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RELATIONSHIP OF BARBITAL DISPOSITION TO AUTO-INDUCED HYPERSUSCEPTIBILITY IN THE RAT*

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Abstract—Two daily doses of barbital induce, in the female rat, a hypersusceptibility to the drug which is present after 18 days, and absent after 38 days of abstinence. Spectrophotometric and radiometric assays of barbital tissue levels, at various times from 10 to 360 min after drug administration, reveal no differences in blood, brain or urinary barbiturate levels among control, hypersusceptible or posthypersusceptible animals. The data support the suggestion that induced hypersusceptibility does not result from changes in the disposition of barbiturate *in vivo*. It appears likely that this phenomenon results from central nervous system alterations in the localization of, or sensitivity to, the drug.

INTRODUCTION

AN EXAGGERATED response to a barbiturate, evoked by prior short-term treatment with the same agent, has been observed in this laboratory in the case of both pentobarbital[1] and barbital.[2] It has been previously demonstrated that the rate of decline of blood pentobarbital concentration is the same in hypersusceptible as in control animals.[3] However, the blood level of such a drug is determined by the interrelationship between processes of distribution, metabolism and excretion. In addition, blood levels of drug alone do not necessarily reflect the distribution of the agent in brain. For these reasons the present study was undertaken to determine the disposition of barbital in blood, brain and urine of naive and hypersusceptible rats. The question of the reversibility of auto-induced hypersusceptibility was also examined.

MATERIALS AND METHODS

Female albino rats of the Sprague–Dawley strain, initially weighing from 130–175 g, were employed in this investigation. The animals were housed in laboratory quarters, automatically provided with alternating 12-hr periods of light and darkness. Room temperature was maintained at 74°F. The rats were kept in their quarters for at least 1 week prior to experimentation. Food and water were allowed *ad lib.*

Sodium barbital in an isotonic concentration (3% aqueous solution) was given in a dose of 200 mg/kg, in terms of the salt, by the intraperitoneal route throughout this study. Control animals received equivalent volumes of physiological saline. In all

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cases in which induction of hypersusceptibility was attempted, rats received two consecutive daily inducing doses of barbital followed, after various abstinence intervals, by a third challenge dose of the drug. All experiments were designed so that challenge doses of barbiturate were given on the same calendar date.

Statistical procedures employed were those outlined by Burn *et al.*[4] The Student *t*-test was employed to determine the significance of differences between means. The 0·05 per cent level of probability was accepted as indicative of significance.

Reversibility of induced hypersusceptibility. Control animals, as well as those exposed to an 8-, 18-, 28- and 38-day abstinence after two inducing doses of barbital, were given a challenge dose of the barbiturate. Sleeping times, measured as the time elapsing between loss and regain of the righting reflex, were recorded and awakening blood levels in each group of animals determined spectrophotometrically as described below.

Spectrometric study of barbital distribution. Blood, whole brain and cumulative urinary barbital levels were measured 45, 90, 180 and 360 min after a challenge dose of the barbiturate in control animals and in animals made abstinent for 18 or 38 days after 2 inducing doses of the drug. Tissue barbiturate was extracted and estimated spectrophotometrically at 260 mμ according to the method of Williams and Zak,[5] using a Beckman model DB spectrophotometer. Blood samples were obtained by cardiac puncture, and whole brains removed after decapitation. Cumulative urine was withdrawn from the exposed urinary bladder of animals in which the urethra was previously ligated.

Radioisotopic study of barbital distribution. Rats received either 200 mg/kg sodium barbital or equivalent volumes of saline 18 days prior to receiving an injection of ^{14}C-labeled barbital. Barbital-2-^{14}C (obtained from Tracerlab, Inc.) with a specific activity of 1·6 mc/mm was used. Immediately before injection, unlabeled sodium barbital and barbital-^{14}C were dissolved in distilled water to yield a 3% solution containing sufficient radioactivity so that the animals received 25 μc of radioactivity/kg body weight in a dose of 200 mg/kg of total drug. At various times from 10 to 360 min after injection of the labeled drug, blood samples were drawn by cardiac puncture and the animals were immediately decapitated. Samples of 0·1 ml of plasma, or 7·9 to 30·5 mg of cerebral cortex, were radio-assayed. Brain tissue was solubilized in 0·2 ml methanolic Hyamin hydroxide (Packard Instrument Co.) at 50° for 12 to 20 hr. Twelve ml of scintillation solvent* was added to each vial containing plasma or solubilized cortex. The samples were counted in a liquid scintillation spectrometer (Packard Instrument Co., model 3310).

RESULTS

Reversibility of induced hypersusceptibility. Table 1 lists the mean barbital sleeping times of animals subjected to various abstinence intervals after two daily doses of the drug.

