Loss of inhibitory motor control following a subanesthetic dose of thiobarbiturate in rhesus monkeys

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An operant paradigm was employed to examine the magnitude and duration of the behavioral excitation induced by a subanesthetic dose of a short-acting thiobarbiturate drug. Thus, .5 h following a single intravenous injection of sodium thiamylal (Surital, P.D.; 12.5 mg/kg), the mean response rate of monkeys previously trained to barpress for food reinforcement on a VI 30-sec schedule was increased threefold. By 3 h postinjection, drug and saline means were statistically indistinguishable and by 4 h, they were virtually identical. Analysis of pauses and interreinforcement response rates from cumulative records showed a tendency for drugged animals to respond in rapid bursts, and undrugged animals to maintain the steady, slow response rate characteristic of performance on a variable-interval schedule. Possible CNS mediation of this behavioral excitation in terms of interference with inhibitory reticular formation processes is discussed.

Despite their total lack of analgesic potency, the barbiturates have accrued a long history of use as a class of extremely valuable anesthetic agents for aiding in surgical procedures in both animals and man (Goodman & Gilman, 1970). The central activity of these compounds is thought to occur in the polysynaptic pathways of the midbrain reticular formation, which structures have been strongly implicated by other means in the regulation of basic motor functions (Magoun, 1950; Ward, 1958), and in mediating sleep and arousal (Lindsley, 1960).

A good deal of evidence now points to the existence of two complementary functions of the reticular formation: thus, the activity of a rostral brainstem/diencephalic activating system (RAS) apparently mediates behavioral arousal, while a more caudal region—possibly the medial bulbar reticular formation (Magoun, 1944; Magoun & Rhines, 1946)—serves to suppress ongoing behavior at least in part by inhibition of the RAS. Normal waking behavior, then, may be viewed as the resultant balance in activity between such systems, and sleep as the result of active inhibition of the RAS by inhibitory centers (Jouvet, 1969; Killam, 1962).

Larrabee and Posternak (1952) have shown the primary neural action of anesthetics to be to interrupt synaptic transmission. Barbiturate-induced anesthesia may thus be thought to obtain when behavioral arousal

is prevented by sufficient depression of RAS activity. However, it is also possible to demonstrate that hyperarousal may be induced by barbiturates under certain conditions. Thus, doses of thiopental insufficient to produce unconsciousness decreased the threshold for the sensation of pain in human patients during induction and recovery phases of anesthesia (Clutton-Brock, 1961).

Other evidence for such hyperarousal has been obtained from animal experiments: Brazier (1961) observed a transitory phase of electroencephalographic arousal shortly after injections of either thiopental or pentobarbital, which reappeared following the usual anesthetic coma. This suggestion of a triphasic effect of barbiturates upon arousal was substantiated by Verhave (1959) in rhesus monkeys trained on a complex operant schedule. In this experiment, subcutaneous secobarbital produced a brief increase in barpressing on a multiple FI 2 min/FR 40 schedule, followed by a longer period of behavioral suppression, and finally by a phase of responding at suprabaseline rates which lasted at least 30 min.

Brodie (1952) has shown that a 5-min delay follows a single intravenous injection of barbiturate before maximal levels of the drug are reached in CSF and, following this maximum, the decline in concentration of barbiturate in this fluid is gradual and parallel to the decline in plasma concentrations. Thus, for a brief interval before, and a longer one after, the point of maximal drug concentration in CSF, intermediate levels of the drug may be expected in brain tissue surrounding the ventricular system. It is precisely during these time intervals that behavioral hyperarousal is manifest in experimental animals and in human surgical patients; thus, by positing that a reticular inhibitory mechanism is

This research was supported by the National Institutes of Health Grant RR-00167 awarded to the Wisconsin Regional Primate Research Center. We gratefully acknowledge the assistance of J. W. Davenport of the Wisconsin Regional Primate Research Center and W. T. McKinney of the University of Wisconsin Primate Laboratory without whose equipment and animals these data would not be available. Publication No. 14-012 of the Wisconsin Regional Primate Research Center. Perry Maloff is now at the New York Downstate Medical Center, State University of Brooklyn, New York.

sensitive to lower concentrations of barbiturate than is the RAS, one can explain the triphasic time course of barbiturate anesthesia on this basis. In effect, hyperarousal occurs when the inhibitory functions of the brainstem, but not the activating functions of the RAS, are interrupted by the drug; anesthesia occurs for the period during which the drug concentration is high enough to disrupt RAS function as well (cf. Brazier, 1961).

