonvulsant intoxication, so the possibility must be considered that such changes may be of more relevance to the production of such toxic effects rather than to anticonvulsant activity.

In another epileptiform disorder, myoclonus, we have also shown the abnormally low CSF 5-HIAA values to be elevated in a dose-dependent manner following clonazepam therapy (Chadwick, Harris, Jenner, Reynolds & Marsden, 1975; Chadwick, Hallett, Harris, Jenner, Reynolds & Marsden, 1977). This action may represent a therapeutic effect in the alteration of 5-HT metabolism.

The clinical action of anticonvulsant drugs and, in particular, benzodiazepines, decreases following chronic administration. It is, therefore, of interest that administration of clonazepam, diazepam and diphenylhydantoin to mice for 8 days leads to a reduction in the elevation of 5-HT and 5-HIAA measured in response to a further drug dose. Tolerance to the increase in homovanillic acid induced by antipsychotic drugs is known (Sayers, Burki, Ruch & Asper, 1975) but such an effect on the 5-HT system has not been previously demonstrated. The effect on chronic administration of diazepam and clonazepam on cerebral tryptophan levels also questions the relevance of this increase to the altered 5-HT and 5-HIAA levels, since the effect of clonazepam on tryptophan is abolished by chronic treatment, while that of diazepam is unchanged, although the effect of both drugs on 5-HT and 5-HIAA is diminished. Why two closely related benzodiazepines should have such different actions on cerebral tryptophan levels remains unclear.

Benzodiazepines and other anticonvulsant drugs, in particular, diphenylhydantoin, have been reported to induce their own metabolism and that of other compounds (Kato & Chiesara, 1962; Gerber & Arnold,



1969; Vallerino, Vessel, Johnson & Aurori, 1973). Such an effect could easily explain the reduced elevation of 5-HT and 5-HIAA observed on chronic administration. The measurement of drug metabolism parameters in this study, however, negates the possibility for diazepam and clonazepam. Diphenylhydantoin, however, produced an expected elevation of cytochrome P<sub>450</sub> and reduction of zoxazolamine paralysis time and hexobarbitone sleeping time in agreement with previous findings (Kato & Chiesara, 1962;

Gerber & Arnold, 1969). These data, therefore, suggest that in the case of clonazepam and diazepam at least, another explanation must be sought, possibly more closely related to the neuronal level of drug action.

This work was supported by the research funds of King's College Hospital and the Maudsley & Bethlem Royal Hospitals, the Medical Research Council and Roche Products Ltd. We thank Miss A. Domeney and Mr L. Disley for their technical assistance.

