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The sensitivity of the brain to barbiturate during chronic administration and withdrawal of barbitone sodium in the rat

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Summary

1. The sensitivity of the central nervous system to barbiturate was determined in rats during the chronic administration of barbitone sodium and after its withdrawal.

2. The brain barbiturate concentration determined on awakening from a hypnotic dose administered intraperitoneally was found to increase throughout the period of barbitone administration.

3. A similar gradual development of central nervous system tolerance was indicated by measuring the duration of anaesthesia following an intraventricular injection of pentobarbitone.

4. The change in sensitivity of the brain which occurred during the period of barbitone administration was not demonstrable from the measurement of sleeping time following intraperitoneal injection of barbitone or pentobarbitone.

5. After withdrawal, the sensitivity of the brain to barbiturate gradually returned to normal.

6. It was concluded that the hypersensitivity to pentobarbitone, but not to barbitone, which develops after withdrawal of barbitone sodium is due to a decreased drug-metabolizing capacity.

Introduction

A recent investigation into the effect of chronic barbitone administration and withdrawal on the activity of hepatic microsomal drug-metabolizing enzymes measured *in vitro* showed that during drug treatment enzyme activity was stimulated but that at some time after withdrawal activity fell to and remained at a lower level than that found normally (Stevenson & Turnbull, 1968). Throughout the period of barbitone administration, tolerance was exhibited to intraperitoneally administered hexobarbitone and after withdrawal a hypersensitivity, indicated by a prolonged sleeping time, was found. This paper presents the results of experiments performed in order to determine the effect of chronic barbitone administration and withdrawal on the sensitivity of the central nervous system to subsequently administered barbitone and pentobarbitone. This information has been obtained by determination of the brain barbiturate concentration on awakening from a hypnotic dose administered intraperitoneally, and in addition, with pentobarbitone, by measure-

ment of the duration of anaesthesia following intraventricular injection. A preliminary report of these results was communicated to the British Pharmacological Society (Stevenson & Turnbull, 1969).

Methods

Animals and barbitone administration

Female Wistar rats, weighing 50 ± 5 g at the beginning of the experiments, were used throughout. Aqueous barbitone sodium solution containing 0·02% w/v saccharin was administered as sole drinking fluid. The dose administered was 100 mg/kg daily during the first week, and the daily dose was increased at weekly intervals by 100 mg/kg until the rats received the maximum daily intake of 400 mg/kg. Barbitone was withdrawn after treatment for 32 days. Withdrawn and control animals received saccharin solution as sole drinking fluid. Except where otherwise indicated, the experimental procedures described below were carried out on animals which had received barbitone for 4, 18 or 32 days or had been withdrawn for 2, 6 or 22 days following 32 days of drug administration.

Radioactive barbiturates

Pentobarbitone-2-^{14}C and barbitone-2-^{14}C were obtained from Tracerlab, G.B. Ltd. and were found to be pure in several thin-layer chromatography systems. The concentration of the pentobarbitone solution used for intraperitoneal injection was 0·08 mmol/ml and that for intraventicular injection 0·1 mmol/ml, the specific activity of both solutions being 66·6 μCi/mmol. The barbitone solution used was 0·54 mmol/ml with a specific activity of 8·0 μCi/mmol.

Determination of tissue barbiturate concentration

Rats were killed on awakening following an intraperitoneal injection of either 40 mg/kg ^{14}C-pentobarbitone sodium or 225 mg/kg ^{14}C-barbitone sodium. The sleeping time was recorded. A sample of blood was obtained by cardiac puncture and 10% w/v aqueous homogenates were prepared of brain and liver.

Tissue barbiturate concentration was determined after extraction at pH 5·5 from the homogenates. ^{14}C-Pentobarbitone and ^{14}C-pentobarbitone metabolites were extracted as described by Kuntzman, Ikeda, Jacobson & Conney (1967) into petroleum ether : iso-amyl alcohol (98·5 : 1·5) and ethyl acetate respectively. ^{14}C-Barbitone was extracted into methylene chloride, but due to poor counting efficiencies in the presence of methylene chloride these extracts were evaporated to dryness and reconstituted in ethyl acetate. Aliquots of the organic layers were counted in a dioxan-naphthalene scintillator in a Nuclear-Chicago counter, efficiency being determined by the Channels Ratio method.

