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A STUDY OF THE FACTORS AFFECTING THE SLEEPING TIME FOLLOWING INTRACEREBROVENTRICULAR ADMINISTRATION OF PENTOBARBITONE SODIUM: EFFECT OF PRIOR ADMINISTRATION OF CENTRALLY ACTIVE DRUGS

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1 Injection of pentobarbitone sodium into a lateral cerebral ventricle of rats produced a loss of righting reflex. The duration of anaesthesia was dose-dependent.

2 The optimum dose of pentobarbitone to allow study of the factors affecting the sleeping time was considered to be 650 μg injected in 25 μl water.

3 In a study of the effect of age and sex on the sleeping time, the youngest rats used (88 g body weight) were found to be the most sensitive to barbiturate. Female rats were more sensitive than male animals.

4 The duration of anaesthesia was not affected by induction or inhibition of hepatic drug-metabolizing enzyme activity.

5 Prior administration (acute) of central nervous system depressant drugs shortened the latent period and prolonged the duration of sleep. Prior administration of stimulant drugs antagonized the effect of pentobarbitone.

6 Animals withdrawn following chronic administration of a number of drugs, barbitone, barbitone/bemegride mixture, Mandrax (methaqualone : diphenhydramine; 10 : 1), chlordiazepoxide, nitrazepam, chlorpromazine or ethanol, exhibited a significant tolerance to intracerebroventricularly administered pentobarbitone.

7 Withdrawal of amphetamine, morphine, methyprylon or diazepam did not result in tolerance to intracerebroventricularly administered pentobarbitone.

8 Chronic administration of all drugs except amphetamine and morphine induced a tolerance to intraperitoneally administered hexobarbitone (100 mg/kg).

9 The usefulness of sleeping time determination following intracerebroventricular administration of pentobarbitone as an assessment of central nervous system excitability is discussed. It is concluded that this method gives a valid indication of the sensitivity of the central nervous system to barbiturate and of the level of excitability in general. The method is particularly applicable in situations where the activity of hepatic drug-metabolizing enzyme activity may be altered.

Introduction

One method of screening compounds for central nervous system activity is to determine their effect on the duration of loss of righting reflex, or sleeping time, produced by a subsequent intraperitoneal injection of a barbiturate. Measurement of barbiturate sleeping time is also commonly used to determine whether repeated drug administration has produced tolerance.

However, there are several ways in which a drug can interact with barbiturates to affect this sleeping time. First, the duration of anaesthesia is lengthened or shortened by central nervous system (CNS) depressant or stimulant drugs respectively. Secondly, acute drug pre-treatment may alter the rate at which the barbiturate is metabolized or may affect the rate of penetration of barbiturate into the brain. Thirdly, chronic drug administration may induce, or more rarely decrease, drug-metabolizing enzyme activity in the liver. Finally, repeated exposure to certain drugs produces

changes within the CNS which brings about a tolerance to their effects. This tolerance may also extend to other pharmacological agents (cross-tolerance) and hence reduce the duration of barbiturate-induced sleep.

Thus, because prior drug administration may affect the sleeping time by several different mechanisms, the use of this particular index provides only a general indication of drug responsiveness and is unsuited to the study of any single factor which may affect the sleeping time. In previous studies, we have been particularly interested in the effect of chronic administration of hypnotic drugs on the sensitivity of the brain to barbiturates and have reported that the determination of sleeping time following intraperitoneal injection of either barbitone or pentobarbitone provided no indication of the change in sensitivity of the brain to barbiturate which developed in rats given barbitone sodium chronically (Stevenson & Turnbull, 1970). However, when sleeping time was determined after an intracerebroventricular injection of pentobarbitone, changes in the sensitivity of the CNS to barbiturates were detected (Stevenson & Turnbull, 1970). Furthermore, the sleeping time determined following this route of administration was not affected by induction of drug-metabolizing processes and it thus appeared to be a particularly useful method of assessing change in brain sensitivity during chronic drug exposure.

This paper reports a study of the factors affecting the duration of anaesthesia produced by intracerebroventricularly administered pentobarbitone and in particular, the way in which this parameter is affected by prior drug administration. The usefulness of this method and its advantages, in certain circumstances, compared with the sleeping time determined after an intraperitoneal injection have been confirmed.

Methods

Intracerebroventricular injection of pentobarbitone sodium

All experiments except those designed to investigate the effect of age, sex and chronic drug administration, were performed on female Wistar rats weighing 180-200 g.

Animals to be injected were lightly anaesthetized with ether and a small burr hole made in the skull at a point 2.5 mm lateral and 1.0 mm posterior to the bregma. After allowing the rat to recover for 10 min, sodium pentobarbitone solution, 650 μg in 25 μl of water, was injected into the left lateral ventricle. This was carried out using a 50 μl Hamilton syringe connected to a size 25G x 5/8 needle fitted with a polythene tubing 'spacer' allowing injection at a depth of 4 mm below the surface of the skull. The time at which animals (a) lost and (b) regained their righting reflex was recorded with a stop watch. From this information the latent period (time between injection and loss of righting reflex) and sleeping time (time between loss and regain of righting reflex) were calculated.

To determine the relationship between dose of pentobarbitone and sleeping time the effect of administering varying amounts of pentobarbitone in a constant injection volume (25 μl) was determined. In this experiment respiratory rate was also recorded during the period of loss of the righting reflex.

