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BARBITONE-INDUCED TOLERANCE TO THE EFFECTS OF SEDATIVE-HYPNOTICS AND RELATED COMPOUNDS ON OPERANT BEHAVIOUR IN THE RAT

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1 Pretreatment doses of barbitone, pentobarbitone, ethanol, and phenytoin (diphenylhydantoin) in non-tolerant rats produced increases in operant responding at low doses and at higher doses resulted in decreases in responding.

2 Daily barbitone injections (100 mg/kg, i.p.) resulted in the development of functional tolerance to both the stimulant and depressant effects of barbitone on responding.

3 Barbitone tolerance development did not result in any change in the brain or plasma pharmacokinetics of barbitone.

4 Barbitone-tolerant rats were cross-tolerant to the behavioural effects of pentobarbitone, ethanol, and phenytoin. The dose-effect curves for all of these drugs were shifted to the right in tolerant rats, compared to non-tolerant rats.

5 Comparison of the brain and plasma levels of these drugs in non-tolerant and tolerant rats provided a means of separating functional cross-tolerance from dispositional cross-tolerance. Barbitone-tolerant rats appeared to be functionally cross-tolerant to ethanol in that there was no change in the brain and blood ethanol levels at times when the degree of behavioural impairment was substantially reduced. In contrast to ethanol, cross-tolerance to phenytoin appeared to be due to a decrease in the brain and plasma levels (dispositional tolerance). Cross-tolerance to pentobarbitone appeared to be comprised of both functional and dispositional cross-tolerance.

6 The usefulness of a multidisciplinary approach in the analysis of sedative hypnotic tolerance and cross-tolerance is discussed. It is concluded that without the concurrent determination of both brain and plasma drug levels it would not be possible to distinguish between functional and dispositional tolerance.

Introduction

The chronic administration of barbiturates, ethanol and related drugs results in the development of tolerance to their behavioural effect (Ebert, Yim & Miya, 1964; Yanagita & Takahashi, 1970). Kalant, LeBlanc, & Gibbins (1971) pointed out that tolerance to sedative hypnotics can be the result of a decrease in drug sensitivity of the CNS ('functional tolerance'), an adaptive change in drug disposition ('dispositional tolerance'), or a combination of the two. Since barbiturates are effective inducers of the liver microsomal drug metabolizing enzymes (Remmer, 1972) it is important to determine blood and brain drug levels in order to distinguish between the functional and dispositional forms of sedative-hypnotic tolerance.

The present study was carried out to show the effects of chronic barbitone injections on sensitivity to the behavioural effects of barbitone, pentobarbitone, phenytoin (diphenylhydantoin) and ethanol. Operant behaviour has been used to describe the development of tolerance to narcotic analgesics (Heifetz & McMillan, 1971), psychomotor stimulants (Schuster, Dockens & Woods, 1966) and Δ^9-tetrahydrocannabinol (Black, Woods, & Domino, 1970; McMillan, 1977). To date, there have not been any studies describing barbiturate-induced tolerance to the effects of sedative-hypnotics on operant behaviour. Disruption of schedule-controlled (operant) behaviour was selected as the behavioural index of the drug effect

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because it is a relatively stable and sensitive preparation for assessment of the progressive changes in drug sensitivity during and after tolerance development. In order to distinguish between the two forms of tolerance and cross-tolerance (functional and dispositional) blood and brain drug levels were determined in parallel treated groups of rats.

Methods

Male Sprague-Dawley rats (Flow Labs, Dublin, Va.) were used. The behavioural studies were conducted with 12 naive rats weighing between 335 and 430 g when allowed free access to food and water. Prior to training, the animals were deprived of food until they reached a level of 80% of their *ad libitum* feeding weights. Animals were housed in individual cages and had free access to water throughout the day, except for the 1 h during the day when experiments were conducted.

The biochemical studies were conducted with rats weighing between 200 and 270 g when allowed free access to food and water. These rats deprived of food as in the behavioural studies; they were housed in groups of six rats per cage. All rats were housed in a central rodent facility (12 h light/dark cycle, 23°C).

Apparatus and procedure for operant studies

Rats were trained to press a lever for 5 s access to a 0.1 ml dipper filled with sweetened milk by reinforcing successive approximations of the lever press response. Only responding on the left lever in the two-lever operant chamber (Coulburn Instruments, No. E10-10) resulted in the presentation of the milk-filled dipper. Responses on the right lever were recorded but had no programmed consequences. After one or two days of reliable responding under a continuous reinforcement schedule for 30 min per day, the schedule was changed to a 15 s Variable Interval (VI 15 s) schedule. Under the VI 15 s schedule the milk dipper was presented contingent on the first response after a variable time interval since the last reinforcement (average interval value = 15 s). The duration of each daily session was 30 min. The operant chamber was illuminated by a 6 W bulb and the chamber was located in a ventilated cubicle to minimize the effects of extraneous light and sound. Schedule contingencies and data recording were controlled by electromechanical and solid-state equipment located in the same room. Experimental sessions were conducted five days a week (Monday-Friday) before daily barbitone injections and every day during daily barbitone injections. Experiments were conducted in the morning between 08 h 30 min and 12 h 00 min.

