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# Similar Development of Tolerance to Barbital-Induced Inhibition of Avoidance Behavior and Loss of Righting Reflex in Rats

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RICHTER, J A , P S HARRIS AND P V HANFORD *Similar development of tolerance to barbital-induced inhibition of avoidance behavior and loss of righting reflex in rats* PHARMAC BIOCHEM BEHAV **16**(3) 467–471, 1982 —In order to determine if tolerance develops to the inhibition of avoidance behavior by the barbiturates, the effects of barbital on avoidance were determined in rats given barbital in their sole source of drinking water for 7 or 33 days For comparison tolerance to the loss of righting reflex was also determined in other rats at the same time All rats were trained by one 60-min session in a one-way active avoidance task, they were then put on the chronic drug administration schedule and then tested on the appropriate day after a single IP injection of 250 mg/kg sodium barbital To assess the degree of tolerance, the brain level of barbital found at the biological endpoint—the loss of avoidance or loss of righting reflex—was compared in the chronic barbital treated rats and controls A similar degree of tolerance developed to both effects of the drug and it appeared to be as great after 7 as after 33 days of chronic barbital treatment

Tolerance Avoidance Righting reflex Barbital Barbiturates Brain drug levels Anxiety Chronic drug administration Behavior Anxiolytics

THE barbiturates as a class have many effects in the whole animal including ataxia, sedation, hypnosis and anesthesia, anti-convulsant activity and antianxiety activity They also disrupt behavior of animals in a wide variety of behavioral paradigms including conditioned avoidance behavior [6] The potency of various barbiturates to cause these effects has usually been described in terms of the injected dose We have been interested in obtaining a more direct measure of potency to use for comparisons among the barbiturates For this purpose we measured the brain concentration of the drug when a particular effect occurs This method is also less subject to variability than the use of injected dose since it bypasses variations in absorption and distribution of the drug

Our second interest is in the rate and degree of CNS tolerance that can be developed to the various effects of the barbiturates If barbiturate levels in the brain determine the drug-induced behavioral changes, tolerance can be defined as an increased level of barbiturate in the brain required to cause a specific behavioral change This method of measuring CNS (functional) tolerance is unaffected by either metabolic tolerance or by the amount of drug remaining in the body from the chronic drug treatment

In previous studies, the time course and degree of CNS tolerance development (alone or in contrast to dependence) has not been fully described for any of the several methods of chronic administration of barbiturates to mice and rats described in the literature [8,9] Okamoto and her group (see [15] and references therein) have done extensive, quantitative studies of tolerance development in the cat using their "maximally tolerated dosing method," but this method for chronic administration is not easily performed and it is not known if the results obtained will apply to other species

In the present studies we compared two effects of barbital—inhibition of avoidance behavior and loss of righting reflex The inhibition of avoidance may be attributed to an antianxiety effect but we have used it primarily as a readily quantitated action of the drug which would be expected (and was found) to occur at brain levels lower than those needed to cause the loss of the righting reflex We then proceeded to determine if functional tolerance developed to the effect of the barbiturates on avoidance behavior and if the degree of tolerance to this measure was different to that observed for the effect on the righting reflex Since Okamoto *et al* [15] have shown in cats that greater tolerance develops to the functions most affected by barbiturates during chronic

