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DETERMINATION OF HALOTHANE-INDUCED SLEEPING TIME IN THE RAT: EFFECT OF PRIOR ADMINISTRATION OF CENTRALLY ACTIVE DRUGS

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1 A method is described for the determination of halothane-induced sleeping time in the rat.

2 The sleeping time exhibited a diurnal variation which was due, at least in part, to a change in the sensitivity of the central nervous system (CNS) to the anaesthetic.

3 Tolerance to halothane did not develop in rats repeatedly exposed to the anaesthetic over a period of over 48 hours.

4 Repeated sleeping time determinations have been used to follow changes in the sensitivity of the CNS to the anaesthetic occurring with time.

5 A tolerance to halothane was induced by pretreatment of rats with doses of amylobarbitone, pentobarbitone or meprobamate sufficient to keep animals anaesthetized for approximately 12 hours. This tolerance was followed by a period of halothane-hypersensitivity.

6 Halothane-tolerant animals awakened with higher brain halothane concentrations and were also tolerant to intracerebroventricularly administered pentobarbitone.

7 Halothane-hypersensitive rats awakened with lower brain halothane concentrations and were also hypersentive to intracerebroventricularly administered pentobarbitone.

8 The possibility that the induction of cross-tolerance to halothane may be indicative of a drug's potential to produce dependence is discussed.

Introduction

Tolerance and cross-tolerance to hypnotic and sedative drugs can be induced in laboratory animals by repeated drug exposure (for a recent review, see Kalant, Le Blanc & Gibbins, 1971). Not surprisingly, the barbiturates have been amongst the most widely studied drugs in this respect. However, in many instances the tolerance observed is almost certainly due to the more rapid metabolism and subsequent elimination of the drug(s) in question and comparatively few attempts have been made to assess the relative contribution to tolerance of increased rate of elimination (dispositional tolerance) and decreased sensitivity of the central nervous system (CNS) (cellular tolerance) (Kato, 1967; Wahlstrom, 1968; Stevenson & Turnbull, 1970; 1974; Ho, Yamamoto & Loh, 1975). In a recent paper, Stevenson & Turnbull (1974) reported that the sleeping time following intracerebroventricular (i.c.v.) administration of pentobarbitone gave a valid indication of the sensitivity of the CNS to barbiturate and that this measure was independent of the activity of hepatic drug-metabolizing enzymes. However, the preparation of animals for i.c.v. injections is time consuming and repeated sleeping time determinations are not possible without the use of cannulae. We have therefore assessed the usefulness of determination of the duration of halothane-induced anaesthesia as a measure of the sensitivity of the CNS to a depressant agent. The results presented in this paper indicate that this sleeping time is a reliable measure of the sensitivity of the brain which has the advantage that it can be determined at intervals of a few hours, thus enabling changes in sensitivity occurring with time to be easily measured. Furthermore, the sleeping time is not affected by stimulation or inhibition of drug-metabolizing enzyme activity.

We have also studied the effect on the sleeping time of pretreatment with very large doses of amylobarbitone, pentobarbitone or meprobamate, in the hope that prior administration of large doses of CNS depressant drugs might induce a cross-tolerance to halothane. It was considered that the induction of such a cross-tolerance might be indicative of those

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drugs which would be likely to induce changes in the sensitivity of the CNS if they were to be given chronically at a lower dose level. We have found that rats kept anaesthetized for approximately 12 h by repeated injections of amylobarbitone, pentobarbitone or meprobamate exhibited a tolerance to halothane the next day and that this tolerance was followed by an increased sensitivity to halothane. In some experiments only, two other measures of CNS excitability, the sleeping time following i.c.v. administered pentobarbitone and the susceptibility to bemegride-induced seizures, indicated similar changes in CNS sensitivity.

Some of these results have been communicated to the British Pharmacological Society (Turnbull & Watkins, 1975a and b)

Methods

Female Wistar rats, weighing approximately 200 g and purchased from A. Tuck and Son, Rayleigh, Essex, were used throughout. The rats were housed in groups of 4 under conditions of 12 h light (06 h 00 min–18 h 00 min) and 12 h darkness (18 h 00 min–06 h 00 min).