Drug injection procedure

Test doses of barbitone, pentobarbitone, ethanol and phenytoin were administered (i.p.) 30 min before the start of the experimental session. After injection, animals were returned to their home cage for 30 min and then transferred to the operant chamber. Test doses were administered once each week (Thursday) and 0.9% w/v NaCl solution (saline) was injected on the day before (Wednesday) injection of the test drug. After dose-effect curves had been determined for the four drugs, daily barbitone injections were instituted. Barbitone 100 mg/kg was injected (i.p.) daily 30 min before the start of the session. After 30 days of daily barbitone injections, the dose-effect curves were redetermined for barbitone, pentobarbitone, ethanol and phenytoin. On the day before testing, saline was substituted for the regularly scheduled barbitone injection to reduce the effects of cumulated barbitone doses. Thus, the animals received test doses 48 h after the last 100 mg/kg barbitone injection.

Determination of barbiturate and phenytoin levels in plasma and brain

On the day of the experiment rats were injected (i.p.) with drug and killed by decapitation at various times after drug administration. Blood was collected into heparinized tubes, and centrifuged at 3000 rev/min for 20 min, and the plasma removed and frozen. Brains were dissected, and excess blood removed by rinsing in saline. The brains were blotted dry, weighed and then frozen until extraction was performed.

In chronic drug administration studies, barbitone (100 mg/kg) was administered once daily for 21 days. Forty-eight h after the last 100 mg/kg barbitone dose, the test drugs were administered (i.p.) and the animals killed at various times after injection as described above.

Drug levels were determined from plasma by a modification of the gas-liquid chromatography (g.l.c.) method of Kupferberg (1970). Plasma samples (1 ml) were acidified with 0.25 N HCl after 50 μg of internal standard was added to the plasma. For barbitone determinations the internal standard was pentobarbitone (P). For phenytoin and pentobarbitone determinations, the internal standards (5-(*p*-methylphenyl)-5-phenylhydantoin (MPPH) or phenobarbitone (Pb), respectively), were dissolved in chloroform and used in the first extraction. After acidifying, the drugs were extracted in 6.0 ml chloroform and after 15 min of shaking, the organic and aqueous layers were separated by means of Whatman PS phase separating paper. The chloroform was evaporated at 50°C in a vacuum evaporator. The resulting residue was resuspended in 2.0 ml methanol and 2.0 ml 0.25 N HCl. This solution was extracted twice with 6.0

ml hexane and then with 6.0 ml chloroform. The final aqueous layer was aspirated and the remaining chloroform was again evaporated. This second residue was resuspended in 50 µl of 0.2 M trimethylphenyl-ammonium hydroxide (6.94 g of silver oxide and 10.52 g of trimethylphenyl-ammonium iodide into 200 ml absolute methanol). Two µl of the sample was injected onto a 5% OV-17 g.l.c. column (6 ft × 2 mm, glass) and flame ionization determination was performed with a Hewlett-Packard 5730 chromatograph. For phenytoin determination g.l.c. conditions were: injection port 250°C, over 215°C isothermal; detector 300°C; air flow-40 ml/min, H_2 flow-20 ml/min, N_2 flow-40 ml/min. Conditions for barbitone and pentobarbitone were: injection port 200°C; oven 170°C, isothermal detector 250°C, air flow-40 ml/min, H_2 flow-20 ml/min, N_2 flow-20 ml/min. The concentrations of drug in the samples were determined by the ratio of the area of the drug peak over the area of the internal standard peak, as compared to a standard curve.

Brain levels of drugs were determined by a modification of the technique of Sherwin, Eisen & Sokolowski, 1973. After brains were dissected and weighed they were homogenized in 3 ml deionized, distilled water with a polytron (Brinkman PCU-2; setting 6 for 8 to 10 s); 1 ml of this homogenate was placed in a clean tube containing 50 µg internal standard (either pentobarbitone, phenobarbitone, or MPPH). The homogenate was acidified with 0.2 ml of 1 N HCl and then extracted with 6.0 ml ethylene dichloride. After shaking gently for 2 min the organic layer was separated from the aqueous by means of Whatman PS phase separating paper. Four ml of 0.5 N NaOH was added to the organic layer and the solution shaken for 2 min. The aqueous layer was removed, reacidified with 0.4 ml 5 N HCl and drugs re-extracted into 6 ml ethylene dichloride. The organic phase was again separated by means of phase-separating paper, and the ethylene dichloride evaporated at 50°C in a vacuum evaporator. The resulting residue was extracted and run on the g.l.c. as described above for plasma samples after the first residue was obtained.

Determination of ethanol concentrations

Plasma was analyzed without further preparation.

Rat brains were rapidly removed from the skull following decapitation. Brains were immediately homogenized in 2 ml of ice-cold deionized water. The homogenate was transferred to a 5 ml volumetric flask and the homogenizer washed with another 2 ml of cold water which was combined with the homogenate. The combined homogenate and wash was made up to 5 ml with cold water.

Ethanol samples were analyzed by an automated head space analysis and a Perkin Elmer gas chromatograph (Multifract F-40). A known aliquot of the sample (500 µl) was combined in a 20 ml vial with 4.5 ml of an aqueous solution of *n*-propanol which served as an internal standard. The vial was sealed and incubated at 58°C for at least 20 min. An aliquot of the head space was automatically injected on a Carbopack C column at 110°C. The ratio of the ethanol peak to the *n*-propanol peak was determined by computer and the concentration of ethanol in the sample calculated by comparison with aqueous standards. The total amount of ethanol in the brain sample thus calculated was divided by the initial brain weight to yield the concentration of ethanol in the brain. The concentration of ethanol in the aqueous standards was determined by a modified Cavett titration. The validity of using aqueous standards for brain samples was verified by the addition of known amounts of ethanol to blank mouse brain and preparing and analyzing them as described. The coefficient of variation for the ethanol analysis procedure was less than 2%.