TABLE 1

BEHAVIOR OF SUBJECTS IN ONE-WAY AVOIDANCE TEST

| | Pretreatment | Injection on Test Day | N | Number of Trials to Criterion (mean ± SEM) | Number of Failures to Avoid (mean ± SEM) | Avoidance Efficiency (%) (mean ± SEM) |
|---|---|---|---|---|---|---|
| (1) | 33 days S | W | 03 | 83 7 ± 0 6 | 3 3 ± 2 33 | 94 ± 0 02 |
| (2) | 7 days S&B | W | 05 | 86 0 ± 1 1 | 1 0 ± 0 31 | 98 ± 0 01 |
| (3) | 33 days S&B | W | 09 | 84 2 ± 0 9 | 0 3 ± 0 17 | 94 ± 0 01 |
| (4) | 7 days S | B | 15 | 23 1 ± 4 4 | 4 9 ± 0 60 | 66 ± 0 05 |
| (5) | 33 days S | B | 13 | 22 0 ± 5 5 | 5 1 ± 1 42 | 63 ± 0 05 |
| (6) | 7 days S&B | B | 12 | 20 3 ± 3 9 | 4 4 ± 0 03 | 68 ± 0 04 |
| (7) | 33 days S&B | B | 10 | 15 5 ± 4 3 | 4 0 ± 1 25 | 54 ± 0 06 |

Rats were randomly assigned to groups, given one hour avoidance training and then put on a drinking schedule with sodium saccharin (S) or sodium saccharin and barbital (S&B) in the drinking water for 7 or 33 days as described in Method section On the test day the rats were given 250 mg/kg sodium barbital (B) or an equivalent volume of distilled water (W) IP and tested in the one-way avoidance behavior for 90 min or until they ceased avoiding (number of trials to criterion, see Method section) The brain levels of barbital were subsequently measured in these rats and those results are included in Table 2 Avoidance Efficiency is the percent of the total time in the chamber that was spent on the platform The number of failures to avoid equals the number of shocks taken Since there was no difference among the water injected groups (Rows 1–3) and the barbital injected groups (Rows 4–7), the data were collapsed The * indicates that the combined data from the water injected groups was significantly different from the combined barbital injected groups at $p < 0\ 05$

treatment, i e , those occurring at lower blood/brain concentrations, we anticipated a greater degree of tolerance to be developed to the inhibition of avoidance than to the inhibition of the righting reflex However this was not the case for these effects of barbital in rats made tolerant by administration of barbital in their drinking water

## METHOD

The experiments were done with male Wistar rats weighing 200–300 g at the beginning of the experiments and housed in a room with controlled lighting (lights on 0600–1800 hrs) The rats were randomly assigned to treatment groups and placed in individual cages with food and water ad lib unless otherwise stated (see below) After 3 days during which the animals were gentled by weighing and handling once each day, the rats were trained in the avoidance behavior

The avoidance training and testing was carried out in two one-way avoidance boxes [1] Standard electromechanical programming and recording equipment located in an adjacent room was used to program the contingencies and record the performance of the subjects in the apparatus To begin a training or testing session a subject was placed on the grid floor and a clock started At the end of 45 sec the grid was charged with a 1 mA electric shock from a Grason Stadlar shock generator When the subject jumped onto the platform breaking the photocell beam (escape), a second clock started At the end of 15 sec on the second clock the motor controlling the shield was energized for one complete cycle pushing the subject off the platform When the shield had returned to the retracted position the next trial started Each trial was one minute in duration If the subject jumped onto the platform before the shock was turned on (avoidance), the second clock did not start until the first clock's 45 sec had elapsed Thus the maximum time that the subject could spend on the platform in each trial was 60 sec

Each rat was trained for 60 min and then returned to its home cage The rats learned the avoidance response rapidly, the mean number of shocks taken per rat during training was 8 0±3 6 (mean±S D N=66) Within a day or two after training, rats were begun on a schedule of chronic barbital administration in their drinking water according to the schedule of Morgan *et al* [14] Barbital (acid form, B) in concentrations rising over 33 days from 1 0 to 4 0 mg/ml was dissolved in water with sodium saccharin (S) in concentrations rising from 20 to 80 $\mu$g/ml and made available to the rats ad lib as their sole source of fluid until they were tested Control rats received the appropriate sodium saccharin solutions without barbital For some experiments drug was provided for 7 days, the concentrations of B and S given on days 4–6 were made available for the additional seventh day