Determination of halothane-induced sleeping time

The gas flow from an anaesthetic apparatus, incorporating a Fluotec Mark 2 vaporizer, was passed through a perspex box (dimensions $35 \times 15 \times 10$ cm) in which up to six 200 g rats could be placed. Oxygen containing 5% v/v halothane was passed through the box at a flow rate of 2 litres min^{-1} for 3 min and the animals were left in the box for a further 5 min after turning off the gas flow. The animals were then removed from the box and placed on cotton wool. Animals were stimulated by picking them up and replacing them on the cotton wool at approximately 20 s intervals and the time at which the rats turned over onto all four legs was noted with a stop-watch for each animal. This degree of exposure to halothane had been found to be optimal; decreasing the anaesthetic concentration, flow rate or total length of exposure markedly shortened the sleeping time, whilst increasing the flow rate or length of exposure only slightly prolonged the sleeping time.

Diurnal variation in halothane sensitivity and effect of repeated sleeping time estimations

As it was our intention to assess changes in the sensitivity of the CNS occurring with time by repeatedly determining the sleeping time at relatively short intervals, it was considered essential to demonstrate that repeated exposure to halothane itself did not influence the results. Accordingly, the sleeping time of a group of rats was determined at intervals of 4–5 h over a period of 48 hours. During the second 24 h period these rats were compared with a second group of previously untreated animals.

Effect of induction or inhibition of hepatic drug-metabolizing enzyme activity

Sleeping times were determined on groups of animals which had been pretreated with either phenobarbitone (10 mg kg^{-1} day^{-1} i.p. for 4 days; induced) or SKF 525A (2-diethylamino-ethyl-2, 2-dipehylvalerate hydrochloride) (15 mg kg^{-1} i.p. 40 min before exposure to halothane; inhibited). These pretreatments are known to cause significant stimulation and inhibition respectively of hepatic drug-metabolizing enzyme capacity.

Effect of prior drug administration on the sleeping time

Single dose. To ascertain whether prior injection of single doses of CNS stimulant or depressant drugs would affect the sleeping time, rats were pretreated with intraperitoneal injections of the following drugs: amphetamine sulphate (1 mg kg^{-1} for 10 min); pentobarbitone sodium (5 mg kg^{-1} for 10 min) and nitrazepam (1 mg kg^{-1} for 15 minutes). In addition, with pentobarbitone sodium (30 mg kg^{-1}) only, repeated sleeping time determinations were made after a single anaesthetic dose. Halothane sleeping time determinations were begun 4 h after injection (approximately 2 h after gaining the righting reflex) and were continued until three successive measurements indicated a return to control values.

Repeated drug administration. Rats were kept anaesthetized for approximately 12 h by repeated intraperitoneal injection of the following drugs: amylobarbitone sodium (225 mg kg^{-1}), pentobarbitone sodium (90 mg kg^{-1}) or meprobamate (800 mg kg^{-1}). The doses given in parentheses are the total amounts which were administered over a 10 h period (10 h^{-1}). The last injection was given 10 h after the first injection and the rats were then allowed to recover from the anaesthetic. Animals recovered their righting reflex approximately 2 h after this last injection.

Typical dose regimes were as follows: amylobarbitone sodium 75 mg kg^{-1} at zero time followed by 37.5 mg kg^{-1} at +3 h, +5.5 h, +8 h and +10 h; pentobarbitone sodium 30 mg kg^{-1} at zero time followed by 20 mg mkg^{-1} at +2 h, +5 h and +10 h; and for meprobamate 300 mg kg^{-1} at zero time followed by 20 mg kg^{-1} at +2 h, +5 h and +10 h; 100 mg kg^{-1} at +10 hours. Preliminary investigation had shown this method of drug administration to be preferable to, and to result in a higher success rate than, maintenance of anaesthesia by constant in-

traperitoneal drug infusion. Control rats received repeated injections of 0.9% w/v NaCl solution (saline). The halothane sleeping time was first determined 4 h after the last drug injection and at intervals thereafter until three successive values indicated a return to normal values.