Drugs

Barbitone sodium, pentobarbitone sodium, and ethanol were dissolved in saline. Phenytoin was dissolved in saline at a pH of 10-11 by the addition of NaOH. Phenytoin was obtained from Parke-Davis & Co. (Ann Arbor, Michigan). All other drugs were purchased commercially. All drug doses are expressed as mg/kg of the base compound.

Statistical analysis of data

Average rates of VI 15 s responding were calculated for each of the rats. Drug-induced changes in responding were expressed as a percentage of the individual animal's non-injection control rate of responding. For the calculation of relative potencies and determination of the degree of tolerance development, the descending limb of the dose-effect curve was subjected to analysis of variance and linear regression. The degree of tolerance was determined by comparison of the dose that would reduce responding to 50% of the control rate as estimated by the regression line equation.

Drug concentrations in brain and plasma were calculated by comparison of the ratio of peak areas of unknown over internal standard to the ratio obtained with known amounts of the drug and the internal standard. Significant difference in plasma and brain levels between non-tolerant and tolerant animals was determined by a one-tailed Student's *t*-test. The half-life of barbitone was calculated by linear regression analysis with the half-life being calculated as the time

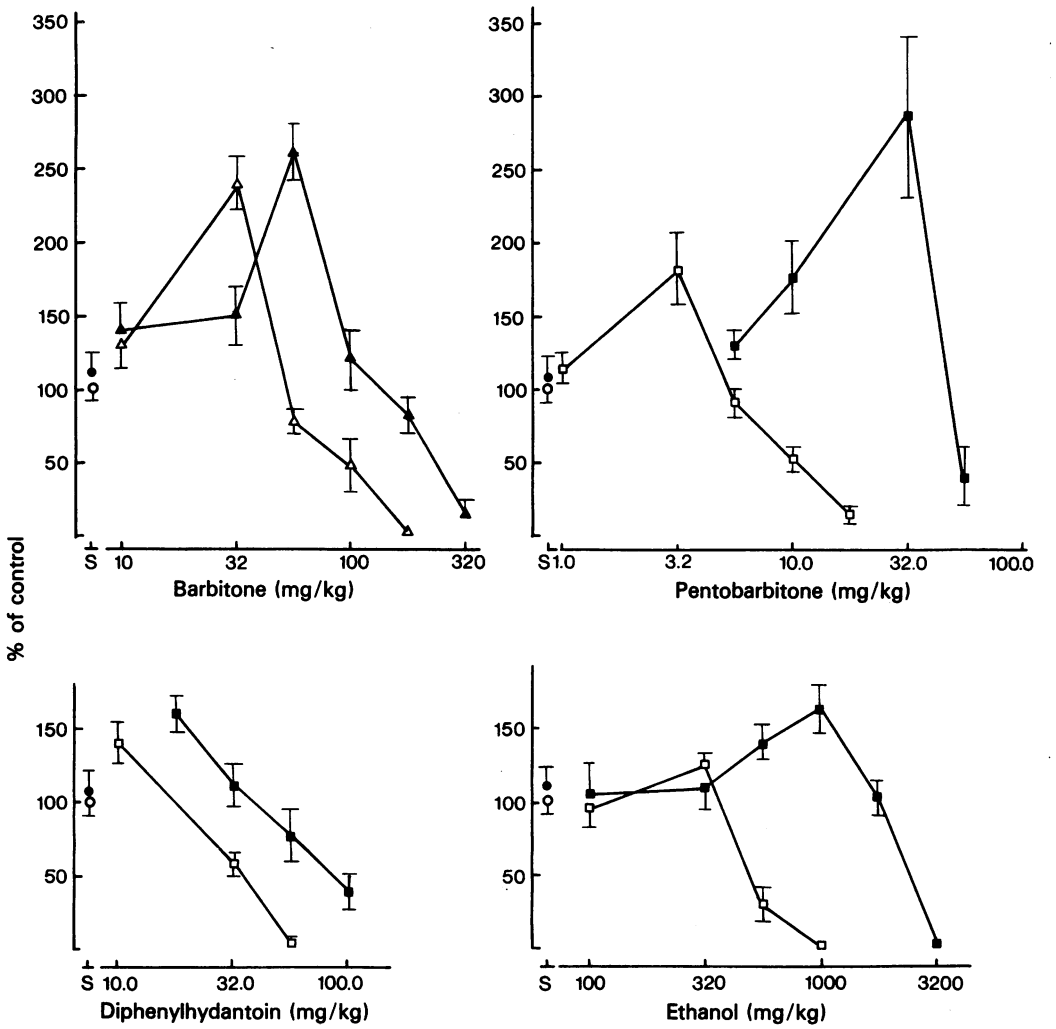

Figure 1. Effects of drug pretreatments on the rate of responding under a variable-interval schedule of liquid-food reinforcement in rats. Each point is the mean result from 6 rats; vertical lines show s.e. Open symbols represent the drug effects before daily barbitone injections. Solid symbols represent the drug effects in the same group of rats after the development of barbitone-tolerance. All drug injections were given (i.p.) 30 min before the start of the session.