Mean sleeping times, observed in response to the inducing doses of barbital, varied from 188·0 to 233·5 min on day 1 of the experiment and from 184·5 to 277·5 on day 2. No significant differences in mean sleeping times were observed either among the four barbiturate-pretreated groups or between the 2 pretreatment days.

* PPO, 8·0 g; POPOP, 0·2 g; Toluene, 1·0 l. Mixed together and added to 1·0 l. of Cellosolve.

On the terminal day of the experimental schedule, the control group exhibited a mean sleeping time of 232 min. The 8- and 18-day abstinent groups provided mean sleeping times of 301·5 and 329·5 min, representing a 30 and a 42 per cent increase over controls. The 28-day abstinent group slept for 240·0 min, which was a significantly lower sleeping time than that observed in the 18-day group, but not different from the control group. The mean sleeping time of the 38-day group (208·5 min) did not differ significantly from either the 28-day or the control groups of animals. With

TABLE 1. RELATIONSHIP OF ABSTINENCE INTERVAL TO TERMINAL DAY SLEEPING TIME AND AWAKENING BLOOD BARBITAL LEVEL IN FEMALE RATS RECEIVING THREE DOSES OF BARBITAL

Abstinence interval (days)	Sleeping + S.E. (min)	Awakening barbital blood level + S.E. (μg/ml)
Control	232·0 ± 27·3 (6*)	208 ± 1·8 (6)
8	301·5 ± 22·8 (6)	190 ± 5·3 (6)†
18	329·5 ± 19·0 (7)†	173 ± 3·7 (6)†
28	240·0 ± 14·8 (6)	196 ± 4·2 (6)
38	208·5 ± 13·8 (6)	255 ± 16·5 (6)

* Numbers in parenthesis indicate number of animals.
† Significantly different from control group.

increasing abstinence intervals, therefore, barbital sleeping times display a progressive increase to a maximum on, or about, the eighteenth day of abstinence, followed by a progressive decline to control values by the twenty-eighth abstinence day.

Awakening blood levels of barbital in these animals display a trend which is the inverse of that of sleeping time, as seen in Table 1. Barbital blood levels exhibit a significant decline from 208 μg/ml in the control group to a minimum of 173 μg/ml in the 18-day abstinent group, and thereafter an increase to control levels in the 28- and 38-day abstinent groups.

Mean blood, brain and cumulative urinary levels of barbital in control, 18-day and 28-day abstinent rats, obtained at various times after barbiturate administration, are listed in Table 2.

Blood barbital content shows a progressive decline between 45 and 360 min in all three groups of rats. Samples taken at corresponding time intervals in the three experimental groups, however, failed to exhibit any significant differences. Whole brain levels of the drug, in all three groups, rose to a peak at 90 min and then declined progressively at 180 and 360 min. As in the case of blood, no differences were observed in brain barbital levels among the three groups of rats at corresponding times. Cumulative urinary barbital levels rose progressively with time in the three groups. No significant differences were observed among these levels in a group to group comparison.

Since the control, the 18-day and the 38-day abstinent groups exhibited no time course differences in blood, brain or urinary barbital content, the tissue levels of the three groups of animals were combined to provide grand-mean values. Analysis of variance demonstrated linearity of the regression of log-blood level on time in min and provided the regression equation, $y = 2{\cdot}50 - 0{\cdot}00060x$. Similar analysis of

TABLE 2. MEAN TISSUE AND URINE BARBITAL LEVELS ± S.E. AT FIXED TIMES AFTER INJECTION IN HYPERSUSCEPTIBLE AND NON-HYPERSUSCEPTIBLE FEMALE RATS

Abstinence interval (days)	Sampling time (min)	Blood barbital (μg/ml)	Whole brain barbital (μg/g)	Cumulative urinary barbital (μg)
Control	45	298 ± 12·7 (4)*	212 ± 2·2 (4)	1286 ± 129 (4)
	90	287 ± 9·6 (4)	228 ± 5·4 (4)	2226 ± 536 (4)
	180	250 ± 4·9 (3)†	201 ± 7·0 (3)†	6577 ± 684 (3)†
	360	214 ± 12·3 (4)†	158 ± 12·3 (4)†	9479 ± 1192 (4)
18	45	315 ± 32·2 (3)	206 ± 14·8 (3)	1019 ± 161 (3)
	90	248 ± 15·8 (4)	236 ± 22·4 (4)	1835 ± 446 (4)
	180	243 ± 7·1 (4)	189 ± 12·3 (4)	5662 ± 946 (4)†
	360	194 ± 18·9 (3)†	139 ± 8·3 (3)†	10314 ± 1686 (3)
28	45	308 ± 12·5 (4)	204 ± 13·3 (4)	1250 ± 145 (4)
	90	277 ± 9·8 (4)	218 ± 5·4 (4)	1519 ± 202 (4)
	180	249 ± 7·1 (4)	186 ± 7·6 (4)†	4808 ± 309 (4)†
	360	176 ± 24·3 (4)†	134 ± 6·0 (4)†	8480 ± 706 (4)†

* Number in parentheses indicate number of animals.
† Significantly different from preceeding value in table.