Effect of age and sex

To investigate the effect of age, three groups of male rats (mean weights 88 g, 235 g and 552 g) were used. Sleeping time was determined both after intraperitoneal and intracerebroventricular injection of pentobarbitone. Brain barbiturate concentration on awakening from the anaesthetic was also measured.

Sleeping time was compared in male and female rats (weighing 180-190 g) after intracerebroventricular injection of labelled pentobarbitone and the brain barbiturate concentration on awakening was also determined. In a separate experiment, the rate of disappearance of intracerebroventricularly administered barbiturate from the brain was determined.

Effect of alteration of drug-metabolizing capacity

Sleeping times were recorded after induction (pretreatment for four days with barbitone sodium 100 mg/kg daily, administered in the drinking water) or inhibition (pretreatment with SKF 525A, 15 mg/kg, injected intraperitoneally 40 min before determination of the sleeping time) of drug-metabolizing processes.

Animals were killed on awakening and 30% w/v homogenates of liver were prepared in alkaline isotonic potassium chloride solution. The extent of metabolism of [^{14}C]-pentobarbitone *in vitro* in the presence of 9,000 g liver supernatants was determined by the method described by Kuntzman, Ikeda, Jacobson & Conney (1967).

Determination of brain barbiturate concentration

In some experiments the sleeping time was determined after an intracerebroventricular injection of [^{14}C]-pentobarbitone sodium solution (0.1 mmol/ml; specific activity 66.6 μCi/mmol).

Animals were killed by decapitation either on awakening or at intervals of 1.5, 3, 5 and 8 min after injection of the radioactive barbiturate solution. The brains were removed as rapidly as possible, weighed, 10% w/v aqueous homogenates prepared and the unchanged pentobarbitone determined by liquid scintillation counting after extraction from the homogenates with petroleum ether : iso-amyl alcohol (98.5 : 1.5) at pH 5.5.

Effect of prior drug administration on the sleeping time

Acute. The effect of an intraperitoneal injection of the following drugs on the sleeping time was determined: pentobarbitone, bemegride, ethanol, morphine, amphetamine, chlorpromazine, chlordiazepoxide, diazepam, nitrazepam, methyprylon and methaqualone : diphenhydramine (10 : 1; Mandrax). The doses, isomers, salts used and pretreatment times are given in Table 3.

Chronic. Sleeping time was determined in rats which had been pretreated for four weeks with gradually increasing doses of the following drugs: barbitone, barbitone/bemegride mixture, morphine, amphetamine, chlordiazepoxide, nitrazepam, diazepam, meprobamate, methyprylon, methaqualone/diphenhydramine mixture (10 : 1; Mandrax) and chlorpromazine. Details of drug dosage and routes of administration are given in Table 4. The rats used weighed 50-65 g at the beginning of the experiment, and were weighed twice weekly throughout. Water-soluble drugs, dissolved in tap water, were administered as the sole drinking fluid together with 0.2% w/v saccharin; control rats received tap water plus saccharin throughout. Ethanol, diluted with tap water, was given for six weeks. Drug concentrations were adjusted twice weekly to allow for increase in body weight and change in fluid intake. Morphine treated rats were given two daily subcutaneous injections of the drug during the last two weeks (see Table 4) in an amount equal, and in addition, to the drug given in the drinking water. The remaining drugs were administered in the food. These drugs were thoroughly mixed with powdered rat pellets before the addition of a small quantity of water to enable the mixture to be made into a paste which was placed in a dish in the rat cage. Control rats received a similarly prepared paste made from powdered pellets.

In some cases, during the last week of drug administration and three days before the animals were to be injected intracerebroventricularly, sleeping time was determined following an intraperitoneal injection of barbiturate. At 0900 h on the morning of such an experiment, the drug, i.e. water bottle or food dish, was removed from the cages of the animals to be injected and 100 mg/kg hexobarbitone sodium was given 2 h later. At the end of the period of chronic drug administration, drugs were removed from the cages at 0900 h and half of each group of animals were injected intracerebroventricularly with pentobarbitone 2 h later. The remaining animals were similarly injected with pentobarbitone 48 h after drug withdrawal (24 h in the case of morphine). In some of these studies, [^{14}C]-pentobarbitone was used and such animals were killed on awakening and the brain barbiturate concentration determined.

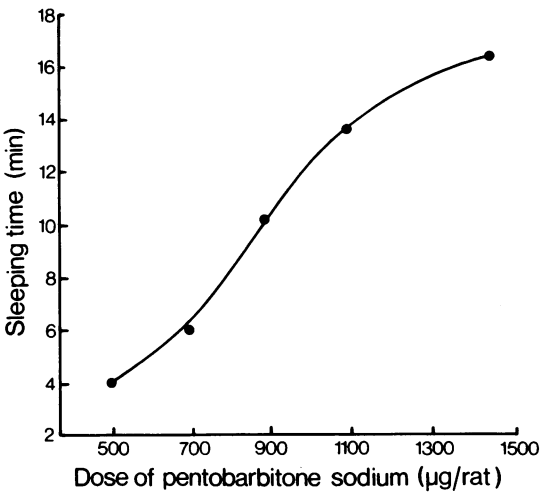

Fig. 1 The relationship between dose of intracerebroventricularly administered pentobarbitone sodium and duration of anaesthesia. Each point represents the mean of five observations.