On the test day, rats were taken from their cages, weighed, and injected IP with either 250 mg/kg Na barbital or an equivalent volume of water (0 5 ml/250 g body weight) and returned to their home cages for 5 min They were then placed in the one-way avoidance box and a session was begun as described above Each subject was returned to the same box in which training had taken place Testing was continued until the subject met one of two criteria If the subject continued to avoid he was removed from the apparatus at 90 minutes If the subject had 3 failures to avoid in 5 trials he was removed from the apparatus However, if at least two of the three failures to avoid did not result in escape, the subject was dropped from the study (Only 2 subjects were dropped for this reason) All rats injected with barbital ceased avoiding within the 90 min test period, all of the rats injected with water continued to avoid for 90 min

To test for loss of righting reflex, the rats were taken from their cages, weighed, and injected with Na barbital as above and then placed in larger plastic cages for observation Tests for righting reflex were done every 15 min by placing the rats on their backs They were considered to have lost the righting reflex when they failed to right themselves 3 times in 30 sec

Animals that had met the criterion for loss of righting reflex or avoidance (or that did not cease to avoid after 90 min) were quickly taken to another room and decapitated Brains were removed rinsed with saline blotted dry

TABLE 2

BRAIN LEVELS OF BARBITAL AT LOSS OR RIGHTING REFLEX AND AT LOSS OF AVOIDANCE BEHAVIOR IN CONTROL AND CHRONICALLY TREATED RATS

| | | Pretreatment | | | | | |
|---|---|---|---|---|---|---|---|
| | | 7 day S | 7 day S&B | | 33 day S | 33 day S&B | |
| | | Brain Barbital Levels | | | | | |
| Behavior | | nmoles/g | nmoles/g | ratio | nmoles/g | nmoles/g | ratio |
| A | Successful Avoidance | | 278 ± 19 7 (5) | | | 480 ± 29 1 ( 9) | |
| B | Loss of Avoidance | 459 ± 12 1 (7) | 626 ± 19 5 (9) | 1 37 | 460 ± 30 3 (13) | 660 ± 49 5 (10) | 1 43 |
| | Loss of Righting Reflex | 574 ± 37 5 (5) | 849 ± 42 1 (5) | 1 48 | 858 ± 49 3 (5) | 1265 ± 53 7 ( 5) | 1 47 |

Rats were randomly assigned to groups, given one hour avoidance training and then put on appropriate drinking schedules with sodium saccharin (S) or sodium saccharin and barbital (S&B) in their drinking water as described in Method section The variable N arises because these data are the combined results of several experiments done testing various combinations of pretreatments and behaviors Nmoles barbital/g brain are given as mean ± SEM (N) A Rats drinking sodium saccharin and barbital were given 0 50 ml/250 g water IP and tested for ability to avoid for 90 min before sacrifice and measurement of brain barbital levels B Rats were given 250 mg/kg sodium barbital IP and decapitated when they ceased to avoid or when they lost their righting reflex and their brains were taken for subsequent assay of barbital as described in Method section Data were not included if the animal did not reach the criterion for loss of avoidance

weighed, and placed in vials for storage at −70°C until assay Barbital was extracted and assayed spectrophotometrically according to the method of Brodie *et al* [2] with minor modifications [16]

The data in each table were subjected to an analysis of variance followed by Fisher's Least Significant Differences Test [11] for comparisons of the means Statements in the Results and Discussion sections to the effect that means are the same or different are based on this test at $p<0$ 05

## RESULTS

Rats drinking barbital solutions consumed as much barbital as reported by Morgan *et al* [14] and as found in earlier experiments in this laboratory [16]—i e , 107±3 6 mg/rat/day (mean±S E M , N=20) at the end of the 33 day period The volume intake for the rats given barbital solutions remained relatively constant over the 33 day period (at about 25–35 ml/day) but the intake of the control animals increased so that the barbital treated rats drank only 61% of the volume of the control rats by the end of the 33 days The drug-treated rats also did not gain weight quite as rapidly as controls, at the end of 33 days the drug-treated rats weighed 89% of controls