Table 1 Control rates of responding for individual rats under the variable-interval 15 s (VI 15 s) schedule of reinforcement

Rat no.	*Resp/s**	(± *s.e.*)	*Rat no.*	*Resp/s*	(± *s.e.*)
1	0.25	(±0.01)	7	0.83	(±0.03)
2	0.65	(±0.03)	8	0.29	(±0.01)
3	0.21	(±0.01)	9	0.68	(±0.03)
4	0.45	(±0.01)	10	0.37	(±0.02)
5	0.59	(±0.02)	11	0.44	(±0.02)
6	0.38	(±0.02)	12	0.75	(±0.03)

* Data for individual rats are the mean ± s.e. of 5 consecutive baseline days.

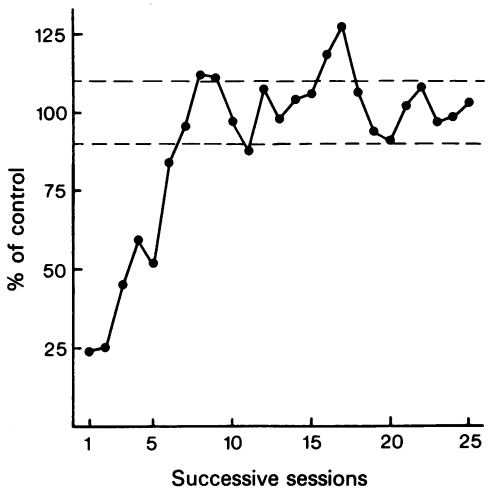

Figure 2 Time course for the development of tolerance to the effects of 100 mg/kg barbitone (i.p.) on VI responding in rats. Each point is the mean of 12 rats. Barbitone injections were given only once per day, 30 min before the start of the session.

to decrease plasma concentration to 1/2 y intercept (concentration at time 0). Analysis of covariance was performed to compare drug half-lives in the non-tolerant and barbitone-tolerant rats (Snedecor & Cochran, 1967).

Results

Drug effects on variable-interval (VI responding in non-tolerant rats)

The average rate of VI 15 s responding was 0.49 ± 0.06 (s.e.) responses/s. Although the range of response rates was relatively large (Table 1), the response rate for each animal was stable across sessions both before and during the initial dose-effect curve determinations. Control response rates did not vary more than 10% of the average rate for each animal during the last 14 days before drug testing was begun. Rates of responding on the inactive (right) lever were at or near zero. Responding on the inactive lever was not affected by drug doses that produced increases in responding on the lever that resulted in dipper presentation (left lever). Drug doses that decreased left lever response rates also decreased right lever responding. All of the drugs produced increases in VI responding at relatively low doses (Figure 1). At the doses tested, barbitone produced the greatest increase in responding in non-tolerant rats. At higher doses all of the drugs produced dose-related decreases in responding. The order of potency for the drug-induced decreases in VI responding was: pentobarbitone > phenytoin > barbitone > ethanol.

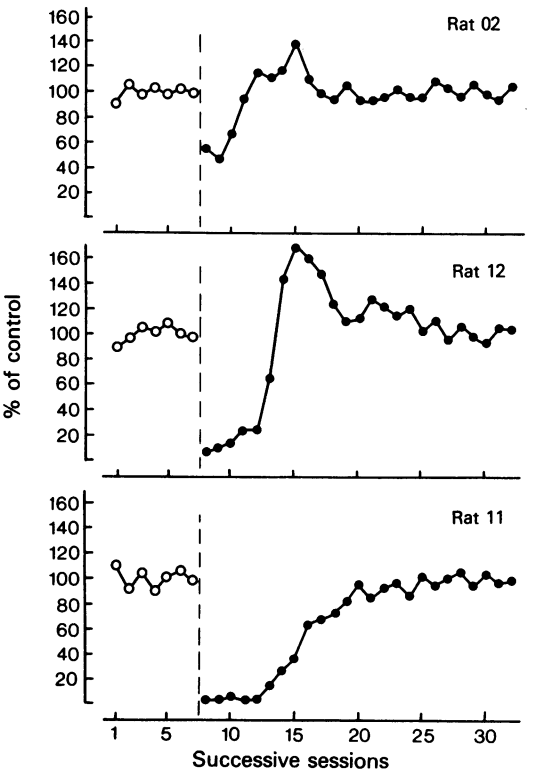

Figure 3 Time course data in rats which were representative of the 3 different patterns of barbitone-tolerance development. Open symbols represent the 7 days of saline pretreatment which immediately preceded daily barbitone injections (solid symbols). Each point represents a single observation in each rat.

Development of tolerance to barbitone

Daily barbitone injections (100 mg/kg, i.p.) initially decreased VI response rates to about 25% of the control rate (Figure 2). Average rates of responding returned to the control range after 7 days of daily barbitone. Following this return, an increase in responding occurred on days 16 and 17. Once tolerance developed to the behavioural stimulant effects of 100 mg/kg barbitone (around day 18) responding remained stable and within the prechronic barbitone range of response rates for the duration of the study. Rats differed in their rate and pattern of tolerance development to barbitone (Figure 3). Six of the 12 rats exhibited about a 50% decrease in VI responding (represented by rat no. 02). Responding returned to the control rate on day 4, increased to 140% of con-