If a low concentration of a short-acting barbiturate anesthetic is introduced into an animal, anesthesia should be incomplete, and "recovery" should proceed immediately. This recovery should be characterized by an initial state of behavioral hyperarousal which should decline with the same time course as that of the concentration of the drug in the CNS.

To quantify the time course of this "recovery," we utilized an operant paradigm in which the rate of barpressing for food reward was taken as a measure of arousal. A simple variable-interval schedule was employed so that changes in the baselines of response rate and response patterning could easily be observed as a function of the time interval following administration of the drug.

METHOD

Subjects

The subjects were six laboratory-reared juvenile rhesus monkeys (*Macaca mulatta*) of both sexes, all of which had received extended training in the conditioning apparatus in prior experiments.

Apparatus

All testing was carried out in a single Lehigh Valley Electronics large primate conditioning chamber (No. 143-40), equipped with a retractable response lever and an illuminable foodwell. Outside the dark, sound-insulated test room which housed the chamber throughout the experiment, automatic relay equipment was programmed to extend and retract the lever, to deliver reinforcers (a random mixture of corn, peanuts, commercial cereal, and chocolate candies) on a VI 30-sec schedule, and to record both the number of responses made by the subject and the number of reinforcers delivered to it. In addition, cumulative records of each animal's performance during each session were kept for analysis of response characteristics under drug and saline conditions.

Design and Data Analysis

The independent variables manipulated here were the drug treatment (drug vs. saline) and the pulse time (PT), or that interval in hours between the time of injection of the drug and the time that the subject was introduced into the conditioning chamber, measured to the nearest minute. A within-subjects repeated-measures design was employed, in which each subject's performance was observed twice at each of six different PTs (.5, 1.0, 1.5, 2.0, 3.0, and 4.0 h), once after the drug and once after saline. The randomly determined order in which the PTs were assigned to each subject was arranged on a single 6 by 6 Latin square with subjects and PTs as factors. In this way, each PT was represented equally often at each stage of the experiment. The experiment was run in six 2-day test blocks, two blocks per

week, such that each block represented one PT for each subject. On one of the 2 days of each block, the subject received drug and on the other, saline; this order too was randomly determined.

Following completion of the experiment, three measures of the effects of the drug treatment were taken: (1) the response rate in responses per minute (R/min) for each subject on each drug condition and at each PT was calculated as the ratio of the total responses it emitted to the total time it responded in the apparatus. Each cumulative record was then scored for (2) the number of response bursts and (3) the frequency of prereinforcement pauses, using the following criteria. A response burst was defined for each animal as any interreinforcement response rate which exceeded by at least two standard deviations the mean response rate of that animal's saline sessions. A prereinforcement pause was scored in a particular subject's session for any time interval which was (a) devoid of responses; (b) preceded by at least one response after delivery of the previous reinforcer; and (c) at least two standard deviations longer than the mean interresponse interval for that animal's saline sessions.

The R/min data were analyzed by a two-way repeated-measures analysis of variance, with drug and PT as factors. The response burst data were subject to the logarithmic transformation $X' = \log(X + 1)$ to reduce the heterogeneity of variance existing between the drug and saline conditions, and were then analyzed by a similar two-way ANOVA. The frequency of prereinforcement pauses was so low within a PT that these data were pooled across PTs and analyzed by a direct-difference t test between the drug and saline means.

Procedure

Prior to the initiation of the experiment, each subject was given a number of sessions (about 12) in the apparatus sufficient to stabilize his response rate on the VI 30-sec schedule. At least six more sessions were then administered following saline injections, to adapt the animals to the stress of the handling involved in the injection procedure. Thereafter, Surital (thiamylal sodium, P.D.), 12.5 mg/kg, or an equal volume (3 to 5 cc) of saline, was administered by slow infusion into the saphenous vein of each subject while it was temporarily restrained in a squeeze cage. This dose (half the nominal anesthetic dose) produced a light anesthetic state in the animals, characterized by muscular flaccidity, reduced respiratory rate, and physical tractability; it did not, however, obviate basic pain or corneal reflexes. The 12.5-mg/kg dose was selected for this study since it was the largest dose which resulted in recovery of gross postural and locomotor abilities adequate to permit the animals to respond appropriately in the conditioning apparatus after all the PTs used in the experiment.