Because of the interesting nature of the changes in sensitivity of the CNS which were indicated by our results, the following additional measurements were made in some instances only: (1) brain halothane concentration on awakening; (2) sleeping time following i.c.v. injection of pentobarbitone, and (3) bemegride-induced seizure threshold. With pentobarbitone only, the rate at which the drug was excreted was determined by measurement of brain and liver pentobarbitone and pentobarbitone metabolite concentrations in groups of animals killed at intervals following repeated intraperitoneal injections of [^{14}C]-pentobarbitone administered in exactly the same way (90 mg kg^{-1} 10 h^{-1}) as in the experiment described above.

Determination of brain halothane concentration

Rats were killed by cervical dislocation on awakening after exposure to halothane and the brain rapidly removed (<1 min) and placed in a previously weighed screwcapped MSE homogenizer bottle (25 ml) containing 2 ml *n*-heptane. The container was reweighed and the brain weight obtained by difference. Further *n*-heptane was then added to give 2 ml g^{-1} tissue and the brain was homogenized for 30 s at full speed with an MSE homogenizer. The homogenates were spun at 5,000 rev/min for 5 min in an MSE minor centrifuge and the superantant solution was then decanted and stored at −20°C in a stoppered tube until required for assay. On each occasion, standards were prepared by homogenization of brain taken from saline pretreated rats with known amounts of halothane in *n*-heptane (2 ml heptane g^{-1} brain tissue). The final halothane concentrations of these standards was between 0.0184 mg ml^{-1} and 0.1472 mg ml^{-1}. All assays were performed within 12 days of preparation of the homogenates, during which time there had been a mean loss of 17.6 ± 2.0% halothane ($n=5$) compared with freshly prepared homogenates. The halothane was assayed by gas chromatography using a PYE series 104 chromatograph with flame ionization detector. Samples (10 µl) were injected onto a 1.5 m column packed with Poropak Q and maintained at a temperature of 170°C. The carrier gas was argon at a flow rate of 60 ml min^{-1}; hydrogen and air flow rates were 60 ml min^{-1} and 200 ml min^{-1} respectively. Halothane concentrations were determined from a calibration curve prepared using the standard supernatant solutions.

Determination of brain and liver [^{14}C]-pentobarbitone concentration

Animals were killed by stunning and subsequent cervical dislocation. The brain and liver were removed, weighed and each tissue was homogenized in 7 ml pH 5.0 phosphate buffer. [^{14}C]-pentobarbitone was extracted by shaking for 30 min with 30 ml petroleum ether (40–60) 98.5%/iso-amyl alcohol 1.5%. After centrifugation at 2,000 rev/min for 10 min (MSE major), 10 ml of the petroleum ether layer was removed for liquid scintillation counting. The remaining petroleum ether layer was aspirated and the aqueous layer was re-extracted with a further 30 ml of petroleum ether/iso-amyl alcohol. After re-centrifugation, the organic layer was aspirated, the surface of the lower (buffer) layer washed twice with 10 ml petroleum ether/iso-amyl alcohol and [^{14}C]-pentobarbitone metabolites were extracted from the remaining aqueous layer by shaking with 30 ml ethyl acetate for 30 minutes. After centrifugation, 10 ml of the ethyl acetate layer was removed for liquid scintillation counting. Standard amounts of [^{14}C]-pentobarbitone were taken through the same extraction procedure. Pentobarbitone metabolite concentrations were determined as the corresponding amounts of unchanged pentobarbitone.

Determination of sleeping time after i.c.v. injection of pentobarbitone

Sleeping time determinations were performed on pentobarbitone and meprobamate-treated rats. Animals were lightly anaesthetized with ether, the surface of the skull was exposed and a small burr hole was made in the skull at a point 2.5 mm lateral and 1.0 mm posterior to the bregma. The rats were allowed to recover for 10 min and 800 µg sodium pentobarbitone in 30 µl water was injected into the left lateral ventricle. Injection was carried out with a 50 µl Hamilton syringe connected to a size 25 g × $\frac{5}{8}$ needle fitted with a polythene tubing 'spacer' allowing injection at a depth of 4 mm below the surface of the skull. The times at which animals lost and regained their righting reflex were recorded with a stopwatch.