Total tissue barbiturate concentration, i.e. unlabelled barbitone together with ^{14}C-pentobarbitone or ^{14}C-barbitone, was determined spectrophotometrically by the method of Brodie, Burns, Mark, Lief, Bernstein & Papper (1953).

Intraventricular injection of pentobarbitone

The duration of anaesthesia following the injection of pentobarbitone sodium (500 μg in 20 μl) into the lateral cerebral ventricle was measured. Barbitone had

been withdrawn from the rats used for 48 h following 1–4 weeks of its administration and for 2, 6 and 22 days following 5 weeks of its use. The method of injection was essentially that described by Noble, Wurtman & Axelrod (1967). The rate of disappearance of pentobarbitone from the brain was determined by killing animals at intervals after the intraventricular injection of 500 μg ^{14}C-pentobarbitone sodium. The whole brain was rapidly removed, homogenized in water and an aliquot equivalent to 100 mg brain tissue taken for estimation of ^{14}C-pentobarbitone (for method see above).

Statistical analysis of results

To economize on animals and radioactive materials, the control animal barbiturate level on awakening was determined at three points only during the experimental period—after 4 and 32 days' barbitone treatment and 22 days after withdrawal. In calculating the significance of the differences between the means of the values obtained with control and treated animals, 4 and 18 day treated rats were compared with 4 day control, 32 day treated and 2 day withdrawn with 32 day treated control and 6 and 22 day withdrawn with controls corresponding to 22 day withdrawn rats.

Results

The tissue concentration of unlabelled barbitone—that is that taken in the drinking water—is shown in Fig. 1. The brain concentration was always between that of the liver and serum and an appreciable concentration remained on the sixth day after withdrawal. No barbitone could be detected by day 22 after withdrawal.

The duration of sleep produced by the intraperitoneal administration of barbitone was the same on all occasions throughout the period of barbiturate administration

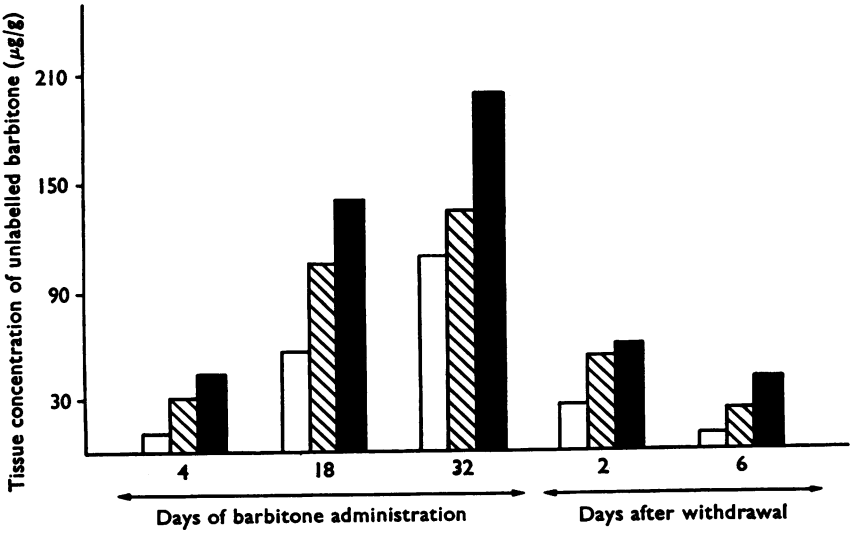

FIG. 1. Tissue concentration of unlabelled barbitone during chronic barbitone administration and after its withdrawal. Each value for brain (hatched columns) is the mean of four observations. Each value for liver (filled columns) and serum (open columns) is the value obtained by pooling four samples.

and withdrawal (Table 1). The tissue concentrations of ^{14}C-barbitone found on awakening were the same in the treated and withdrawn rats as in the controls. Fig. 2a illustrates this for the brain. However, the sensitivity of the central nervous system to barbitone was not the same on all occasions, for when unlabelled barbitone is taken into account (Fig. 2a) it is obvious that the chronically treated animals awakened with a higher total brain barbitone level. The total barbitone concentration in liver and serum was also found to be higher in the treated animals. After withdrawal this tolerance was gradually lost (Fig. 2a).