Results

Effect of dose

Figure 1 shows the relationship between the dose of pentobarbitone and duration of anaesthesia. The smallest dose which consistently caused a loss of righting reflex was 500 μg and the largest dose which regularly produced anaesthesia with no fatalities was 1400 μg. Some animals that received 1600 μg survived (two out of five) but all five animals that were injected with 1800 μg died due to respiratory arrest. Smaller doses, e.g. 1400 μg, regularly produced a transient respiratory arrest (Figure 2). A dose of 650 μg, which resulted in a sleeping time of between 5-6 min was considered

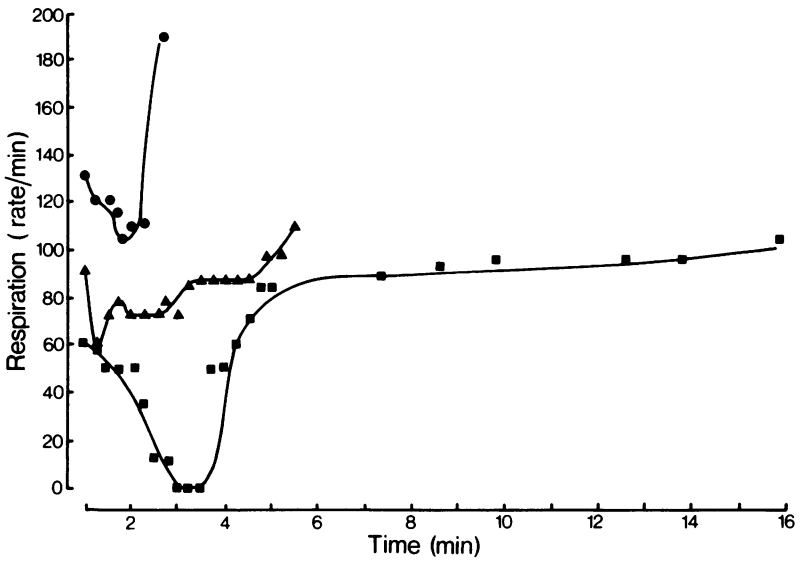

Fig. 2 Effect of various doses of intracerebroventricularly administered pentobarbitone sodium on respiratory rate. (■), 1,400 μg; (▲), 800 μg; (●), 500 μg. Each point represents the mean of three observations.

to be optimum and was used in all the remaining experiments.

Effect of age and sex

Table 1 presents the results of the experiments designed to investigate the effect of age on the sensitivity to barbiturate. Group II (235 g body weight) was the least sensitive and group I (88 g body weight) the most sensitive to intraperitoneally injected pentobarbitone, administered on a body weight basis. There was an inverse relationship between liver drug-metabolizing enzyme activity and this sleeping time. The same order of sensitivity was indicated by both sleeping time and brain barbiturate concentration on awakening following intracerebroventricularly administered pentobarbitone. However, a different order of sensitivity was indicated when the latent period alone was considered.

Female rats were more sensitive to pentobarbitone than male animals (Table 2). This was indicated by a shorter latent period, longer sleeping time and the suggestion of lower brain barbiturate concentration on awakening, although this difference was not statistically significant. The rate of disappearance of pentobarbitone from the brain following its intracerebroventricular administra-

Table 1 Effect of age on the sensitivity to barbiturate

	Group I	*Group II*	*Group III*
Body weight (g)	88 ± 5	235 ± 10	552 ± 42
Pentobarbitone sodium (40 mg/kg i.p.)			
Sleeping time (min)	82 ± 15	53 ± 7*	60 ± 11**
Pentobarbitone sodium (650 μg i.c.v.)			
Latent period (min)	1.13 ± 0.49	1.07 ± 0.17	1.46 ± 0.56
Sleeping time (min)	6.9 ± 2.0	4.2 ± 1.15**	6.20 ± 2.74
Brain barbiturate level on awakening	19.1 ± 4.0	33.6 ± 12.6***	22.6 ± 10
Liver drug-metabolizing enzyme activity†	16.2 ± 5.1	30.1 ± 8.5***	22.1 ± 5.5

Figures represent mean ± s.d. of six observations. * $P < 0.01$; ** $P < 0.02$; *** $P < 0.05$.
† mμ moles pentobarbitone metabolites formed/g liver/30 minute.
Significance calculated using Student's *t* test, by comparison with Group I.

