Pharmacology Biochemistry & Behavior, Vol. 15, pp. 583–586, 1981. Printed in the U.S.A.

Pentobarbital Induces a Naloxone-Reversible Decrease in Mesolimbic Self-Stimulation Threshold

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Received 20 May 1981

SEEGER, T. F., K. R. CARLSON AND J. M. NAZZARO. *Pentobarbital induces a naloxone-reversible decrease in mesolimbic self-stimulation threshold.* PHARMAC. BIOCHEM. BEHAV. 15(4) 583–586, 1981.—The effects of sodium pentobarbital and naloxone were tested on intracranial self-stimulation (ICSS) in rats implanted with electrodes in the ventral tegmental area. Threshold for ICSS was determined using a rate-independent current titration paradigm. A low dose of pentobarbital (5 mg/kg) did not have a significant effect on ICSS thresholds, while a high dose (20 mg/kg) rendered the subjects too ataxic to respond reliably in the operant task. An intermediate dose (10 mg/kg) induced a highly significant lowering of threshold (17% below saline baseline levels) without apparent deterioration in response capability. The concurrent administration of naloxone (2 mg/kg) significantly reversed the pentobarbital-induced threshold decrease, while naloxone treatment alone had no effect on the ICSS threshold.

Intracranial self-stimulation (ICSS) Pentobarbital Naloxone Ventral tegmentum

DRUG abuse in humans takes the form of self-administration of a variety of agents, belonging to several broad drug classes. The spectrum of abused drugs (including opiates, stimulants, depressants and sedative-hypnotics, minor tranquilizers, hallucinogens, and dissociative anesthetics) includes agents which differ markedly in terms of their perceived drug effects and in their primary mechanisms of action but which presumably act on common final reward substrates.

One of the animal behavioral models with which the effects of these agents on reinforcement mechanisms can be studied is intracranial self-stimulation. Increases in response rate for ICSS or decreases in the current level required to sustain ICSS behavior (reflecting a sensitization of central reward processes) have been demonstrated for a number of commonly abused drugs, including opiates [4, 11, 15] amphetamine and cocaine [5,20], chlordiazepoxide and alcohol [12], and phencyclidine [14].

We have report the effects of pentobarbital, another agent of common abuse potential, on ICSS behavior, using rats implanted in the dopaminergic ventral tegmental area, and performing a rate-independent threshold titration task to determine changes in reward sensitivity.

With regard to the possible mechanisms of reinforcement which may be activated by drugs of abuse, the enhancements in ICSS response produced by a number of these agents have been shown to be antagonized by concurrent treatment with the opiate antagonist, naloxone, although no effect is seen under the same test conditions when naloxone is given alone [5, 9, 12, 14, 15].

Naloxone and naltrexone have been shown to antagonize a number of barbiturate effects, such as the loss of righting reflex and acute toxicity in the rat [6] and the loss of flexor reflex and consciousness in the spinal dog [8]. Other barbiturate effects, such as increase in pulse rate and decrease in respiratory rate, are not blocked by opiate antagonist treatment [8]. The possibility that any barbiturate effects on ICSS may be mediated by endogenous opiate peptide systems was tested via concurrent administration of naloxone with pentobarbital.

METHOD

The experimental subjects were 17 male Sprague-Dawley rats, weighing approximately 350 grams at the time of surgery. The animals were anesthetized with sodium pentobarbital (45 mg/kg IP) and implanted with bipolar stainless steel electrodes (Plastic Products, type MS303) using standard surgical and stereotaxic techniques. The electrodes were aimed at the ventral tegmental (A10 cell body) area at coordinates: AP +2.6, L ±1.2 and H −3.5 according to the atlas of Pellegrino [17]. ICSS training and testing were conducted

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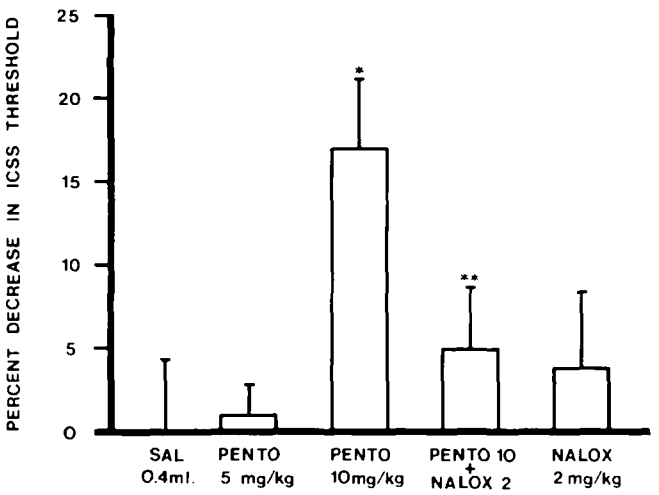