## References

- BEDARD, P. & PYCOCK, C. (1977). Wet-dog shake behaviour in the rat; a possible quantitative model of central 5-hydroxytryptamine activity. *Neuropharmacology* (in press).
- BOGGAN, W.O. (1973). Serotonin and convulsions. In *Serotonin and Behavior*. ed. Barchas, J. & Usdin E. pp. 167-172. New York: Raven Press.
- BONNYCASTLE, D.D., GIARMAN, N.J. & PAASONEN, M.K. (1957). Anticonvulsant compounds and 5-hydroxytryptamine in rat brain. *Br. J. Pharmac. Chemother.*, **12**, 228-231.
- CHADWICK, D., HALLETT, M., HARRIS, R., JENNER, P., REYNOLDS, E.H. & MARSDEN, C.D. (1977). Clinical, biochemical and physiological factors distinguishing myoclonus responsive to 5-hydroxytryptophan plus a monoamine oxidase inhibitor and clonazepam. *Brain*, (in press).
- CHADWICK, D., HARRIS, R., JENNER, P., REYNOLDS, E.H. & MARSDEN, C.D. (1975). Manipulation of brain serotonin in the treatment of myoclonus. *Lancet*, **ii**, 434-435.
- CHADWICK, D., JENNER, P. & REYNOLDS, E.H. (1975). Amines, anticonvulsants and epilepsy. *Lancet*, **i**, 473-476.
- CHADWICK, D., JENNER, P. & REYNOLDS, E.H. (1977). Serotonin metabolism in human epilepsy. *Ann. Neurol*, **1**, 218-224.
- CHASE, T.N., KATZ, R.I. & KOPIN, I.J. (1969). Effect of anticonvulsants on brain serotonin. *Trans. Am. Neurol. Ass.*, **94**, 236-238.
- CHASE, T.N., KATZ, R.I. & KOPIN, I.J. (1970). Effect of diazepam on the fate of intra-cisternally injected serotonin-<sup>14</sup>C. *Neuropharmacology*, **9**, 103-108.
- CORNE, J.J., PICKERING, R.W. & WARNER, B.T. (1963). A method for assessing the effects of drugs on the central actions of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **20**, 106-120.
- CURZON, G. & GREEN, A.R. (1970). A rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in small regions of rat brain. *Br. J. Pharmac.*, **39**, 653-655.
- CURZON, G. & KNOTT, P.J. (1974). Fatty acids and the disposition of tryptophan. In *Aromatic Amino Acids in the Brain*. Ciba Foundation Symposium, **22**, pp. 217-229. Amsterdam: Elsevier.
- DENCKLA, W.D. & DEWEY, H.K. (1967). The determination of tryptophan in plasma, liver and urine. *J. Lab. clin. Med.*, **69**, 160-169.
- DOMINIC, J.A., JINHA, A.K. & BARCHAS, J.D. (1975). Effect of benzodiazepines on brain amine metabolism. *Eur. J. Pharmac.*, **32**, 124-127.
- ECCLESTON, E.G. (1973). A method for the estimation of free and total acid-soluble plasma tryptophan using an ultrafiltration technique. *Clin. Chim. Acta*, **48**, 269-272.
- FERNSTROM, J.D., SHABSHELOWITZ, H. & FALLER, D.V. (1974). Diazepam increases 5-hydroxyindole concentrations in rat brain and spinal cord. *Life Sci.*, **15**, 1577-1584.
- GERBER, N. & ARNOLD, K. (1969). Studies on the metabolism of diphenylhydantoin in mice. *J. Pharmac. exp. Ther.*, **167**, 77-90.
- GESSA, G.L. & TAGLIAMONTE, A. (1974). Serum free tryptophan: control of brain concentrations of tryptophan and of synthesis of 5-hydroxytryptamine. In *Amino Acids in the Brain*. Ciba Foundation Symposium, **22**, pp. 207-216. Amsterdam: Elsevier.
- GRAHAME-SMITH, D.G. (1971). Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J. Neurochem.*, **18**, 1053-1066.
- GREEN, A.R. & GRAHAME-SMITH, D.G. (1975). The effect of diphenylhydantoin on brain 5-hydroxytryptamine metabolism and function. *Neuropharmacology*, **14**, 107-113.
- HAMON, M. & GLOWINSKI, J. (1974). Regulation of serotonin synthesis. *Life Sci.*, **15**, 1538-1548.
- IWATA, H., OKAMOTO, H. & KOH, S. (1975). Effect of various drugs on serum free and total tryptophan levels and brain tryptophan metabolism in rats. *Jap. J. Pharmac.*, **25**, 303-310.
- JENNER, P., CHADWICK, D., REYNOLDS, E.H. & MARSDEN, C.D. (1975). Altered 5-HT metabolism with clonazepam, diazepam and diphenylhydantoin. *J. Pharm. Pharmac.*, **27**, 707-710.
- KATO, R. & CHIESARA, E. (1962). Increase of pentobarbitone metabolism induced in rats pre-treated with some centrally acting compounds. *Br. J. Pharmac. Chemother.*, **18**, 29-38.
- KLAWANS, H.L., GOETZ, B.A. & WEINER, W.J. (1973). 5-hydroxytryptophan-induced myoclonus in guinea-pigs and the possible role of serotonin in infantile myoclonus. *Neurology (Minneapolis)*, **23**, 1234-1240.
- LIDBRINK, P., CORRODI, H. & FUXE, K. (1974). Benzodiazepines and barbiturates: turnover changes in central