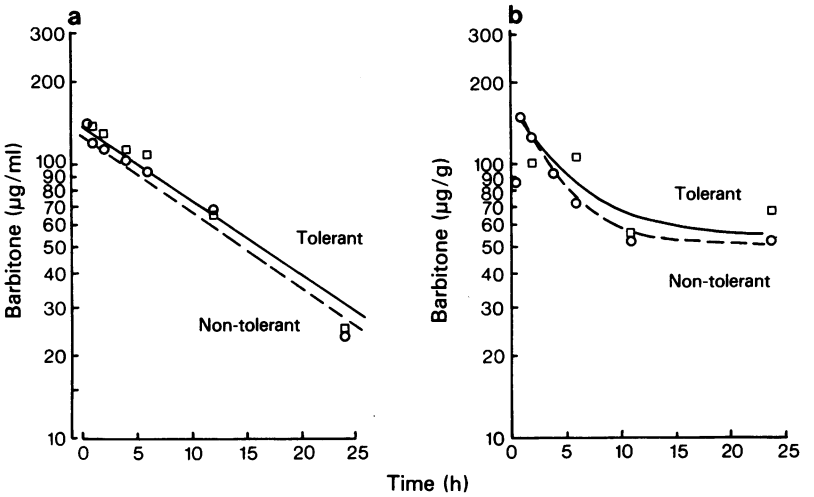

Figure 4 Plasma (a) and brain (b) decay curves for barbitone in non-tolerant and barbitone-tolerant rats. Each point represents the mean plasma or brain level of barbitone in 2 to 8 animals. Drug is administered to tolerant animals 48 h after last 100 mg/kg dose. The decay curves for tolerant (solid line) and non-tolerant animals (dashed line) are not significantly different. The plasma half-life of barbitone in non-tolerant animals was 18.9 h and in tolerant 21.0 h.

trol on day 8, and then returned to control rates on day 10. A somewhat similar pattern of tolerance development was seen in 4 of the remaining 6 rats (represented by rat no. 12). Responding was initially suppressed to about 8% of control on the first day of daily barbitone. Responding returned to about 65% of control rates by day 6. These rats showed a marked stimulation of responding (maximum of 165% of control) on days 9 to 12 of chronic barbitone injections. As was the case for the first group of rats (represented by rat no. 02) responding returned to control rates after a period of response rate increases and by day 18 of daily barbitone, responding had returned to control values. The remaining 2 rats (represented by rat no. 11) exhibited an initial suppression of responding which was followed by a recovery to control rates by day 13 of daily barbitone. These two rats did not show the response rate increases during daily barbitone that was observed for the other 10 rats.

Effects of chronic administration of barbitone on sensitivity to the effect of barbitone, ethyl alcohol, pentobarbitone and phenytoin

Daily barbitone injections (100 mg/kg) resulted in the development of tolerance to barbitone and cross-tolerance to the behavioural effects of pentobarbitone, ethanol and phenytoin (Figure 1). Barbitone tolerance was indicated by an approximately 3-fold shift to the right of the entire barbitone dose-effect curve. Barbitone-induced cross-tolerance was indicated by a shift to the right of the dose-effect curves for pentobarbitone (about 6 fold), ethanol (about 6 fold), and phenytoin (about 3 fold). Cross-tolerance developed to both the response rate increasing and decreasing effects of these drugs. In some cases the maximum drug-induced increases in responding was greater in tolerant rats compared to the maximum increases obtained in the same rats before the development of barbitone tolerance. For example, the maximum increase in responding produced by pentobarbitone was about 175% of control (3.2 mg/kg) in non-tolerant rats, compared to an increase in responding to 275% of control (32.0 mg/kg, pentobarbitone) in barbitone-tolerant rats. The same was true for ethanol, where a greater increase in responding occurred in barbitone tolerant rats, compared to the same rats before tolerance development.

Pharmacokinetics of barbitone in non-tolerant and barbitone-tolerant rats

No significant change in the plasma half-life of barbitone was observed when rats received daily drug injections for 21 days (Figure 4). The half-life for non-tolerant rats ($n = 40$, group of 2 to 8 rats/time point) was 18.9 h and in tolerant rats was 21.0 h ($n = 35$ rats, groups of 2 to 8 rats/time point). However, the barbitone-tolerant rats had significantly higher ($P < 0.01$) plasma levels. No significant change was seen in the brain concentration decay curves of barbitone between the tolerant and non-tolerant groups. Two kinetic components were observed for the decay curve of brain barbitone levels. The initial fast com-

ponent lasted for approximately 12 h. This was followed by a slower component which stabilized the drug concentration for at least the next 12 h. Even though the absolute drug concentration in the brain of animals receiving barbitone for 21 days was slightly higher than in animals injected once, no significant differences in either of the components of the decay curves were observed. A comparison of the brain and plasma barbitone levels showed that from 1 to 4 h following injection, the plasma level paralleled the brain barbitone level. The barbitone brain/plasma ratio at 30 min (0.5 h) was substantially lower than the 1 h point and probably represents part of the absorption portion (α phase) of the pharmacokinetic function for barbitone. Four hours after injection, brain barbitone levels began to fall at a faster rate than did plasma levels (Figure 4 and Table 2). However, at 24 h post injection there was a dramatic reversal of the brain/plasma barbitone ratio such that it appeared that barbitone was being preferentially retained in the brain. This reversal of the brain/plasma ratio at 24 h post injection occurred both in the non-tolerant and in the barbitone-tolerant rats.