Determination of bemegride-induced seizure threshold

The bemegride seizure threshold was determined in groups of pentobarbitone-treated rats. The CD_{50} for bemegride was calculated by treating groups of 6 rats at 4 dose levels according to the method of Weil (1952). Animals failing to convulse within 5 min of intraperitoneal injection were scored as negative. The results were calculated as the mean with 95% confidence limits.

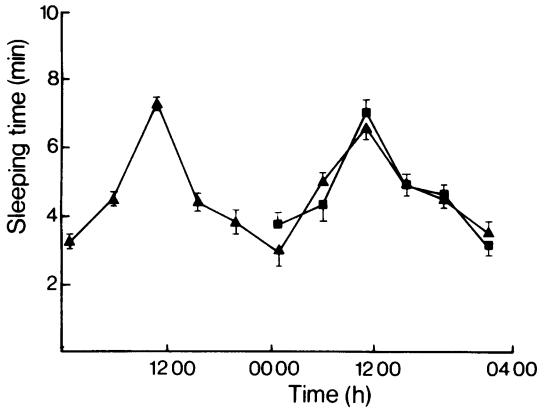

Figure 1 Diurnal variation in, and effect of repeated exposure to halothane on, halothane-induced sleeping time. Each point represents the mean of 6 observations. Vertical lines show s.e. mean. (▲) Group 1, exposed repeatedly for 50 h; (■) Group 2, exposed only on day 2.

Table 1 Diurnal variation in sensitivity to halothane

Time	*Sleeping time (min)*	*Brain halothane conc. on awakening ($\mu g\ g^{-1}$)*
01 h 00 min	2.50 ± 0.14 (6)	167 ± 7 (6)
11 h 00 min	4.45 ± 0.10 (6)	126 ± 11 (6)
	$P < 0.001$	$P < 0.01$

Figures represent mean ± s.e. mean with number of observations in parentheses.

Results

Under the conditions adopted, there was only a small intra- and inter-group variation in sleeping time on any one occasion. The end-point, i.e., awakening from the anaesthetic, was very sharp. Animals passed from the flacid immobile state to one of considerable locomotor activity within a few seconds. However a marked diurnal variation in sleeping time was observed (Figure 1). There was a considerable decrease in sensitivity to halothane during the night (dark phase) compared with during the day (light phase) and this diurnal variation in sleeping time was due, at least in part, to an altered sensitivity of the CNS to the anaesthetic. This was indicated by the observation that the brain halothane concentration found in rats awakening from the anaesthetic at 11 h 00 min was lower than the concentration found in animals awakening at 01 h 00 min (Table 1). Repeated sleeping time determinations on the same group of animals did not induce a tolerance, since the sleeping time on the eleventh exposure to halothane within a 48 h period was not significantly different from the sleeping time on the first occasion (Figure 1). Furthermore, the sensitivity of the second group of rats exposed only during the second 24 h period was the same as that of animals repeatedly exposed over the whole 48 h period (Figure 1). In a separate experiment, brain halothane concentrations found on awakening confirmed that repeated exposure to halothane did not affect the sensitivity of the CNS to the anaesthetic (Table 2). Thus, animals exposed on the twelfth occasion during a 50 h cycle awakened with the same brain halothane concentration as rats exposed only twice during the same period.

To determine whether pretreatment with small doses of CNS stimulant or depressant drugs would affect the sleeping time, rats were injected with amphetamine, pentobarbitone or nitrazepam (Table 3). Amphetamine pretreatment shortened and pentobarbitone and nitrazepam pretreatment lengthened the sleeping time (Table 3). With pentobarbitone only, the sleeping time was determined at

Table 2 Effect of repeated halothane inhalation on halothane-induced sleeping time and brain halothane concentration on awakening

	Number of exposures to halothane	*Time (h)*	*Sleeping time (min)*	*Brain halothane conc. on awakening ($\mu g\ g^{-1}$)*
Group 1	1	0	4.35 ± 0.13 (6)	—
Group 2	1	0	4.20 ± 0.15 (6)	—
Group 1	2	50	5.46 ± 0.22 (6)	120 ± 6 (6)
Group 2	12	50	5.81 ± 0.20 (6)	111 ± 10 (6)

Figures represent mean ± s.e.mean with number of observations in parentheses.