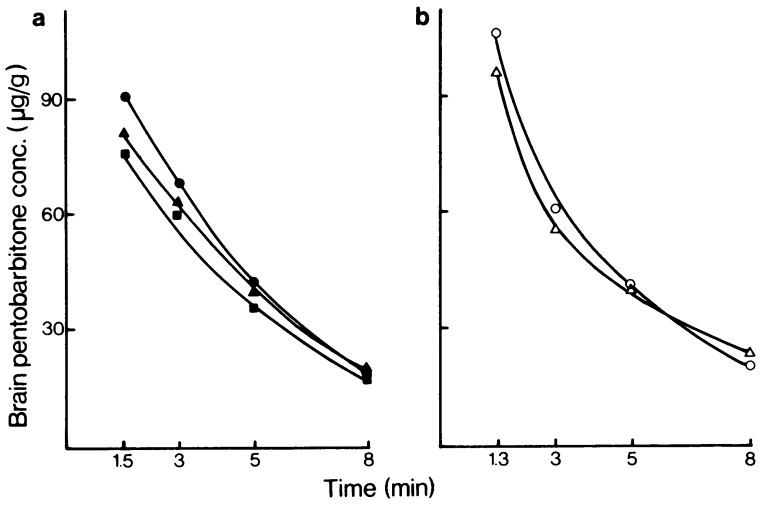

Fig. 3 Rate of disappearance of [^{14}C]-pentobarbitone sodium after intracerebroventricular injection (650 µg in 25 µl): (a) effect of induction or inhibition of drug-metabolizing processes; (b) comparison of male and female rats. (●), Control; (▲), inhibited; (■), induced; (○), female; (△), male. Each point represents the mean of three observations.

tion did not differ significantly between males and females (Figure 3).

Effect of induction or inhibition of drug-metabolizing capacity

Pretreatment with SKF 525A or barbitone caused a significant inhibition or induction of drug metabolism respectively (Table 2). Although pretreatment with SKF 525A did not affect the latent period, brain barbiturate level on awakening or the rate of disappearance of barbiturate from the brain (Fig. 3), it did tend however to prolong the sleeping time (Table 2). We have found this to be so on several different occasions. Induction of drug-metabolizing processes did not affect any of the parameters measured.

Effect of acute drug pretreatment on sleeping time

The effect of prior administration of a number of centrally active compounds on the sleeping time is shown by the results in Table 3. Pretreatment with the CNS stimulant drugs bemegride and amphetamine prolonged the latent period and shortened the sleeping time. Larger doses completely antagonized the effect of the pentobarbitone i.e. animals failed to lose their righting reflex. Depressant

Table 2 Effect of sex and rate of drug metabolism on the sensitivity to intracerebroventricularly administered pentobarbitone sodium

Pretreatment	*Sex*	*Latent period (min)*	*Sleeping time (min)*	*Brain barbiturate level on awakening (µg/g)*	*†Liver drug-metabolizing enzyme activity*
Saline	M	1.10 ± 0.17	3.54 ± 0.96	31.4 ± 8.56	–
Saline	F	0.78 ± 0.09*	4.79 ± 0.99**	26.45 ± 4.46	–
Saline	F	1.0 ± 0.16	4.86 ± 0.65	30.5 ± 6.0	16.2 ± 3.4
SKF-525A	F	0.93 ± 0.06	5.57 ± 1.39	30.7 ± 8.5	11.0 ± 4.6*
Barbitone	F	0.82 ± 0.18	4.92 ± 0.70	32.0 ± 12	29.3 ± 2.1*

Figures represent mean ± s.d. of six observations. * $P < 0.01$; ** $P < 0.05$.
† nmol pentobarbitone metabolites formed per g liver in 30 minutes.
Significance calculated using Student's *t* test, by comparison with saline treated rats.

Table 3 Effect of acute drug pretreatment on the sleeping time produced by intracerebroventricularly administered pentobarbitone sodium

Pretreatment	*Dose (mg/kg)*	*Pretreatment time (min)*	*Latent period (min)†*	*Sleeping time (min)†*
Saline	–	15	1.03 ± 0.15 (12)	5.4 ± 0.70 (12)
Bemegride	2.5	5	No loss of R.R.	–
Bemegride	1.25	5	1.42 ± 0.25 (4)**	2.95 ± 0.65 (4)**
Pentobarbitone sodium	5	10	0.71 ± 0.25 (6)*	12.22 ± 1.45 (6)**
Morphine sulphate	10	15	1.0 ± 0.01 (3)	5.7 ± 1.1 (3)
Morphine sulphate	5	15	1.0 ± 0.01 (3)	5.4 ± 0.56 (3)
Ethanol	1000	30	1.17 ± 0.20 (4)	9.50 ± 1.16 (4)**
(+)-Amphetamine sulphate	2.5	15	No loss of R.R.	–
(+)-Amphetamine sulphate	1	15	1.19 ± 0.38 (4)	2.74 ± 1.18 (4)**
Chlorpromazine hydrochloride	1.0	15	0.85	12.5 (2)
Chlorpromazine hydrochloride	0.5	15	1.06	6.0 (2)
Chlordiazepoxide	10	15	0.42	18.5 (2)
Chlordiazepoxide	5	15	0.50	16.75 (2)
Chlordiazepoxide	2.5	15	0.79 ± 0.15 (4)***	9.19 ± 0.64 (4)**
Nitrazepam	10	20	0.40	19.0 (2)
Nitrazepam	5	20	0.50	18.0 (2)
Nitrazepam	1	20	0.75	10.0 (2)
Diazepam	10	20	0.15	37 (2)
Diazepam	2.5	20	0.40	12.4 (2)
Methaqualone/diphenhydramine hydrochloride (10 : 1)	20	20	0.40	22 (2)
Methyprylon	20	20	0.40	15 (2)

† Figures denote mean ± s.d. with numbers of observations in parentheses. * $P < 0.01$; ** $P < 0.001$; *** $P < 0.02$.
R.R., righting reflex.
Significance calculated using Student's *t* test, by comparison with saline treated rats.

drugs shortened the latent period and prolonged the sleeping time. Doses larger than those shown in Table 1 resulted either in an undue prolongation of the sleeping time, making the end point difficult to determine, or depressed respiration to such an extent that the subsequently injected pentobarbitone caused respiratory arrest. Morphine at doses of 5 and 10 mg/kg did not significantly affect the sleeping time. Animals pretreated with larger doses of morphine died due to respiratory arrest following administration of the pentobarbitone.