Plasma and brain concentrations of ethyl alcohol, pentobarbitone and phenytoin in non-tolerant and barbitone-tolerant rats

Table 3 summarizes the time-dependent changes in drug levels in both non-tolerant and barbitone-tolerant rats. When 56.0 mg/kg of phenytoin was administered, the plasma level in tolerant animals was significantly reduced at both 30 and 60 min after administration. These plasma levels in tolerant animals are not significantly different from the levels obtained when 32.0 mg/kg phenytoin was administered to non-tolerant rats. In non-tolerant animals 32.0 mg/kg phenytoin produced approximately the same decrease in VI responding as 56 mg/kg in barbitone-tolerant animals. With pentobarbitone, significant reductions in the plasma drug levels were observed 30 and 60 min after 32.0 mg/kg compared to the non-tolerant group of rats (Table 3). Even though 3.2 mg/kg of pentobarbitone in non-tolerant animals produced a similar effect on VI responding to that of 32.0 mg/kg in tolerant rats, the plasma drug levels were significantly different at both 30 and 60 min. No significant difference in plasma ethanol levels were observed between non-tolerant and barbitone-tolerant rats 30 or 60 min after 1000 mg/kg ethanol (Table 3).

Similar results were observed for drug levels in the brain (Table 3). Brain drug levels of phenytoin and pentobarbitone were significantly reduced at both 30 and 60 min after administration in barbitone-tolerant rats, compared to non-tolerant rats. No changes in brain ethanol levels were observed between non-tolerant and tolerant rats receiving 1000 mg/kg ethanol.

Loss of tolerance to barbitone

Barbitone deprivation resulted in a gradual loss of tolerance (Figure 5). Sensitivity to the effects of barbitone had not returned to control values even 40 days after the termination of chronic barbitone injections. This slow loss of barbitone-tolerance is in contrast to the relatively rapid development of barbitone-tolerance seen at the beginning of the study (Figure 2).

Table 2 Distribution of barbitone in brain and plasma in non-tolerant and barbitone-tolerant rats

	Time after adminstration						
	0.5 h	1 h	2 h	4 h	6 h	12 h	24 h
Non-tolerant							
Brain	86.9* ±8.3	150.0 ±11.7	125.4 ±6.6	94.6 ±19.1	71.6 ±4.1	53.0** ±6.6	53.5 ±4.7
Plasma	141.8 ±5.0	119.2 ±5.0	115.4 ±1.5	105.7 ±3.8	95.6 ±3.4	68.9 ±6.4	23.1 ±2.8
Brain/plasma ratio	0.61	1.27	1.09	0.89	0.75	0.77	2.31
Barbitone-tolerant							
Brain	—	149.4 ±23.8	107.0 ±17.5	—	106.5 ±11.6	55.2 ±3.1	67.9 ±6.5
Plasma	—	138.2 ±3.5	131.8 ±3.8	115.2 ±2.9	109.1 ±5.3	66.8 ±6.8	25.7 ±2.9
Brain/plasma	—	1.08	0.81	—	0.98	0.83	2.64

* Data presented as the mean ± s.e. (μg/g wet weight or ml fluid) for 6 rats at each time point.
** Data for 2 rats only.

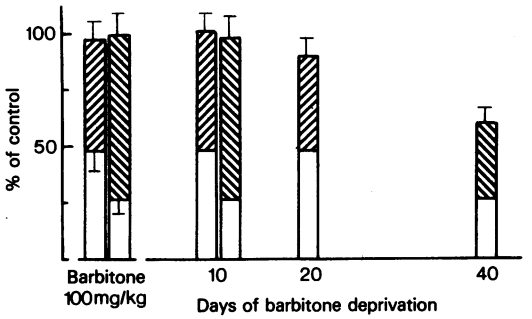

Figure 5 Time course of the loss of barbitone tolerance in rats responding under a VI schedule of liquid-food reinforcement. Open portions of the columns represent the initial barbitone sensitivity for each of two groups of 6 rats. The hatched columns represent the amount of barbitone tolerance in each of the two different groups of 6 rats at the different time points. The amount of tolerance with daily barbitone injection is shown at the left of the figure (barbitone 100 mg/kg).

Discussion

The effects of pentobarbitone and ethanol on food-reinforced responding were similar to those previously reported (Leander & McMillan, 1974; Barrett, 1976). Both pentobarbitone and ethanol had a biphasic effect on VI response rates. Relatively low doses increased response rates above control values, while higher doses decreased response rates. Barbitone and phenytoin (diphenylhydantoin) pretreatment resulted in increases and decreases in VI responding that were qualitatively similar to those described for pentobarbitone and ethanol. Domino & Olds (1972) reported that phenytoin produced only decreases in self-stimulation behaviour of rats. However, the dose-range of phenytoin which they studied was between 25 and 75 mg/kg. Compared to the data obtained in the present study, it appears that Domino & Olds (1972) examined only the descending limb (high dose end) of the phenytoin dose-effect curve. Their data showing that response rate decreases following phenytoin pretreatment are similar to the effects of the relatively high phenytoin doses used in the present study. Biphasic effects of sedative hypnotics on behaviour are not limited to schedule-controlled (operant) behaviour. Read, Cutting & Furst (1960) reported that the effects of phenobarbitone on the spontaneous locomotor activity of mice also were biphasic. Quigley, Barlow & Himmelsbach (1934) found that relatively low doses of barbitone and phenobarbitone produced excitation in dogs, while higher doses produced excitement less frequently.