TABLE 1. *Sleeping time (min) following intraperitoneal injection of 225 mg/kg barbitone or 40 mg/kg pentobarbitone into barbitone treated and withdrawn rats*

		Days of barbitone administration			Days after withdrawal		
		4	18	32	2	6	22
Barbitone	Control	172±22		152±23			167±20
	Treated	182±16	161±8	180±14	146±15	146±17	157±29
	Significance (*P*)	NS	NS	NS	NS	NS	NS
Pento-barbitone	Control	117±13		134±18			176±23
	Treated	32±13	33±18	29±5	38±11	107±41	233±21
	Significance (*P*)	<0·001	<0·001	<0·001	<0·001	<0·05	<0·02

All values given are the means ± S.D. of four observations. NS, Not significant.

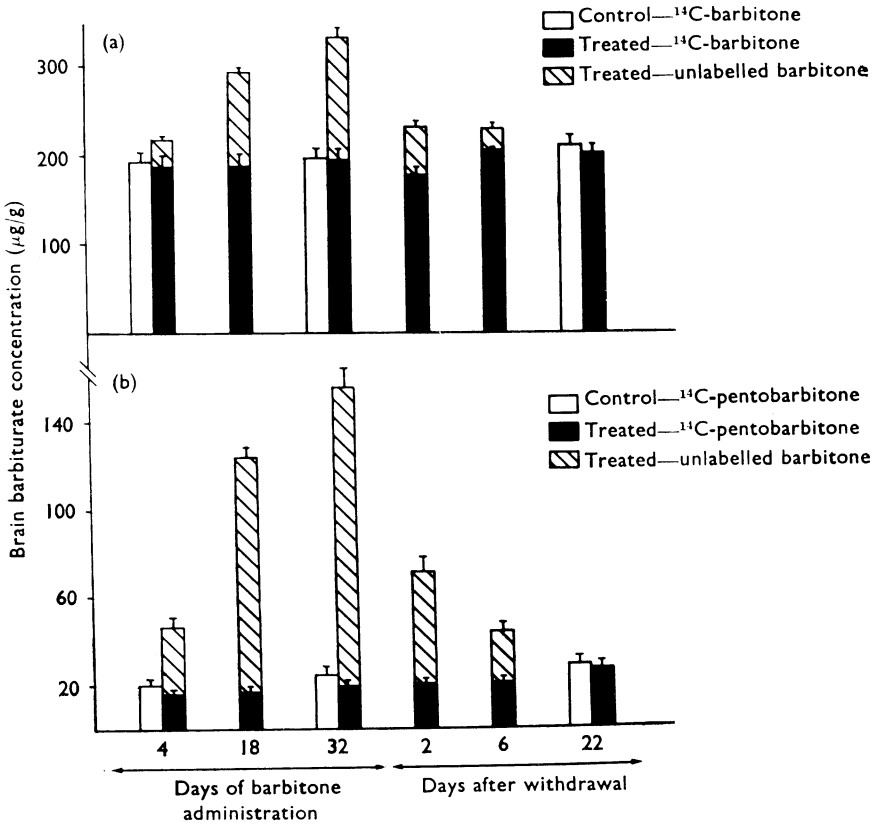

FIG. 2. Brain barbiturate concentration found on awakening following an intraperitoneal injection of (a) 225 mg/kg ^{14}C-barbitone sodium and (b) 40 mg/kg ^{14}C-pentobarbitone sodium. Each value is the mean ± S.D. of four observations.