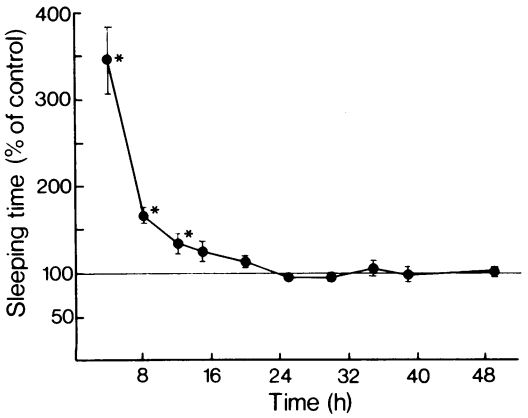

Figure 2 Halothane-induced sleeping time at intervals following a single intraperitoneal injection of pentobarbitone sodium (30 mg kg^{-1}). Each point represents the mean of 6 animals in comparison with 6 saline pretreated rats. Vertical lines show s.e. mean. The asterisks denote $P < 0.05$ in comparison with control animals (Student's t test).

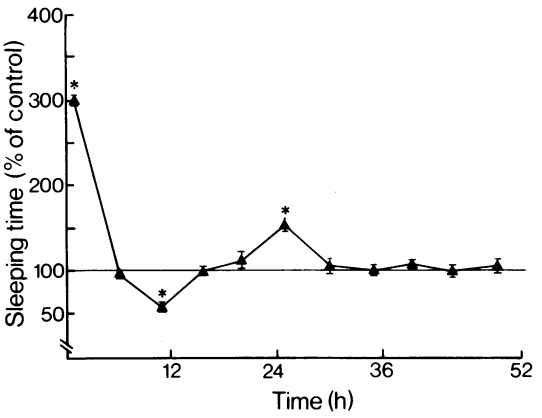

Figure 3 Halothane-induced sleeping time at intervals after repeated injections of amylobarbitone sodium (225 mg kg^{-1} 10 h^{-1}). Each point represents the mean of 6 animals in comparison with 6 saline pretreated rats. Vertical lines show s.e. mean. Zero time on the horizontal axis is 4 h after the last injection of amylobarbitone. Asterisks denote $P < 0.05$ in comparison with control animals (Student's t test).

intervals after recovery from a single intraperitoneal injection of an anaesthetic dose (30 mg/kg) in order to follow the time course of effect on halothane sleeping time (Figure 2). (In Figures 2, 3, and 4 the sleeping times are expressed as a percentage of control values, thus eliminating the diurnal variation and facilitating the comparison between drug pretreated and control animals. Each point on the graphs represents a comparison between drug and saline pretreated rats which had been exposed to halothane on the same occasion.) Our results show that the sleeping time was initially prolonged and that it gradually decreased to reach control values after 24 hours.

The sensitivity to halothane was not affected by the stimulation of hepatic drug-metabolizing enzyme activity produced by pretreatment of rats for four days with phenobarbitone or by the inhibition of enzyme activity produced by a single intraperitoneal injection of SKF 525A (Table 3).

The effect of repeated drug administration was determined on groups of rats which had been kept anaesthetized for approximately 12 h by repeated injection of amylobarbitone, pentobarbitone or meprobamate. The effect on halothane sleeping time was similar with all three drugs (Figures 3 and 4). The first sleeping time, determined 4 h after the last

Table 3 Effect of drug pretreatment on halothane-induced sleeping time

Drug	*Dose*	*Duration of pretreatment*	*Sleeping time (min)*
Control			6.9 ± 0.2 (4)
Amphetamine sulphate	1 mg kg^{-1}	10 min	2.6 ± 0.2 (3)*
Pentobarbitone sodium	5 mg kg^{-1}	5 min	9.2 ± 0.1 (3)*
Nitrazepam	1 mg kg^{-1}	15 min	10.8 ± 1.2 (3)*
Control			5.3 ± 0.1 (5)
Phenobarbitone sodium	10 mg kg^{-1} day^{-1}	4 days	5.4 ± 0.2 (4)
S.K.F. 525A	15 mg kg^{-1}	40 min	5.7 ± 0.3 (4)

Figures represent mean ± s.e. mean with number of observations in parentheses.
* $P < 0.05$ compared with control values (Student's t test).