- 5-hydroxytryptamine pathways. *Eur. J. Pharmac.*, **26**, 35-40.
- LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.
- McMENAMY, R.H. & ONCLEY, J.L. (1958). The specific binding of L-tryptophan to serum albumin. *J. biol. Chem.*, **233**, 1436-1447.
- MELDRUM, B.S., BALZAMO, E., WADA, J.A. & VUILLON-CACCIUTTOLO, G. (1972). Effects of L-tryptophan, L-3,4-dihydroxyphenylalanine and tranlycypromine on the electroencephalogram and on photically-induced epilepsy in the baboon, *Papio papio*. *Physiol. Behav.*, **9**, 615-621.
- MILLER, G.L. (1959). Protein determination for large numbers of samples. *Anal. Chem.*, **31**, 964.
- MOIR, A.T.B. & ECCLESTON, D. (1968). The effects of precursor loading in the cerebral metabolism of 5-hydroxyindoles. *J. Neurochem.*, **15**, 1093-1108.
- MULLER, W.E. & WOLLERT, U. (1975). Benzodiazepines: Specific competitors for the binding of L-tryptophan to human serum albumin. *Naunyn-Schmiedebergs Arch. Pharmac.*, **288**, 17-27.
- NAKAMURA, M. & FUKUSHIMA, H. (1976). Head twitches induced by benzodiazepines and the role of biogenic amines. *Psychopharmac.(Berl)*, **49**, 259-261.
- OMARA, T. & SATO, R. (1964) The carbon monoxide binding pigments of liver microsomes. *J. biol. Chem.*, **239**, 2330-2378.
- SAYERS, A.C., BURKI, H.R., RUCH, W. & ASPER, H. (1975). Neuroleptic-induced hypersensitivity of striatal dopamine receptors in the rat as a model of tardive dyskinesias. Effects of clozapine, haloperidol, loxapine and chlorpromazine. *Psychopharmac. (Berl)*, **41**, 97-104.
- SJÖHOLM, I. & SJÖDIN, T. (1972). Binding of drugs to human serum albumin. I. Circular dichroism studies on the binding of some analgesics, sedatives and antidepressive agents. *Biochem. Pharmac.*, **21**, 3041-3052.
- TAGLIAMONTE, A., BIGGIO, G., VARGIU, L. & GESSA, G.L. (1973). Increase of brain tryptophan and stimulation of serotonin synthesis by salicylate. *J. Neurochem.*, **20**, 904-912.
- VALLERINO, D.M., VESSEL, E.S., JOHNSON, A.O. & AURORI, J.C. (1973). Effects of various centrally active drugs on hepatic microsomal enzymes: a comparative study. *Drug Metab. Disp.*, **1**, 669-678.
- WADA, J.A., BALZAMO, E., MELDRUM, B.S. & NAQUET, R. (1972). Behavioural and electroencephalographic effects of L-5-hydroxytryptophan and D,L-parachlorophenylalanine on the epileptic Senegalese baboon (*Papio papio*). *Electroenceph. Clin. Neurophysiol.*, **33**, 520-526.
- WURTMAN, R.J. & FERNSTROM, J.D. (1972). L-Tryptophan, L-tyrosine and the control of brain monoamine biosynthesis. In *Perspectives in Neuropharmacology*. ed. Snyder, S.H. pp. 143-193. Oxford University Press.
- YOUNG, J.N., LAL, S., FELDMULLER, F., SOURKES, T.L., FORD, R.M., KIELY, M. & MARTIN, J.B. (1976). Parallel variation of ventricular C.S.F. tryptophan and free serum tryptophan in man. *J. Neurol. Neurosurg. Psychiat.*, **39**, 61-65.

(Received May 31, 1977.  
Revised August 2, 1977.)