The brain concentrations (Fig. 2b) and liver and serum concentrations (Fig. 3) of ^{14}C-pentobarbitone on awakening were significantly lower in the barbitone treated rats than in control animals, indicating that even though the sleeping time following intraperitoneal injection of pentobarbitone was greatly reduced in the treated animals (Table 1) more pentobarbitone had been eliminated. The higher liver and serum concentration of labelled pentobarbitone metabolites (Fig. 4) in the barbitone treated rats would indicate that increased metabolism was responsible. The extent to which tolerance, as indicated by reduction in sleeping time, had developed to intraperitoneally administered pentobarbitone (Table 1) was approximately the same at the end of the period of barbitone administration (day 32) as on day 4. However, as was the case with barbitone, the sensitivity of the central nervous system to barbiturate was not the same on these occasions, chronically treated animals awakening with a higher brain total barbiturate level (Fig. 2b).

After withdrawal, tolerance to pentobarbitone was rapidly lost, as indicated by a return towards control values of both duration of anaesthesia (Table 1) and brain total barbiturate concentration on awakening (Fig. 2b). However, by the twenty-second day after withdrawal a hypersensitivity to intraperitoneally administered pentobarbitone had developed (Table 1), the sleeping time being significantly longer in these animals than in controls. This hypersensitivity was not associated with an increased brain sensitivity to pentobarbitone because the tissue levels of pentobarbitone and its metabolites on awakening were the same in the withdrawn and control rats (Figs. 3 and 4).

The duration of anaesthesia following the intraventricular injection of 500 μg pentobarbitone is shown in Table 2. Animals which had received barbitone for only one week had a slightly reduced sleeping time, but the difference was not

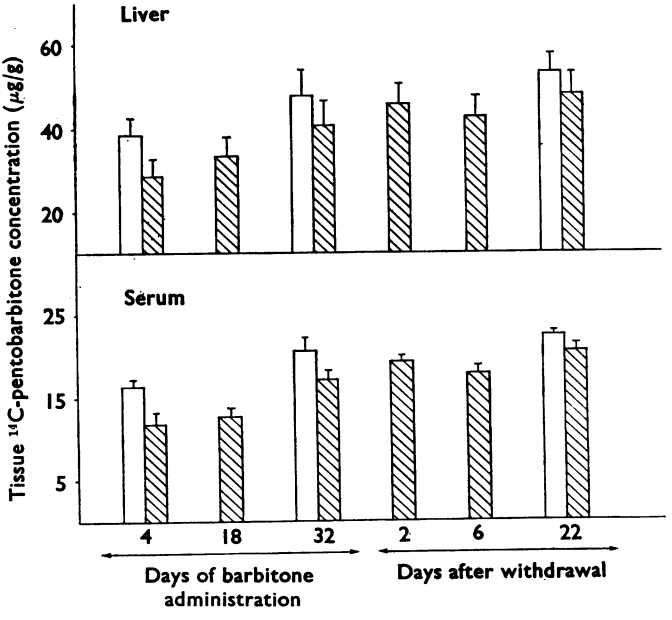

FIG. 3. Liver and serum concentration of ^{14}C-pentobarbitone found on awakening following an intraperitoneal injection of 40 mg/kg ^{14}C-pentobarbitone sodium. Each value is the mean ±S.D. of four observations. Open columns, control; hatched columns, treated.

statistically significant. On all other occasions the treated animals had a significantly reduced sleeping time. Some animals which had been treated for 4 or 5 weeks did not lose their righting reflex at all. That this tolerance to pentobarbitone was not due to the barbiturate diffusing more rapidly from the brains of the barbitone treated rats was shown (Fig. 5) by the fact that the rate of disappearance of intraventricularly administered ^{14}C-pentobarbitone was the same in the two groups of animals, the half-life as calculated from Fig. 5 being 2·9 min in each case. The brain pentobarbitone level found on awakening in control animals was the same after intraventricular injection as after intraperitoneal injection. After withdrawal,