Effect of chronic drug pretreatment and withdrawal on sleeping time

The results of experiments employing the pretreatments shown in Table 4 are given in Table 5. All animals except those treated with amphetamine and morphine exhibited tolerance to intraperitoneally administered barbiturate. A number of animals were so tolerant that they failed to lose their righting reflex after injection of the barbiturate, e.g. nitrazepam. Tolerance to intracerebroventricularly administered barbiturate i.e. a sleeping time lower than that produced in control animals, was observed in animals withdrawn from barbitone, barbitone/bemegride, methaqualone/diphenhydramine, chlordiazepoxide, nitrazepam, chlorpromazine and ethanol. Some animals failed to lose the righting reflex. Tolerance was present both before and after withdrawal of meprobamate and ethanol. Withdrawal of the following drugs did not result in the development of cross tolerance to barbiturate: amphetamine, methyprylon, diazepam and morphine.

The body weights of rats determined during the administration of these drugs are shown in Table 6. The body weight of rats receiving barbitone was significantly increased, this being evident after only one week of drug administration. Chlordiazepoxide treated rats were also heavier at the end of the four-week period than their controls. However, rats which received the barbitone/bemegride mixture were not significantly heavier than controls. Animals receiving amphetamine, methaqualone/diphenhydramine, ethanol and chlorpromazine had significantly lower body weights compared with untreated rats after various periods of drug administration.

Discussion

The two most important variables determining the sensitivity of laboratory animals to intraperitoneally administered barbiturate are the activity of hepatic drug-metabolizing enzymes and the

Table 4 Doses and method of administration of drugs given chronically

Drug	*Method of administration*	*Dose (mg kg^{-1} d^{-1}) in week:* 1	2	3	4	5	6
Amphetamine sulphate	D.W.	2.5	7.5	15	25		
Barbitone sodium	D.W.	100	200	300	400		
Barbitone/bemegride	D.W.	100/50	200/100	300/150	400/200		
Methyprylon	D.W.	100	300	500	700		
Nitrazepam	F.	20	50	100	200		
Diazepam	F.	20	50	100	200		
Meprobamate	F.	250	750	1500	2000		
Chlorpromazine hydrochloride	D.W.	10	20	40	80		
Chlordiazepoxide	F.	10	30	50	100		
Methaqualone/diphenhydramine hydrochloride (10 : 1)	F.	100	300	600	600		
Morphine sulphate	D.W.	1-4	8-16	30-60	90-120		
	s.c. injections	—	—	30-60	90-120		
				% in drinking water			
Ethanol	D.W.	5	10	12.5	15	17.5	20

D.W., administered in the drinking water; F, administered in the food. (See methods section for details.)

Table 5 Effect of chronic drug administration on sensitivity to barbiturate

	Intraperitoneal hexobarbitone sodium		*Intracerebroventricular pentobarbitone sodium*			
	Sleeping time (min)	*No. failing to lose R.R.*	*Latent period (min)*	*Sleeping time (min)*	*No. failing to lose R.R.*	*Brain pentobarbitone concentration on awakening (μg/g)*
Control	61 ± 16 (6)	0	0.96 ± 0.18 (19)	5.53 ± 1.08 (19)	0	22 ± 5 (17)
Amphetamine	70 ± 21 (5)†	0	1.25 ± 0.32 (4)†*	5.32 ± 1.78 (3)	1	–
,, withdrawn	–	–	1.20 ± 0.38 (6)**	8.21 ± 2.77 (6)*	0	–
Barbitone	24 ± 10 (3)*	3	0.82 ± 0.11 (6)	6.57 ± 1.14 (6)**	0	30 ± 13 (6)**
,, withdrawn	–	–	0.94 ± 0.13 (4)	3.69 ± 1.59 (4)*	2	40 ± 3 (4)***
Barbitone/bemegride	12.3 ± 3 (3)***	3	0.87 ± 0.32 (5)	4.32 ± 1.29 (5)	1	–
,, withdrawn	–	–	–	–	5	–
Meprobamate	32 ± 14 (6)**	0	0.86 ± 0.18 (5)	2.57 ± 1.09 (5)***	1	40 ± 10 (5)***
,, withdrawn	–	–	1.15 ± 0.32 (4)	2.44 ± 0.65 (4)***	2	36 ± 7 (4)***
Methyprylon	20 ± 3 (5)***	1	0.80 ± 0.14 (5)†	6.81 ± 3.62 (5)	–	–
,, withdrawn	–	–	0.90 ± 0.18 (4)†	6.56 ± 2.0 (4)	–	–
Methaqualone/diphenhydramine (10 : 1)	11 ± 2 (6)***	0	0.68 ± 0.18 (6)*	5.24 ± 1.63 (6)	0	30 ± 15 (6)
,, withdrawn	–	–	1.06 ± 0.14 (4)	2.66 ± 1.35 (4)***	2	40 ± 4 (4)***
Chlordiazepoxide	21 ± 0.6 (4)***	2	0.84 ± 0.23 (6)	5.57 ± 1.49 (6)	0	18 ± 2 (6)
,, withdrawn	–	–	0.97 ± 0.18 (6)	2.32 ± 0.82 (6)***	0	53 ± 23 (6)***
Nitrazepam	48 ± 4 (3)	3	0.53 ± 0.15 (6)***	11.39 ± 3.87 (6)***	0	14 ± 6 (6)*
,, withdrawn	–	–	0.73 ± 0.40 (5)**	3.0 ± 0.56 (5)***	1	44 ± 10 (5)***
Control	59 ± 11 (11)	0	0.93 ± 0.29 (11)	7.9 ± 1.8 (11)	0	–
Diazepam	25 ± 2 (5)***	1	1.04 ± 0.38 (6)	7.19 ± 2.69 (6)**	0	–
,, withdrawn	–	–	1.13 ± 0.29 (5)	5.0 ± 1.49 (5)*	1	–
Chlorpromazine	49 ± 8 (11)**	0	1.17 ± 0.19 (6)	9.0 ± 3.1 (6)	0	–
,, withdrawn	–	–	1.33 ± 0.08 (6)*	5.6 ± 1.07 (6)*	0	–
Ethanol	45 ± 11 (9)**	0	1.07 ± 0.16 (6)	3.92 ± 1.85 (6)*	0	38 ± 14 (6)***
,, withdrawn	–	–	1.15 ± 0.43 (5)	1.93 ± 0.68 (5)	1	49 ± 8 (5)***
Morphine	75 ± 18 (8)**	0	1.03 ± 0.20 (6)	9.1 ± 0.67 (6)	0	–
,, withdrawn	–	–	1.37 ± 0.32 (6)**	8.7 ± 0.07 (6)	0	–