Following the appropriate time interval, the subject was introduced into the conditioning chamber and allowed to respond until it had earned all 36 available reinforcers or until 30 min had elapsed. (In only one session of the 72 run did an animal fail to earn all 36 reinforcers.) Each animal was then returned to its home cage and fed. Each subject was tested once per day, and had been deprived of food for at least 20 h prior to testing.

RESULTS

Figure 1 shows the mean R/min values, plotted as a function of PT, for the drug and saline conditions. A threefold increase in response rate can be seen at .5 h, and a steady decline in rate until baseline performance is reached at 4 h. Analysis of variance on these data

Figure 1. Mean R/min values plotted as a function of PT for saline and drug conditions. Starred points differ significantly from the saline mean at the 5% level.

Figure 2. Mean frequency of response bursts as a function of PT for saline and drug conditions.

confirmed the suggestions of a main effect of drug ($F = 15.42$, $df = 1/5$, $p < .025$) and Drug by PT interaction ($F = 2.65$, $df = 5/25$, $p < .05$). In addition, Dunnett's (1955) procedure for comparing experimental means to a control value (Winer, 1962) was applied to the data with the mean saline value (6.38 R/min) as the control. With $\alpha = .05$, the drug R/min values at .5, 1.0, 1.5, and 2.0 h differed significantly from all saline trials, while drug R/min values at the longer PTs did not.

Drug treatment did not affect the frequency of prereinforcement pauses: the mean frequency of pauses under Surital was nearly identical to that occurring under saline (Surital, 8.17 ± 4.24 ; saline, 10.67 ± 5.53)¹. This difference is clearly nonsignificant ($t = .725$).

By contrast, the frequency of bursts in all drug sessions exceeded that found in all saline sessions (Surital, 81.00 ± 11.96 ; saline, 12.00 ± 2.45)¹; Figure 2 shows the mean frequencies plotted as a function of PT. Analysis of variance on the (log transform) data confirmed the apparent effects of drug ($F = 307.14$, $df = 1/5$, $p < .001$) and of PT ($F = 3.58$, $df = 5/25$, $p < .025$). Furthermore, the frequency of bursts in the drug condition dropped significantly between 2 and 3 h ($t = 3.09$, $df = 5$, $p < .05$), that is, at the same PT at which the overall response rates declined to control levels (Figure 1).

DISCUSSION

Clearly, facilitation of operant responding follows from treatment with a subanesthetic dose of thiobarbiturate, in confirmation of previous reports of hyperarousal during induction to, and recovery from, anesthesia (Brazier, 1961; Clutton-Brock, 1961; Verhave, 1959). Moreover, the present technique permits a fairly precise quantification of the magnitude and the time course of this effect. The significant, threefold increase in response rate seen at the .5-h PT declines monotonically to statistical insignificance at 3 h and to virtual identity with the saline mean at 4 h. This decline in response rate parallels the decrease in plasma thiamylal concentration as determined by Brodie (1952) in dogs and humans, and furthermore follows nearly precisely the same time course. Unfortunately, Brodie (1952) does not provide data on the levels of thiamylal in CSF, but indications from his data with thiopental are that clearance of this drug from CSF and plasma are essentially parallel functions, and that plasma levels can therefore be expected to provide a good indication of CSF levels as well. If such be the case, then Brodie's finding that Surital concentrations in plasma reach relatively stable levels (due to the drug's sequestration in body fat) by 4 h is in substantial agreement with the present data, which indicate that behavioral

hyperarousal returns to normal levels with the same latency.

Under the neural model proposed in the introduction, subanesthetic levels of barbiturate interfere with activity in a reticular inhibitory system to a greater degree than in the RAS. Such loss of inhibitory function should lead to a concomitant loss of control of motor activity, particularly in view of the importance of the medial bulbar portion of the reticular formation as a modulator of motor function (Magoun, 1950). Observations of the monkeys in this experiment during recovery from the drug effects are consistent with such a model: typically, such animals cannot control their movements, and they commonly lunge and thrash about their cages when stimulated by the presence of an observer. If left alone, by contrast, they tend to sit quietly and do not appear strikingly different from untreated animals. It appears that control of movement, rather than its initiation, is impeded under these circumstances.