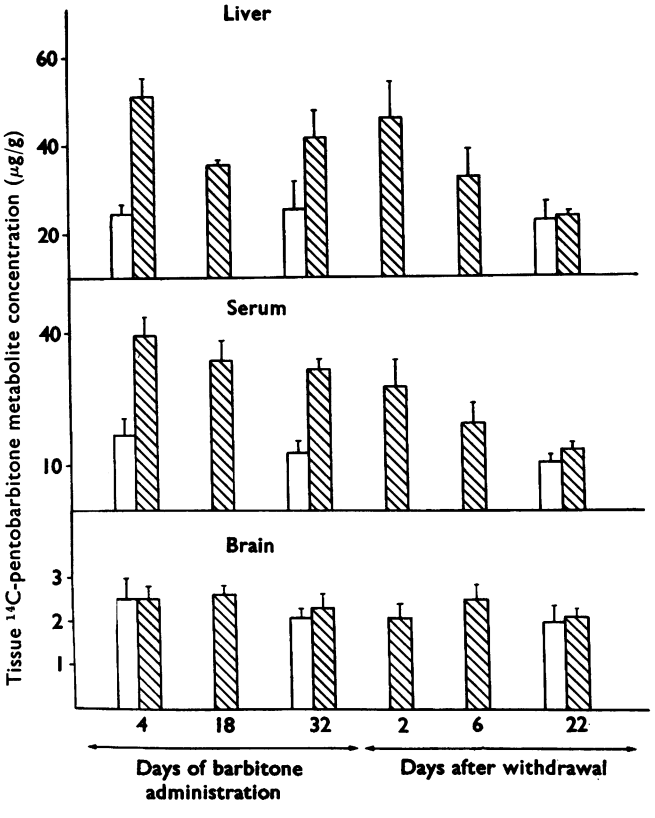

FIG. 4. Tissue concentration of ^{14}C-pentobarbitone metabolites found on awakening following an intraperitoneal injection of 40 mg/kg ^{14}C-pentobarbitone sodium. Each value is the mean ±S.D. of four observations. Open columns, control; hatched columns, treated.

TABLE 2. *Duration (min) of anaesthesia following intraventricular administration of pentobarbitone*

	Weeks of barbitone administration*					Weeks after withdrawal†	
	1	2	3	4	5	1	3
Control	13·1 ±1·4(5)	11·5 ±2·5(5)	7·2 ±0·8(4)	7·3 ±1·3(6)	7·1 ±0·3(6)	5·4 ±0·5(4)	4·3 ±0·5(5)
Treated	11·0 ±3·5(6)	6·2 ±1·5(6)	3·8 ±0·8(4)	4·4‡ ±0·4(3)	5·0§ ±0·6(4)	4·6 ±0·5(4)	4·2 ±0·6(5)
Significance (*P*)	NS	<0·01	<0·001	<0·01	<0·001	NS	NS

All values given are means±S.D. with number of observations in parentheses. * Animals withdrawn for 48 h following the period of barbitone administration indicated. † Time after withdrawal following 5 weeks of barbitone administration. ‡ Three out of six rats failed to sleep. § Two out of six rats failed to sleep. NS, Not significant.

the sensitivity of the central nervous system to intraventricularly administered pentobarbitone returned to normal, there being no evidence of an increased sensitivity to pentobarbitone.

Discussion

It was previously reported that the barbiturate tolerance developing during chronic barbitone administration and the hypersensitivity found after its withdrawal correlated well with changes in hepatic drug-metabolizing enzyme activity (Stevenson & Turnbull, 1968). The possibility that changes in the sensitivity of the central nervous system might also have contributed to the altered response was not ruled out by this study however. The barbitone dosage schedule used both then, and in the present study, was one known to produce physical dependence (Crossland & Leonard, 1963 ; Stevenson & Turnbull, 1968). Abrupt withdrawal of the drug precipitated a withdrawal syndrome characterized by weight loss, decreased convulsant threshold and the development of susceptibility to audiogenic seizures. The effect of such chronic drug administration and its subsequent withdrawal on the response to centrally acting drugs has not been closely studied.