† One died. * $P < 0.01$; ** $P < 0.05$; *** $P < 0.001$. R.R., righting reflex.
Figures denote mean ± s.d. with number of observations in parenthesis.
Significance calculated using Student's *t* test, by comparison with control values. For details of drug administration etc. (see methods section).

Table 6 Body weight of rats during chronic drug administration

Drug	*No.*	*Weeks of drug administration* 0	1	2	3	4	5	6
Control	12	66 ± 3	99 ± 7	133 ± 8	153 ± 11	171 ± 13		
Amphetamine	12	63 ± 6	96 ± 10	123 ± 9*	141 ± 13**	152 ± 16*		
Methyprylon	12	64 ± 6	99 ± 8	127 ± 7	147 ± 6	166 ± 16		
Nitrazepam	12	63 ± 5	98 ± 3	131 ± 6	158 ± 7	179 ± 11		
Meprobamate	12	67 ± 5	101 ± 8	132 ± 12	142 ± 14***	166 ± 16		
Methaqualone/diphenhydramine (10 : 1)	12	66 ± 4	99 ± 6	128 ± 9	135 ± 14*	157 ± 15***		
Chlordiazepoxide	12	68 ± 3	104 ± 5	133 ± 6	155 ± 8	185 ± 12**		
Barbitone	12	64 ± 5	106 ± 6**	147 ± 8****	168 ± 11*	189 ± 22**		
Control	6	50 ± 4	88 ± 10	132 ± 9	155 ± 9	171 ± 9	191 ± 8	210 ± 10
Ethanol	12	51 ± 4	88 ± 7	120 ± 12	144 ± 12	156 ± 8*	165 ± 11****	176 ± 13****
Chlorpromazine	12	50 ± 3	85 ± 6	123 ± 7***	147 ± 9	151 ± 9*		
Diazepam	12	52 ± 4	95 ± 5	124 ± 7	149 ± 14	164 ± 10		
Morphine	12	51 ± 3	87 ± 10	124 ± 12	145 ± 15	159 ± 12		
Barbitone/bemegride	12	50 ± 3	94 ± 6	135 ± 10	165 ± 9	183 ± 14		

* $P < 0.01$; ** $P < 0.02$; *** $P < 0.05$; **** $P < 0.001$.
Figures denote means ± s.d.
Significance calculated using Student's *t* test, by comparison with control values.
For details of drug dosage and method of administration, see Table 4.

sensitivity of the CNS. Such factors as age, sex, strain, diet, the presence of stress and prior or concomitant drug administration have been reported to influence the duration of barbiturate-induced anaesthesia. Thus infant (five-day) rats have been reported to have a lower ability to metabolize hexobarbitone and an increased brain sensitivity to this drug compared with 44-day-old rats (Kalser, Forbes & Kunig, 1969). An increase in the activity of drug-metabolizing enzymes (oxidation of hexobarbitone) from weaning to early maturity has also been reported in mice (Lee, Hospador & Manthei, 1967). Mature male rats have a higher level of enzyme activity than female animals and this is correlated with shorter duration of action of drugs that are metabolized (Kato & Gillette, 1965; Jori, Pescador & Pugliatti, 1971). This difference has been attributed to the differing effect of male and female sex hormones on liver enzymes. Immature female rats do not metabolize drugs more rapidly than males (Furner, Gram & Stitzel, 1969) yet their sensitivity to barbiturates is greater than that of males (Moir, 1937). This is perhaps indicative of an increased drug sensitivity in female rats. Strain differences in drug-metabolizing enzyme activity have been reported with rabbits (Cram, Juchau & Fouts, 1965) and rats (Mitoma, Neubauer, Badger & Sorich, 1967; Jori *et al.*, 1971). Stress has been reported to stimulate metabolism of, and reduce the response to, hexobarbitone, pentobarbitone and meprobamate. However, stress did not apparently affect the sensitivity of the CNS since no effect was reported with compounds such as phenobarbitone or barbitone which are excreted largely in the unchanged form (Driever, Bousquet & Miya, 1966).