A similar lack of control may explain the increased response rates observed following drug treatment. Responding on a VI schedule normally involves the subjects' emitting a steady train of evenly spaced responses at a relatively low rate (6.38 R/min in this experiment). After treatment with Surital, however, such steady, paced responding is replaced by the initiation of response bursts, characterized by a rapid rate and persistence for the duration of the time interval, i.e., until a reinforcer is delivered (Figure 3).

The analyses of the response burst and pause data serve to quantify this change in response topography. Pauses invariably follow delivery of a reinforcer, since, in this experiment, the lever was retracted at the time of reinforcement, and the animal took a short time to ingest the food pellet. However, pauses also occasionally occurred after some number of responses but prior to reinforcement (prereinforcement pauses); such pauses always preceded initiation of another train of responses which typically terminated at the end of the time interval, concurrent with reinforcement delivery. Thus, the total number of pauses (prereinforcement plus postreinforcement) may be considered to reflect the number of response trains that an animal generated over the course of a test session. Since the frequency of postreinforcement pauses was constant (36 reinforcers were delivered during each session) any change in this response characteristic must be reflected in a change in the frequency of prereinforcement pauses. The near identity of the mean frequencies of prereinforcement pauses in the Surital (8.17) and saline (10.67) conditions suggests that drug treatment did not alter the animals' initiation of response trains.

On the other hand, the number of response bursts emitted by the animals following treatment with Surital far exceeded that emitted by animals after saline

Figure 3. Representative cumulative records from three animals at three PTs, demonstrating the characteristics of VI responding under saline (left) and Surital (right). Note steeper slopes and postreinforcement pauses in Surital records in contrast to the steady, paced responding apparent under saline.

injections (Figure 2). For the first four PTs, a nearly eightfold increase in this measure characterized the responding of the drugged animals; the drop in this frequency at 3 h parallels the drop in mean response rate to control levels (Figure 1). In this regard, it may be noted that, while the overall response rates of the drugged and undrugged animals were indistinguishable by 3 h postinjection, the frequency of response bursts remained at an elevated level for as long as 4 h ($t = 5.06$, $p < .01$), suggesting this latter measure to be a more sensitive index of the effects of the drug than is the overall response rate.

This analysis of the response topographies is, of course, limited by the accuracy with which time intervals and slopes can be measured from cumulative records. However, the difference in burst frequency from saline to drug is so great, and the similarity of pause frequency so close, that whatever error may have accrued in the process of measurement is unlikely to interfere with its interpretation. We, therefore, conclude

that a subanesthetic dose of Surital affects the ability of these monkeys to inhibit responses once a response train has been initiated, but does not affect the initiation of such trains per se. In other words, once a drugged animal begins responding, he tends to continue to do so at an elevated rate until interrupted by delivery of a reinforcer. Thus, the increased overall response rate produced by Surital treatment may be attributed to a suppression of the ability to inhibit unnecessary motor responses, rather than to a general excitation of an arousal system.

The model proposed to account for the facilitation of response rate on behavioral grounds is consonant with accepted ideas regarding the nature of the effects of barbiturate drugs on the CNS (see Introduction). Interference with synaptic transmission in an inhibitory motor control center (e.g., the bulbar reticular formation) should lead to the effects described at the behavioral level. That a subanesthetic dose of barbiturate produces behavioral excitation implies that the RAS is more resistant to the action of the drug than is the inhibitory portion of the reticular formation; elucidation of the neural mechanism mediating this sensitivity difference requires further experimentation.

Some implications of this work may be mentioned. First, the combination of barbiturate treatment with the operant paradigm and data analysis used may provide a useful analytical tool for investigating reticular control of behavior. For example, pretreatment with drugs affecting neurotransmitter metabolism may yield valuable information regarding neurochemical mediation of behavioral arousal.

Second, it is imperative that any behavioral experimentation requiring treatment with barbiturates take into account this persistent drug-induced hypermotility. A sufficient time interval between administration of the drug and testing must be maintained to avoid contamination of the data with these prolonged (and probably unexpected!) effects of the anesthetic. The 3- to 4-h time course observed on the

rate data with Surital could well be taken as a *minimum* duration of barbiturate effect, as this drug is one of the fastest acting barbiturates commonly available. Moreover, burst frequency analysis (Figure 2) indicates that some effects of the drug may persist beyond 4 h and be evident under such a response topography analysis, despite the return to baseline performance on a simpler response-rate measure.

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NOTE

1. Mean $\pm$ standard error of the mean.

(Received for publication November 5, 1974;
accepted December 9, 1974.)