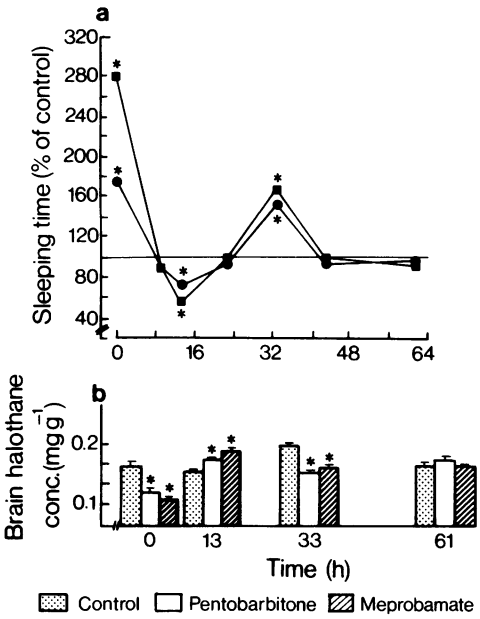

Figure 4 (a) Halothane-induced sleeping time at intervals after repeated intraperitoneal injections of pentobarbitone sodium (●) (90 mg kg^{-1} 10 h^{-1}) and meprobamate (■) (800 mg kg^{-1} 10 h^{-1}). Each point represents the mean of 6 animals in comparison with 6 saline pretreated rats. Zero time on the horizontal axis is 4 h after the last injection of hypnotic. Asterisks denote $P < 0.05$ in comparison with control animals (Student's t test). (b) Brain halothane concentration found on awakening at various times after exposure to pentobarbitone or meprobamate. Each histogram represents the mean ± s.e. mean of 6 observations. Zero time on the horizontal axis is 4 h after the last injection of hypnotic. Asterisks denote $P < 0.05$ in comparison with control animals (Student's t test).

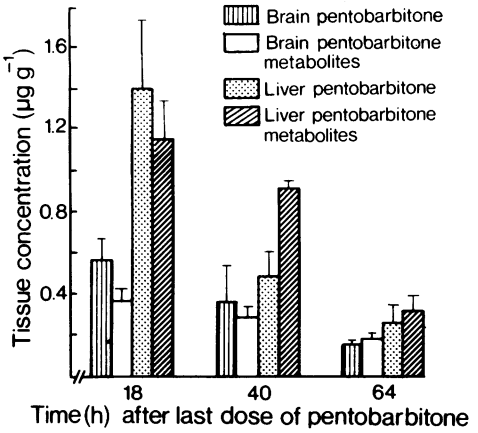

Figure 5 Brain and liver pentobarbitone and pentobarbitone metabolite concentrations following repeated intraperitoneal injections of [^{14}C]-pentobarbitone sodium (90 mg kg^{-1} 10 h^{-1}). Each histogram represents the mean ± s.e. mean of at least 4 observations.

injection of depressant, was significantly prolonged compared with that in saline pretreated rats. However, the response then rapidly fell to below control values, this tolerance lasting approximately 8 h in the case of amylobarbitone (Figure 3) and 16 h in the cases of pentobarbitone and meprobamate (Figure 4a). With all three drugs the tolerance was followed by a hypersensitivity to halothane which was maximal approximately 28 h after the last injection of amylobarbitone (Figure 3) and 36 h after pentobarbitone or meprobamate (Figure 4a). Thereafter the sensitivity returned to normal. This pattern of changing sensitivity to halothane, a tolerance followed by a hypersensitivity, was in marked contrast to the gradual return to normal found after a single injection of pentobarbitone (Figure 2). In order to correlate the sleeping times with brain halothane concentrations on wakening, groups of rats were killed on awakening at various times after pretreatment with either pentobarbitone or meprobamate. The brain halothane concentrations on awakening are shown in Figure 4b. Initially, when the sleeping time was prolonged, pretreated animals awakened with significantly lower brain halothane concentrations. At the times of halothane tolerance

Table 4 Sleeping time to i.c.v. administered pentobarbitone in pentobarbitone and meprobamate-treated rats at times of tolerance and hypersensitivity to halothane