Experiments to test possible interactions of weight loss and barbital on loss of righting reflex and inhibition of avoidance behavior indicated that there was no effect of weight loss on either effect of the drug Animals which were brought to 70–80% of the weight of a control group by reducing the food available lost their righting reflex at 968±44 nmoles barbital/g brain compared with 862±31 for the controls Similarly deprived rats ceased avoiding at 637±58 nmoles barbital/g brain compared with 561±90 in the controls (mean±S E M , N=5–7, neither pair of means was significantly different by Student's $t$-test at $p<0$ 05)

The first three rows of Table 1 present behavioral data for the control groups which were tested following water injection Rats in these groups continued to avoid throughout the 90 minute session Only one subject failed to avoid on more than one trial of the 82-plus trials that they performed This subject was one of the 3 in row 1, and his performance caused the high variability in the number of failures to avoid for this group Rats in all three groups showed the same high level of avoidance efficiency, 94%–98%, which was determined by dividing the time on the platform by the total session time This indicates that the latency to jump onto the platform was uniformly short, and that the subjects spent most of their time in the apparatus on the platform

The next four rows of Table 1 present the data of the subjects that were injected with barbital prior to the test session If the barbital injected rats are compared as a group with the water-injected rats two differences immediately become apparent First, the subjects of the barbital-injected groups met the criterion of failure to avoid before the 90 minute criterion was reached This effect on performance is seen both in the number of shocks taken and in the avoidance efficiency Avoidance efficiency was considerably and significantly lower for the barbital-injected groups compared to the water-injected groups This indicates that it took the barbital-injected groups longer to jump onto the platform Indeed on many trials the saline-injected rats would jump onto the platform before the shield which had brushed them off had completely receded to the wall This resulted in a zero time for the latency measure and a 100% time spent on the platform These data give an indication of the aversiveness of the grid

The rats which were given barbital and saccharin in their drinking water for 7 or 33 days and then tested for avoidance after a water injection did not cease to avoid They were decapitated and the levels of barbital in their brains remaining at the end of the 90 min period were measured (Table 2A) As expected the brain barbital levels in these rats were lower than the levels in rats injected with barbital and decapitated when they ceased avoiding (compare 278 vs 626

and 480 vs 660 nmoles/g, Table 2) The brain barbital level after 33 days of drinking and 90 min of avoidance behavior, 480±29 1 nmoles/g, was lower than that found in an earlier study for animals decapitated immediately after 30–33 days of drinking (700–850 nmoles/g, [16]) The lower values in the present experiment may be the result of a number of factors (a) the result of declining drug levels during the 90 minutes without intake, (b) the task—avoidance behavior—may somehow have reduced the brain levels or (c) the rats in this study did not achieve as high a brain concentration of barbital compared to those in earlier studies At the present time we have no data to distinguish among these factors

The brain level of barbital in control animals given a single injection of barbital and killed when they ceased to avoid, was relatively constant (459 and 460 nmoles/g, Table 2B) In control animals given a single injection of barbital and killed at the loss of righting reflex, the brain barbital level was always higher than the brain level at loss of avoidance behavior (574 vs 459 and 858 vs 460, Table 2B) The brain barbital level at loss of righting reflex was also more variable than the brain barbital level at loss of avoidance (574 and 858 nmoles/g, Table 2B)

Tolerance, defined as a higher brain level required to cause a given action, developed to both effects of barbital being measured The degree of tolerance can be expressed quantitatively as the ratio of the brain level of the drug at the behavioral endpoint in the animals receiving the chronic drug treatment and then a test dose, over the brain levels at the behavioral endpoint in the control animals given only the test dose A similar degree of tolerance developed to loss of avoidance and loss of righting reflex (1 37 vs 1 48 and 1 43 vs 1 47) In addition, there seems to be no increase in the degree of tolerance developed to either drug effect between 7 and 33 days of barbital drinking (compare 1 37 with 1 43 and 1 48 with 1 47) (Table 2B)