TABLE 3. TISSUE RADIOACTIVITY (dpm × 10^{-3}/ml) ± S.E. AT VARIOUS TIMES AFTER BARBITAL-^{14}C ADMINISTRATION IN CONTROL AND HYPERSUSCEPTIBLE FEMALE RATS

	Plasma		Cortex	
Time	Control	Hypersusceptible	Control	Hypersusceptible
10	62·6 ± 1·63 (4)*	61·1 ± 5·84 (4)	46·0 ± 19·6 (4)	34·5 ± 9·1 (4)
30	66·7 ± 2·90 (4)	68·7 ± 5·22 (4)	32·6 ± 11·3 (4)	23·4 ± 13·3 (4)
60	64·6 ± 5·41 (4)	64·3 ± 10·34 (4)	18·3 ± 14·3 (4)	29·9 ± 4·4 (4)
90	56·8 ± 3·73 (4)	56·8 ± 4·64 (5)	45·5 ± 2·4 (4)	47·1 ± 3·2 (5)
180	53·6 ± 4·92 (4)	55·4 ± 4·83 (4)	36·6 ± 2·4 (3)	47·0 ± 7·9 (4)
360	42·3 ± 3·23 (4)	38·7 ± 2·38 (4)	34·0 ± 8·5 (4)	25·8 ± 4·0 (4)

* Number in parentheses indicate number of animals.

brain barbital levels between 90 and 360 min provided the regression equation, $y = 2{\cdot}42 - 0{\cdot}00073x$. The tissue barbital half-life was estimated to be 505·0 min in blood and 409·5 min in brain. The regression of log urinary barbital content on time proved to be nonlinear.

Radioisotopic study of barbital distribution. Plasma and cortical levels of radioactivity at various times after administration of barbital-^{14}C are given in Table 3. No significant differences in either plasma or cortical radioactivity are evident between the control (saline pretreated) and the hypersusceptible (barbital pretreated) rats at any time interval up to 360 min after drug administration.

The data from control and pretreated animals were pooled, and grand-mean plasma and brain radioactivity were calculated at each time interval. Plasma levels of radioactivity exhibit a nonsignificant increase between 10 and 30 min to a maximum of 67·7 dpm × 10^3/ml followed by a continuous decline to 40·5 dpm × 10^3/ml at 360 min after injection. Grand mean cortical radioactivity, unlike that of plasma, displays a triphasic trend, declining between 10 and 60 min to a minimum of 24·1 dpm × 10^{-3}/g, rising to a maximum of 46·4 dpm × 10^{-3}/g at 90 min, and thereafter declining continuously to 29·9 dpm × 10^{-3}/g at 360 min.

The log grand-mean radioactivity levels for plasma between 30 and 360 min, and for cortex between 90 and 360 min, were subjected to analysis of variance and, in both cases, linearity and a significant slope were established. The calculated regression equations were $y = 1{\cdot}84 - 0{\cdot}00064$ for plasma, and $y = 1{\cdot}75 - 0{\cdot}00085$ for cortex. The biological half-life of the radioactivity was found to be 402·2 and 355·5 min for plasma and for cortex respectively.

DISCUSSION

Auto-induced hypersusceptibility to barbital has been observed in the female Sprague–Dawley rat after two consecutive daily doses of the drug. This state of exaggerated responsiveness has been shown, in the present study, to develop progressively to a peak level on or about the eighteenth day of abstinence. From this point the hypersusceptible response appears to wane until, by the twenty-eighth abstinence day, it has returned to the level observed in naive animals.

Hypersusceptibility may be due to an alteration in the disposition *in vivo* of a barbiturate, allowing the drug to remain at its site of action in the body for prolonged periods of time. Such a mechanism could involve a delay in absorption, distribution, metabolism or excretion of the agent given.