	Control	*Pentobarbitone (90 mg kg^{-1} 10 h^{-1})*	*Meprobamate (800 mg kg^{-1} 10 h^{-1})*
At time of halothane tolerance	5.5 ± 0.3 (6)	3.2 ± 0.6 (4) $P < 0.02$	3.2 ± 0.5 (6) $P < 0.01$
At time of halothane hypersensitivity	4.6 ± 0.5 (8)	6.8 ± 0.5 (8) $P < 0.01$	6.2 ± 0.1 (8) $P < 0.01$

Figures represent mean ± s.e. mean with number of observations in parentheses.

and hypersensitivity, the brain halothane concentrations found on awakening were significantly higher and lower respectively compared with saline pretreated rats. There was no difference between the brain halothane concentrations on awakening when sleeping times had returned to normal. With pentobarbitone only, brain and liver pentobarbitone and pentobarbitone metabolite concentrations were determined at intervals after repeated intraperitoneal injection of [^{14}C]-pentobarbitone (90 mg kg^{-1} 10 h^{-1}). Brain pentobarbitone levels at the time of halothane tolerance (18 h after last dose of pentobarbitone) and hypersensitivity (40 h after last dose of pentobarbitone) were 0.55 μg g^{-1} and 0.35 μg g^{-1} respectively (Figure 5). The liver pentobarbitone concentrations were higher than those found in brain but the overall low levels of unchanged drug and metabolites (Figure 5) indicated that most of the drug had been eliminated even by the time of the first measurements.

The same pattern of results as was indicated by the halothane sleeping time determinations, an increase followed by a decrease in CNS excitability, was also found when the sleeping times following i.c.v. administered pentobarbitone and sensitivity to bemegride-induced seizures were considered. At the time of halothane tolerance, pentobarbitone and meprobamate pretreated animals were also tolerant to i.c.v. administered pentobarbitone (Table 4) and pentobarbitone-treated rats tended ($0.1 < P < 0.2$) to be more sensitive to intraperitoneally administered bemegride (control 15.2 (13.6–16.9); treated 12.2 (9.1–16.5) mean mg kg^{-1} (95% confidence limits)). In contrast, a decreased excitability of the CNS was indicated at the time of halothane hypersensitivity since pentobarbitone and meprobamate pretreated rats were more sensitive to i.c.v. administered pentobarbitone (Table 4) and the threshold for bemegride-induced seizures tended to be higher ($0.05 < P < 0.1$) in pentobarbitone-treated animals (control 13.8 (12.2–15.6); treated 16.3 (14.3–18.5) mean mg kg^{-1} (95% confidence limits)).

Discussion

Our objectives in this study were as follows: firstly, to determine whether halothane-induced sleeping time could be used as a valid index of the sensitivity of the CNS to a depressant drug; secondly, to determine whether repeated sleeping time estimations on the same group of animals could be reliably used to follow the changes in sensitivity to halothane occurring with time, and thirdly, to ascertain whether a cross-tolerance to halothane would be induced by pretreatment of animals with other CNS depressant drugs, and if so, to determine the time course of such changes.

We have found that the halothane-induced sleeping time shows only a small inter- and intra-group variation when determined at any particular time but that there is a pronounced diurnal variation in sensitivity to the anaesthetic. A circadian variation in anaesthetic effectiveness is well documented (Moore Ede, 1973), and in the present experiments we have demonstrated that this is due, at least in part, to a variation in the sensitivity of the CNS to halothane. The diurnal variation in hepatic drug-metabolizing enzyme activity (Radzialowski & Bousquet, 1968), which is largely responsible for the variation in sensitivity to non-gaseous anaesthetics and hypnotic agents, does not appear to be involved in the change in sensitivity to halothane since we have found that inhibition or induction of drug-metabolizing enzyme activity does not affect the halothane-induced sleeping time. However, changes in absorption, distribution and excretion of the anaesthetic could also be involved.

Repeated determination of the sleeping time on the same group of rats over a period of 48 h did not induce a tolerance to halothane. This was indicated by the finding that after repeated exposure to anaesthetic the sleeping time and brain halothane concentration on awakening were the same as those found in control rats. Nevertheless, in all further experiments, control rats which had been exposed to halothane on each and every occasion were always included.