Tolerance to the behavioural effects of barbitone developed rapidly under the once-daily injection schedule. On the first day the 100 mg/kg barbitone dose produced a substantial decrease in responding. Tolerance development to the rate decreasing effects of barbitone was evident as early as the 3rd day of chronic dosing. As tolerance developed to depressant effects of barbitone, the behavioural stimulant properties became apparent. Subsequently, tolerance developed to the stimulant properties and by the 18th day tolerance to the behavioural effects of 100 mg/kg barbitone appeared to be complete.

Since barbitone is excreted unchanged (Dorfman & Goldbaum, 1947; Maynert & Van Dyke, 1950; Burns, Evans & Trousof, 1957; Ebert, *et al.*, 1964), the observed tolerance development is most likely due to an adaptive change in the sensitivity of the CNS to the effects of barbitone. Determination of the brain and plasma barbitone pharmacokinetics supported the hypothesis that barbitone-tolerance was due to an adaptive change in the CNS and not due to a change in the rate of excretion or metabolism. The brain and plasma half-lives for barbitone in tolerant rats were not different from those observed for non-tolerant rats. This lack of a difference in barbitone elimination between tolerant and non-tolerant rats is similar to that previously reported for the barbitone-tolerant rat (Ebert *et al.*, 1964).

Determinations of brain and plasma drug levels for the other drugs tested made it possible to distinguish between functional cross-tolerance and dispositional cross-tolerance (Kalant *et al.*, 1971). Barbitone-tolerant rats appeared to be functionally cross-tolerant to ethanol, in that the depressant effect of 1,000 mg/kg seen in non-tolerant rats was not observed in barbitone-tolerant rats. In fact, this dose of ethanol produced a stimulation of responding in tolerant rats. In contrast to these changes in the behavioural effect of ethanol in tolerant rats, there was no significant difference in the brain and plasma ethanol levels of tolerant rats, compared to non-tolerant rats.

Cross-tolerance to phenytoin appeared to be due to the development of dispositional tolerance. Phenytoin doses of 56 mg/kg in tolerant rats and 32.0 mg/kg in non-tolerant rats produced comparable behavioural effects and resulted in similar plasma and brain drug levels. Since a complete pharmacokinetic profile was not determined, it is not possible to assess the roles of increased metabolism, changes in drug distribution or changes in drug excretion. However, it is likely that the chronic barbitone administration resulted in an induction of the hepatic microsomal enzyme system which is responsible for the metabolism of phenytoin to 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (Kutt & Fouts, 1971). In support of this hypothesis, Conney & Burns (1962) and Schnell, Stoll & Prosser (1976) have shown that barbitone pretreatment can result in increases in the metabolism of drugs that are metabolized by hepatic microsomal enzymes. Cross-tolerance to pentobarbitone was

probably the result of the development of both functional and dispositional cross-tolerance.

In contrast to the relatively rapid rate of barbitone-tolerance development, the decline of barbitone-tolerance appeared to occur at a very slow rate. A substantial amount of barbitone-tolerance could be demonstrated even after 40 days following cessation of daily barbitone injections. Based on the data obtained in the present study, it is estimated that it would take between 60 and 90 days for the complete loss of barbitone-tolerance. Cox, Ginsburg & Willis (1975) reported that the loss of morphine tolerance also occurs very slowly. In their study, morphine tolerance was produced with a morphine pretreatment schedule which lasted for 3 days. However, the loss of morphine tolerance required 20 to 30 days. Because of the relatively long time required for the loss of barbitone-tolerance, it seems unlikely that the continued presence of barbitone is necessary to maintain the degree of tolerance observed. This long recovery time may indicate that chronic exposure to sedative-hypnotics results in a major change in the neurochemistry and metabolic economy of the CNS, and further analysis of the recovery of normal drug sensitivity may greatly increase our understanding of the development of tolerance and dependence.

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Table 3 Drug levels of various CNS depressants in non-tolerant and barbitone-tolerant rats

Drug	*Dose (mg/kg)*	*Time (min)*	*Plasma level (μg/ml)*		
			Non-tolerant	*Tolerant*	P *value*
Phenytoin	32.0	30	20.60 ± 3.74*	—	—
		60	12.29 ± 1.88	—	—
	56.0	30	27.42 ± 1.17	18.53 ± 1.54	<0.005
		60	22.16 ± 1.18	12.32 ± 1.94	<0.005
Pentobarbitone	3.2	30	3.75 ± 0.73	—	—
		60	3.06 ± 0.46	—	—
	32.0	30	35.48 ± 5.27	19.23 ± 5.43	0.05
		60	24.30 ± 4.40	10.02 ± 1.99	0.05
Ethanol	1000.0	30	0.93 ± 0.06	1.05 ± 0.05	NS
		60	0.78 ± 0.04	0.82 ± 0.04	NS
			Brain level (μg/g)		
Phenytoin	32.0	30	15.89 ± 2.6	—	—
		60	8.83 ± 1.12	—	—
	56.0	30	22.85 ± 1.30	11.04 ± 1.08	<0.005
		60	18.24 ± 1.22	13.10 ± 1.81	<0.05
Pentobarbitone	32.0	30	12.87 ± 1.43	9.33 ± 0.91	<0.05
		60	11.33 ± 1.95	5.37 ± 1.51	<0.05
Ethanol	1000.0	30	0.86 ± 0.11	0.94 ± 0.12	NS
		60	0.65 ± 0.09	0.77 ± 0.07	NS
			Brain/plasma ratio		
Phenytoin	56.0	30	0.83	0.59	
		60	0.82	1.06	
Pentobarbitone	32.0	30	0.35	0.48	
		60	0.47	0.49	
Ethanol	1000.0	30	0.92	0.94	
		60	0.83	0.77	

* Data presented as the mean ± s.e. (μg/g wet weight or ml fluid) for 6 rats at each time point.