It is apparent from the results presented in this paper that the sensitivity of the brain to barbiturate decreases throughout a period of chronic barbitone administration. However, the determination of sleeping time alone provided no indication of this changing sensitivity. The inadequacy of sleeping time determinations in assessing brain sensitivity is emphasized by the fact that sleeping time to barbitone and

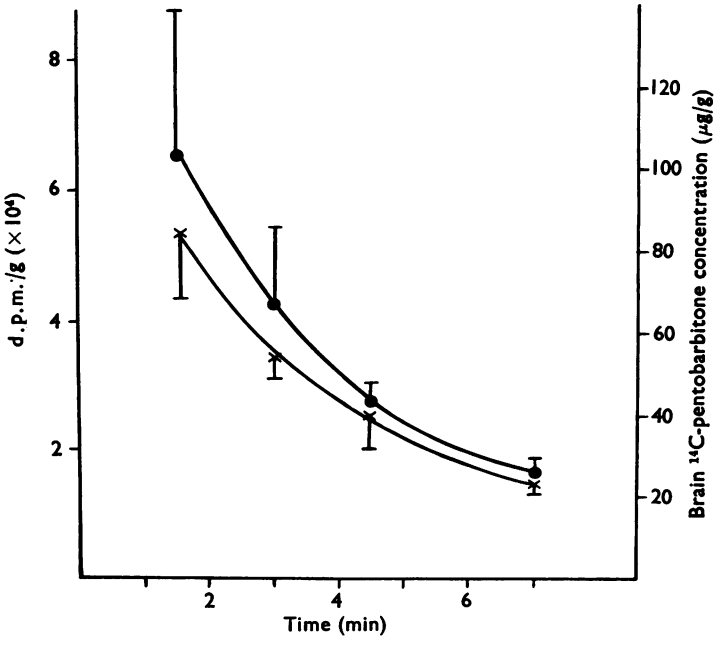

FIG. 5. Rate of disappearance of ^{14}C-pentobarbitone sodium after injection (500 μg in 20 μl) into the left lateral cerebral ventricle. Each value is the mean ± S.D. of three observations. ×, Control ; ●, treated.

the extent to which tolerance was exhibited to pentobarbitone was the same on day 4 as on day 32. By the end of the period of drug treatment, however, physical dependence had developed to such an extent that physical signs of withdrawal were produced if the drug was withheld. Only when more direct indices of central nervous sensitivity were employed such as the determination of brain total barbiturate level on awakening, or duration of anaesthesia following intraventricular administration of barbiturate, did it become apparent that the response to barbiturate decreased gradually throughout the period of barbiturate exposure. These parmeters, unlike the measurement of sleeping times, are not influenced by alteration of hepatic drug-metabolizing enzyme activity. Furthermore, as well as reflecting brain sensitivity, the estimation of tissue levels of both unchanged barbiturate and its metabolite(s) provides information on the *in vivo* rate of barbiturate metabolism. Previously, *in vivo* metabolizing capacity has been extrapolated from results obtained *in vitro* with hepatic microsomal preparations. The activity of drug-metabolizing enzymes as indicated by tissue levels in the present experiments correlate well with our previously reported *in vitro* findings (Stevenson & Turnbull, 1968). The fact that the tissue levels of pentobarbitone or its metabolites in the 22 day withdrawn rats do not appear to indicate a reduced activity of drug-metabolizing enzymes may be due to the control and withdrawn animals being killed at different times after injection. Thus control animals were killed 176 min and 22 day withdrawn animals 233 min after injection. A second apparent anomaly with tissue levels of pentobarbitone metabolites occurs with brain, where, in contrast to liver and serum, the level of pentobarbitone metabolites is not increased during barbitone treatment. As the absolute level of metabolites in this tissue is very much lower than that of serum, it may reasonably be concluded that the metabolites pass the blood brain barrier with difficulty.

Following withdrawal of barbitone, the sensitivity of the central nervous system as indicated by intraventricular sleeping times and brain barbiturate level on awakening increased again. The return to normal was gradual and was complete by day 22 after withdrawal. At this time, however, a hypersensitivity to intraperitoneally administered pentobarbitone was found. Liver microsomal preparations from withdrawn rats have previously been shown to have reduced capacity to oxidize barbiturates (Stevenson & Turnbull, 1968) and it would seem that this is the sole mechanism involved in the hypersensitivity response. This is in contrast to the findings of Aston (Aston, 1966 ; Aston & Hibbeln, 1967), who found that rats which had 4 weeks previously been injected with only two hypnotic doses of pentobarbitone or barbitone exhibited a hypersensitivity to barbiturates which was apparently not due to decreased drug metabolizing capacity.

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