Thus, having established that the sleeping time was directly related to the sensitivity of the CNS to the anaesthetic, and was unaffected by alteration of drug-metabolizing capacity or by prior repeated exposure to the anaesthetic, we considered that such sleeping time determinations could be reliably used to study the changes in CNS sensitivity produced by various drug pretreatment regimes.

Anaesthetization of rats for approximately 12 h by repeated intraperitoneal injections of pentobarbitone, amylobarbitone or meprobamate induced a cross-tolerance to halothane and with all three drugs the tolerance was followed by a rebound hypersensitivity to the anaesthetic. That the halothane tolerance represented a true cellular tolerance induced by the prior exposure to the hypnotic drugs was indicated by the fact that such animals awakened with higher brain halothane concentrations, were also tolerant to i.c.v. administered pentobarbitone and, in the case of pentobarbitone, tended to be more sensitive to bemegride. Thus the 12 h period of anaesthesia appeared to have induced a withdrawal hyperexcitability of the CNS. No such hyperexcitability was produced by the 2 h period of anaesthesia induced by the single injection of pentobarbitone.

With the same methodology, the period of halothane hypersensitivity was found to coincide with a period of decreased CNS excitability since such animals were more sensitive to i.c.v. administered pentobarbitone and, with pentobarbitone pre-

treatment, were less sensitive to bemegride. The possibility that the post-tolerance hypersensitivity could have been due to an additive effect of halothane and residual hypnotic drug was shown to be extremely unlikely, since it was found in a separate experiment that the brain pentobarbitone concentration at the time of hypersensitivity was only 0.55 $\mu g\ g^{-1}$. As animals normally awaken from pentobarbitone anaesthesia with brain concentrations of approximately 20 $\mu g\ g^{-1}$ (Stevenson & Turnbull, 1970) it would appear unlikely that the additional 0.55 $\mu g\ g^{-1}$ could cause a significant prolongation of halothane sleeping time.

We have also considered the possibility that the observed changes in sleeping time could have been due to a shift in the circadian rhythm of halothane sensitivity. However, the following (unpublished) findings suggest that this is not the case. First, we have found the same pattern of change in sleeping time, a tolerance followed by a hypersensitivity, regardless of whether the drug pretreatment is given during the light or dark phase; secondly, although at first disrupted by drug pretreatment, the circadian rhythm of locomotor activity returns to normal well before the return of normal halothane sensitivity; thirdly, not all hypnotics and tranquillizers induce a tolerance to halothane when administered in doses which cause a loss of righting reflex for the same time as used in the present experiments; and finally, quantitative differences in the magnitude of the tolerance can be obtained by pretreatment with various doses of pentobarbitone or by pretreatment on successive days.

As far as we are aware, this is the first report of a post-tolerance hypersensitivity of this type. Although Aston and co-workers have reported a hypersensitivity to barbiturate (Aston, 1966; 1973), the hypersensitivity in their experiments occurred at a much later stage after barbiturate administration. We have also reported on a barbiturate hypersensitivity which occurred some 3 weeks after stopping barbitone administration (Stevenson & Turnbull, 1968) but showed that a decreased drug-metabolizing capacity was the most likely explanation in that particular instance. In the present experiments our results clearly indicate that the prolonged halothane sleeping time is due to an increased sensitivity of the CNS to depressant drugs. The two most likely reasons why such a hypersensitivity has not been previously found are that the sensitivity of the CNS has been too infrequently assessed and also, that in most tolerance studies the methods used to measure changes in drug sensitivity are so affected by alteration of drug-metabolizing capacity that the more subtle changes occurring in the CNS tend to be masked.

It remains to be seen whether the induction of cross-tolerance to halothane by acute pretreatment with large doses of an hypnotic is indicative that such a drug would cause CNS changes, e.g. those responsible for the production of dependence, if it were to be given chronically and at a lower dose level. This possibility is currently being investigated. It is more than likely that the mechanisms responsible for the production of the cross-tolerance to halothane seen in the present acute experiments are the same as those which are responsible for the withdrawal phenomena seen in chronically pretreated animals. If this were to be the case, then the methodology that we have described above could be used to screen compounds for their dependence liability.

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