References

BARRETT, J.E. (1976). Effects of alcohol, chlordiazepoxide, cocaine and pentobarbital on responding maintained under fixed-interval schedules of food or shock presentation. *J. Pharmac. exp. Ther.*, **196**, 605–615.

BLACK, M.B., WOODS, J.H., & DOMINO, E. (1970). Some effects of (−)-Δ^9-*trans*-tetrahydrocannabinol and other cannabis derivatives on schedule-controlled behavior. *Pharmacologist*, **12**, 258.

BURNS, J.J., EVANS, C., & TROUSOF, N. (1957). Stimulatory effect of barbital on urinary excretion of L-ascorbic acid and non-conjugated O-glucuronic acid. *J. biol. Chem.*, **277**, 785–794.

CONNEY, A.H., & BURNS, J.J. (1962). Factors influencing drug metabolism. *Adv. Pharmac.*, **1**, 31–58.

COX, B.M., GINSBURG, M., & WILLIS, J. (1975). The offset of morphine tolerance in rats and mice. *Br. J. Pharmac.*, **53**, 383–391.

DOMINO, E.F. & OLDS, M.E. (1972). Effects of *d*-amphetamine, scopolamine, chlordiazeporide and diphenylhydantoin on self-stimulation behavior and brain acetylcholine. *Psychopharmacologia (Berl.)*, **23**, 1–6.

DORFMAN, A. & GOLDBAUM, L.R. (1974). Detoxification of barbiturates. *J. Pharmac. exp. Ther.*, **90**, 330–337.

EBERT, A.G., YIM, G.K.W., & MIYA, T.S. (1964). Distribution and metabolism of barbital-^{14}C in tolerant and intolerant rats. *Biochem. Pharmac.*, **13**, 1267–1274.

HEIFETZ, S.A. & MCMILLAN, D.E. (1971). Development of behavioral tolerance to morphine and methadone using schedule controlled behavior of the pigeon. *Psychopharmacologia, (Berl)*, **19**, 40–52.

KALANT, H., LEBLANC, A.E., & GIBBINS, R.J. (1971). Tolerance to and dependence on, some non-opiate psychotropic drugs. *Pharmac. Rev.*, **23**, 135–191.

KUPFERBERG, H.J. (1970). Quantitative estimation of diphenylhydantoin, primidone, and phenobarbital in plasma by gas-liquid chromatography. *Clin. Chem. Acta.*, **29**, 283–288.

KUTT, H. & FOUTS, J.R. (1971). Diphenylhydantoin metabolism by rat liver microsomes and some of the effects of drug or chemical pretreatment on diphenylhydantoin metabolism by microsomal preparation. *J. Pharmac. exp. Ther.*, **176**, 11–26.

LEANDER, J.D. & MCMILLAN, D.E. (1974). Rate-dependent effects of drugs. I. Comparisons of d-amphetamine, pentobarbital and chlorpromazine on multiple and mixed schedules. *J. Pharmac. exp. Ther.*, **188**, 729–739.

MAYNERT, E.W. & VAN DYKE, H.B. (1949). The metabolism of barbiturates. *Pharmac. Rev.*, **1**, 217–242.

MCMILLAN, D.E. (1977). Behavioral pharmacology of the tetrahydrocannabinols. *Adv. Behav. Pharmac.*, **1**, 1–34.

QUIGLEY, J.P., BARLOW, O.W., & HIMMELSBACH, C.K. (1934). Correlation of visceral and somatic activity following administration of hypnotics. *J. Pharmac. exp. Ther.*, **50**, 425–439.

READ, G.W., CUTTING, W., & FURST, A. (1960). Comparison of the excited phases after sedatives and tranquilizers. *Psychopharmacologia (Berl.)*, **1**, 346–350.

REMMER, H. (1972). Enzymatic mechanisms of drug tolerance. In *Drug Addiction: Experimental Pharmacology*, Vol. 1. ed. Singh, J. M., Miller, L. & Lal, H. pp. 377–392. Mount Kisco, New York: Futura Pub. Co., Inc.

SCHNELL, R.C., STOLL, R.E. & PROSSER, T.D. (1976). Barbital alteration of central nervous system sensitivity to hexobarbital in the rat. *Psychopharmac.*, **47**, 93–96.

SCHUSTER, C.R., DOCKENS, W.S., & WOODS, J.H. (1966). Behavioral variables affecting the development of amphetamine tolerance. *Psychopharmacologia, (Berl.)*, **9**, 170–182.

SHERWIN, A.L., EISEN, A.A., & SOKOLOWSKI, C.D. (1973). Anticonvulsant drugs in human epileptogenic brain. *Arch. Neurol.*, **29**, 73–77.

SNEDECOR, E. & COCHRAN, E. (1967). *Statistical Methods.* Ames, Iowa: Iowa State University Press.

YANAGITA, T. & TAKAHASHI, S. (1970). Development of tolerance to and physical dependence on barbiturates in rhesus monkeys. *J. Pharmac. exp. Ther.*, **172**, 163–167.

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