Since sleeping time values after intracerebroventricular pentobarbitone administration were found to be independent of the rate of drug metabolism, this method was employed in the present study to investigate the effect of age and sex on the sensitivity of the CNS to barbiturate. Rats in the intermediate weight range (235 g) were the least sensitive to barbiturate as indicated by sleeping time and brain barbiturate level on awakening. The youngest animals (88 g) were the most sensitive to barbiturate. The order of sensitivity was the same when sleeping time was determined after an intraperitoneal injection of pentobarbitone but part of this difference in response would be accounted for by the different activities of the liver drug-metabolizing enzymes. Female rats were more sensitive to barbiturate than male animals. Although drug-metabolizing enzyme activity was not determined in these particular animals, it is well known that female animals in this weight range also have a lower drug-metabolizing capacity and sleep for much longer after an intraperitoneal injection of barbiturate. The present results indicate that this increased sensitivity to barbiturate is also due in part to an increased sensitivity of the CNS to the drug.

However, the area of research which has received the most attention during the last decade has been the effect of drugs themselves on the effectiveness of subsequent drug action, and in particular on drug metabolism (Conney, 1967; Remmer, 1972). When the interval between administration of two drugs is short, a simple additive effect between drugs having similar pharmacological actions or antagonistic effect between drugs having opposite pharmacological action, is to be expected. Also, the biological half-life of drugs may be prolonged through competition for the same liver drug-metabolizing enzymes. However, when the interval is extended, or repeated administrations are given, interactions of a different type can occur. These are of two main kinds. First a drug may inhibit or more usually, induce hepatic drug-metabolizing capacity leading to an increased or decreased effect respectively of the subsequently administered drug. Secondly, a drug may alter the sensitivity of the CNS to that or other drugs. Such interactions are more likely to be found after repeated drug administration (Stevenson & Turnbull, 1969; Stevenson & Turnbull, 1970) but can be detected after only two drug administrations (Aston & Hibbeln, 1967). The usual result is a decrease in drug sensitivity, although an increase has been reported in some circumstances (Aston, 1966; Aston & Hibbeln, 1967).

Thus drug tolerance may be brought about by increased metabolic degradation, decreased sensitivity of the CNS or a combination of both these factors. With the opiates, chronic drug administration does not induce hepatic drug-metabolizing enzyme activity, the tolerance being due entirely to adaptive changes (Bousquet, Rupe & Miya, 1964; Adler, 1967). In the case of the barbiturates, enzyme induction is usually responsible for the observed tolerance, but after prolonged exposure to high drug concentrations a central cellular tolerance may also be a contributory factor. In such studies with the barbiturates it has been usual to test for tolerance by measuring the duration of anaesthesia produced by an intraperitoneal injection of a barbiturate. However, we have shown that even in rats which have developed physical dependence on barbiturate determination of the sleeping time following intraperitoneal injection of either barbitone or pentobarbitone provides no indication of the sensitivity of the brain to barbiturate (Stevenson & Turnbull, 1970). Only

when the brain barbiturate level on awakening or sleeping time following an intracerebroventricular injection of pentobarbitone was determined was the gradual development of CNS tolerance produced by chronic exposure to a barbiturate detected (Stevenson & Turnbull, 1970). The validity of the measurement of sleeping time following intracerebroventricular injection of pentobarbitone was indicated by the fact that the brain barbiturate level on awakening was the same after this route as after an intraperitoneal injection. The finding that the rate of disappearance of labelled pentobarbitone from the lateral ventricles was not affected by induction of hepatic drug-metabolizing processes indicated that this method would be particularly applicable in a situation where the rate of drug metabolism was likely to be altered (Stevenson & Turnbull, 1970). This finding has now been confirmed. Thus we have shown that the latent period, sleeping time and brain barbiturate level on awakening is not significantly affected by induction or inhibition of liver drug-metabolizing capacity. The disappearance of labelled barbiturate from the ventricles was also the same in control and rats whose drug-metabolizing enzyme capacity was inhibited.

Acute drug pretreatment produced the expected effect on both the latent period and the duration of anaesthesia. This method appeared to be sensitive in that small doses of CNS depressants or stimulants affected the parameters measured. For example, small doses of pentobarbitone (5 mg/kg) or amphetamine (1 mg/kg) resulted in an approximate doubling or halving respectively of the duration of anaesthesia. Opposite effects were produced on the latent period. Large doses of depressant drugs had an additive effect with the subsequently injected pentobarbitone which resulted in respiratory arrest. Large doses of stimulant drugs antagonized pentobarbitone to the extent that animals failed to lose their righting reflex. Such interactions must be at the level of the CNS, since, as discussed above, the intracerebroventricular sleeping time is unaffected by alteration in hepatic drug-metabolizing capacity. Morphine did not affect the sleeping time. This observation is of particular significance when considering the applicability of this method to the detection of altered drug sensitivity in animals treated chronically with drugs of dependence.