The degree of tolerance to the loss of righting reflex following 33 days of barbital drinking reported here (1 4–1 5 fold, Table 2) was less than the approximately 2-fold tolerance observed previously [16] Even though the drinking schedule was the same and the amounts of drug consumed by the rats were very similar, the brain levels of barbital achieved in the earlier experiments were apparently higher (see above) and the degree of tolerance to the hypnotic effect was higher than in the present experiments These results might support the reasonable notion that if higher brain levels are produced more tolerance will be developed

## DISCUSSION

We have observed that tolerance develops to the effect of barbital on avoidance behavior and the degree of tolerance achieved is quite similar to that found for the hypnotic effect The tolerance we have measured is functional tolerance and not metabolic tolerance, since barbital is not extensively metabolized and in any case we measured brain levels of the unchanged compound Further, it is not behavioral tolerance which will not be developed in our studies because each rat, after training in the absence of drug, was tested only once (see [7]) Other studies of barbiturates on avoidance behavior have examined the effect of chronic drug administration, or withdrawal from chronic administration, on the *acquisition* of this task [5, 10, 12, 13, 18] In our approach we looked at the effect of the drug, acutely or chronically, on the already-learned behavior

Our finding that the same degree of CNS tolerance developed to the two effects of barbital was not what we had expected on the basis of the work of Okamoto *et al* [15] in cats They have shown that greater CNS tolerance develops to those functions which are most affected by the drug during chronic treatment Since inhibition of avoidance behavior occurs at lower brain barbital levels than loss of righting reflex, it is reasonable to suggest that the avoidance behavior is more sensitive to the drug and would therefore have been more affected during the chronic treatment Thus we expected a greater degree of tolerance to develop to the inhibition of avoidance behavior Even if the inhibition of avoidance behavior we measured is really ataxia (which we don't think, see below), greater tolerance to this effect should be developed In fact, the finding that greater tolerance did not develop might be an argument that we are measuring an effect of the drug on some other aspect of the (learned) behavior

There are many differences in the procedures used in the present studies compared with those of Okamoto *et al* [15] including different species, different method of chronic drug administration, and different method of tolerance assessment Further studies will be necessary to determine if the "rule" for the degree of tolerance development described by Okamoto *et al* [15] in cats also applies to rats, and if it does, to determine why the effect of barbital on avoidance behavior doesn't follow this pattern

The inhibitory effects of barbital on avoidance could be explained by a primary action of the drug to cause ataxia and sedation or to a drug-induced loss of anxiety Cook and Weidley have shown that the barbiturates tend to inhibit avoidance and escape behavior at similar doses (in contrast to the phenothiazines) and suggested that the effects of the barbiturates on avoidance were nonspecific [4] However our data suggest that this may not be the case The criterion for inclusion in the study was that on the three trials in which the subject failed to avoid, the subjects must escape on two of them Of the 52 subjects that were injected with barbital prior to the test, only 2 subjects were dropped for not meeting the escape criterion although a number of subjects did fail to escape once This indicates that when the subject ceased avoiding they were still capable of jumping onto the platform, even though they did appear somewhat ataxic

Barbiturates and benzodiazepines but not neuroleptics seem to have clinical anxiolytic actions and the anxiolytic effects correlated with suppression of punished responding [3] However, since phenothiazines are able to inhibit avoidance fairly selectively, yet are not specifically effective in punished responding [3], it is possible that inhibition of avoidance is not a good measure of anxiolytic action Our demonstration that tolerance develops to the anti-avoidance effect of barbital might also suggest that this action is not related to an anxiolytic action if it is true that tolerance does not develop to the anti-anxiety effects of barbiturates or benzodiazepines [17]

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