A delay in the absorption of the drug would likely result in a lengthened time of induction of sleep. However, previous studies have shown that induction periods tend to be shorter in hypersusceptible than in naive animals, both in the case of pentobarbital[1] and barbital.[2] A reduction in the rate of metabolism of barbiturates in hypersusceptible animals does not appear to be the mechanism through which these animals develop an augmented response to the drugs, since it has been reported previously that the rate of decline of the blood level of pentobarbital is the same in naive and in hypersusceptible animals.[3] In addition, the fact that hypersusceptibility can be elicited to barbital appears to preclude a biotransformative basis for this phenomenon, since barbital is metabolized, in this species, only to the extent of 3·7 per cent of a given dose.[6]

The present study was designed to investigate the possible role of the distributive and excretive aspects of barbital disposition in the development of auto-induced hypersusceptibility. It was observed that the barbital concentration in blood, obtained from animals at the time of awakening, bore an inverse relationship to the sleeping time in rats subjected to varying abstinence periods after barbital pretreatment (Table 1). Maximum hypersusceptibility occurred in the 18-day abstinent group which also exhibited a minimum awakening blood barbital level compared to control rats. These results indicate that the prolonged sleeping time in hypersusceptible animals results from an increased sensitivity of the central nervous system to barbital, rather than to a change in the rate of distribution of the drug in the body.

In order to substantiate the latter conclusion, experiments were performed to determine whether the rate of change of blood, brain and urinary barbital levels, determined both spectrophotometrically and radiometrically, in hypersusceptible animals differed from that seen in control animals. The data presented in Tables 2 and 3 confirm that the rate of distribution of barbital in blood and to brain is the same, up to 360 min after drug administration, in both control and hypersusceptible animals. These studies, however, do not preclude a possible change in the differential distribution of the drug within subcortical areas of brain among hypersusceptible, as

compared to control animals, particularly in view of the suggestion by Lal *et al.*[7] that barbital is differentially localized within the central nervous system. It is evident that brain to blood ratios do not differ between the two groups of rats and, hence, that hypersusceptibility cannot be ascribed to alteration in the permeability of the blood–brain barrier. In addition, the similarity in excretory patterns of barbital in hypersusceptible and control animals (Table 2) shows that hypersusceptibility does not entail any changes in the rate of excretion of the drug.

The peculiar initial rapid decline and increase in cortical radioactivity recorded in Table 3 are likely related to a fall and subsequent rise in plasma pCO_2 which, in turn, result from an initial locomotor stimulation of the rats by barbital, followed, at the time of loss of righting reflex, i.e. about 40 to 50 min after drug administration, by hypoventilation and hypercarbia. An increased venous pCO_2 in rats 75 min after barbital administration has been observed previously.[8] Such shifts in plasma pCO_2 could influence brain barbital content by changing cerebral blood flow.

The present studies provide a picture of barbiturate hypersusceptibility which is induced by brief exposure to the drug, i.e. two doses, is of relatively short duration and is mediated centrally. A phenomenon with similar characteristics was described by Adler[9] who found an enhanced inhibition of locomotor activity in the rat after the second of two doses of tetrabenazine when the interval between doses was 1 or 2, but not 4 weeks. Such alterations in central barbiturate threshold may bear some relationship to the observations of other authors[10, 11] that the sensitivity of the rat central nervous system to such drugs is considerably greater in very young, compared to more mature animals.

A second type of induced hypersusceptibility to barbiturates has been reported in the literature, which, it appears, is manifested after prolonged exposure to the drug, is of extended duration and is mediated hepatically. This response has been reported by Wahlstrom[12] to occur in rats after 35 daily doses of barbital. Such animals exhibited an enhancement of hexobarbital sleeping time which was greater at 5 than at 3 weeks after barbital pretreatment. Central nervous system sensitivity (measured as the dose of hexobarbital required to produce a 1-sec burst suppression in the EEG) was unchanged from control levels in hypersusceptible rats. Stevenson and Turnbull[13] gave barbital in drinking water to rats for 32 days and found a subsequent increase in hexobarbital sleeping time which persisted for up to 4 months. These hypersensitive animals possessed a reduced hepatic hexobarbital-metabolizing enzyme activity. The phenomenon observed by these authors likely parallels that reported in 1937 by Moir.[14] This author found an enhanced sensitivity to pentobarbital in rats occurring 20 and 38, but not 14, days after 8 to 18 doses of the drug had been given every second day.

This latter type of induced hypersusceptibility may bear some relation to the fact that a decreased barbiturate sleeping time occurs in rats, after the second of two shortly-spaced doses of drug, as a result of microsomal enzyme induction.[13] The use of a chronic dosage regimen with barbital, as in the studies mentioned above, might eventually deplete the metabolic pool of essential precursors, thus reducing the rate of microsomal drug metabolism, as observed by these authors.

Induced hypersusceptibility, therefore, like tolerance to barbiturates seems to be mediated through two distinct mechanisms, one at a central nervous system and the other at a periperal hepatic site.

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