We have included in our results the body weights of animals to which drugs were administered chronically. A previous observation has been that rats given barbitone sodium chronically increase in body weight more rapidly than litter mate control animals (Crossland & Turnbull, 1972). This was again observed in the present experiment. The only other drug to increase body weight significantly was chlordiazepoxide. Administration of several drugs, namely amphetamine, methaqualone/diphenhydramine, ethanol and chlorpromazine resulted in animals gaining weight less rapidly than controls. Interestingly, rats given barbitone/bemegride mixture did not gain weight more rapidly than controls. However, although bemegride antagonized this particular effect of barbitone, rats so treated still exhibited a marked tolerance to pentobarbitone on withdrawal.

We have previously reported that animals withdrawn following chronic barbitone sodium administration exhibit a CNS tolerance to barbiturates (Stevenson & Turnbull, 1970). This finding has now been confirmed and the observation extended to other centrally active compounds. Rats withdrawn for 48 h following chronic administration of barbitone sodium, barbitone sodium/bemegride mixture, Mandrax, chlordiazepoxide, nitrazepam, chlorpromazine and ethanol all exhibited tolerance to intracerebroventricularly administered pentobarbitone i.e. a central nervous cross tolerance to pentobarbitone. In some instances, animals were so tolerant that they failed to lose their righting reflex after the injection. Except in the case of meprobamate and ethanol, this tolerance was not present before withdrawal. This may have been because chronic administration of meprobamate and ethanol is particularly liable to produce cross tolerance to barbiturate. Since these animals awakened with a significantly higher brain pentobarbitone level than control rats an alternative explanation, that the presence of either of these drugs facilitated the passage of barbiturate from the brain, seems unlikely. The development of such CNS tolerance to pentobarbitone suggests that dependence on these drugs has developed during their chronic administration and that the observed tolerance is a measure of the rebound withdrawal excitability.

No withdrawal-induced barbiturate tolerance was detected after chronic administration of amphetamine, methyprylon, diazepam or morphine. Although physical dependence and tolerance develop on the opiates, there is no evidence in the literature for the development of cross tolerance or cross dependence with the barbiturates. Similarly, there is no reason to expect withdrawal from amphetamine to produce tolerance to barbiturate. Indeed, in the present experiment an increased sensitivity to pentobarbitone was found, perhaps indicating a rebound depression of nervous excitability following chronic administration of this CNS stimulant drug.

Thus there is a reasonably good correlation between the occurrence of withdrawal excitability as indicated in the present experiments and the published results of other workers, for both animal

and man. Withdrawal phenomena or CNS tolerance have been reported to occur in laboratory animals after administration of barbiturates (Essig & Flanary, 1959; Ebert, Yim & Miya, 1964; Jaffe & Sharpless, 1965; Leonard, 1968), ethanol (Le Blanc, Kalant, Gibbins & Berman, 1969; Ratcliffe, 1972), meprobamate (Kato, 1961) and chlorpromazine (Boyd, 1960) and in man after withdrawal of chlordiazepoxide (Hollister, Motzenbecker & Degan, 1961), methyprylon (Berger, 1961), meprobamate (Essig, 1964), nitrazepam (Oswald & Priest, 1965) and diazepam (Isbell & Chruschiel, 1970). However, no evidence was found in our experiments for a withdrawal excitability following methyprylon or diazepam administration. It may have been, although it appears unlikely, that the dose administered in these cases was insufficient to allow the development of dependence. Alternatively, it may be that for some drugs there is a poor correlation between the results obtained in different animal tests. Thus chlorpromazine is not self administered by laboratory animals (Collier, 1972), although withdrawal phenomena have been observed (Boyd, 1960). Again there may be a poor correlation between the incidence of drug abuse on the one hand and certain indices of the development of dependence on the other. Oswald, Lewis, Dunleavy, Brezinova & Briggs (1971) have termed fenfluramine and imipramine 'drugs of dependence though not of abuse', and although in an earlier series of experiments Oswald found the effect and subsequent withdrawal of nitrazepam and amylobarbitone on sleep to be similar (Oswald & Priest, 1965), there seems little doubt that the risk of abuse is much less with nitrazepam than with the barbiturates (Bethune, Burrell, Culpan & Ogg, 1966; Glatt, 1968).

Other methods which have been adopted to detect and quantitate the hyperexcitability produced by withdrawal from CNS depressant drugs have been estimation of the threshold to chemically or electrically induced seizures (Jaffe & Sharpless, 1965; Leonard, 1968); determination of the amount of intravenously infused hexobarbitone required to produce a burst suppression of 1 s or more on the electroencephalogram (Wahlstrom, 1968); correlation of drug effect with serum or brain concentrations (Kato, 1967; Stevenson & Turnbull, 1970); a scoring of the abstinence symptoms (Yanagita & Takahashi, 1970) or observation of the occurrence of spontaneous (Essig & Flanary, 1959) or sound induced convulsions (Crossland & Leonard, 1963). We suggest that determination of the duration of anaesthesia following an intracerebroventricular injection of pentobarbitone is another method which could be usefully added to this list, being especially applicable when the drugs administered are thought to affect the activity of hepatic drug-metabolizing enzymes.

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