FIG. 1. Effects of pentobarbital and naloxone on intracranial self-stimulation (ICSS) thresholds in rats. Data is expressed in percent decrease below average saline baseline of 140 μA ± SD. *$p<0.001$ vs saline tests, **$p<0.02$ vs pentobarbital tests.

in a clear Plexiglas cage measuring 22.5×20×17.5 cm high equipped with two 5×2 cm levers mounted side by side 6.5 cm apart on one wall. Each depression of the stimulating lever delivered a 250 msec stimulus train through the implanted electrode, consisting of 60 Hz bipolar rectangular pulses of 0.3 msec duration, with a 0.15 msec delay between the pulses of opposite polarity. Maximum stimulating current was 250 μA, which was decremented by 15 μA after every lever press (N=8) or after every third lever press (N=9). The animal could reset the current to the maximum level at any time by pressing the second lever, which also activated a signal tone but did not deliver stimulating current. The current step at which the animal chose to reset was automatically recorded and used to generate a reset frequency distribution for analysis. The mean of this bell-shaped reset distribution was defined as the ICSS current threshold for the test session, while the standard deviation was taken as a relative measure of response integrity (that is, an increase in the standard deviation for the drug trials would be an indication of a deterioration of performance or ataxia) [20].

After each animal had reached stable performance levels in the threshold paradigm, baseline levels were determined on three consecutive test days fifteen minutes after injection of 0.4 ml of 0.9% saline. Test sessions were 15 min each, preceded by a 3 min warm-up period during which data were not recorded. On succeeding drug test days, the 17 rats were treated by intraperitoneal injection with either sodium pentobarbital (10 mg/kg) or a combination of naloxone HCl (2 mg/kg) and sodium pentobarbital (10 mg/kg) in random order, all drugs being dissolved in 0.9% saline and administered 15 min before the ICSS test was begun. In addition, subgroups of the same set of animals were also given other doses of sodium pentobarbital (5 mg/kg: N=6, 20 mg/kg: N=8), or naloxone alone (2 mg/kg: N=9).

Following the completion of testing, verification of electrode placement was performed on sixteen animals. The rats were perfused intracardially with 10% buffered Formalin.

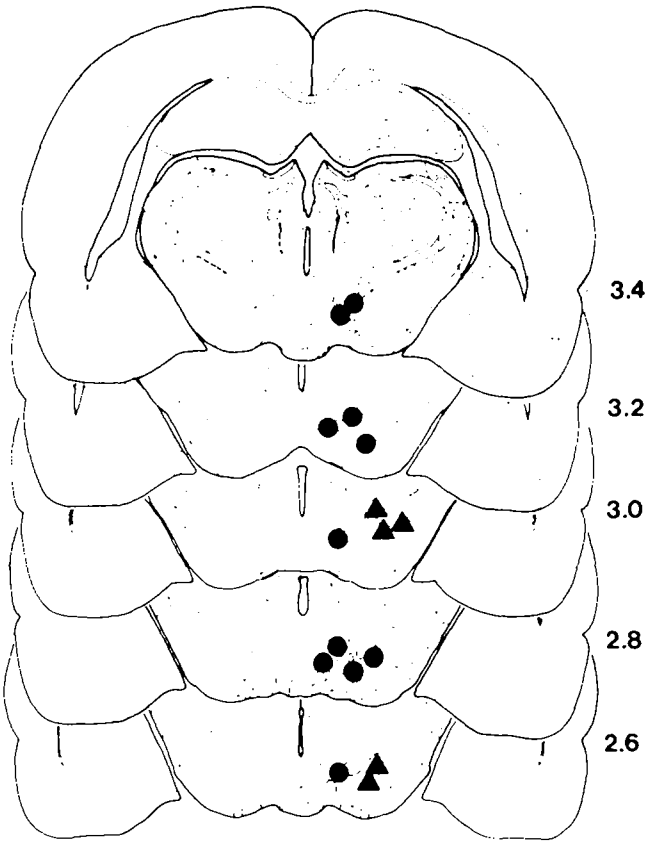

FIG. 2. ICSS electrode placements according to the atlas of Pellegrino *et al.* [17]. Placements which showed a naloxone-reversible threshold decrease are indicated by circles (●). Placements which did not decrease due to pentobarbital or did not reverse upon naloxone treatment are indicated by triangles (▲). Vertical plane coordinates are in mm anterior to the interaural line.

Frozen sections surrounding the electrode track area were cut at 40 micron thickness and stained with cresyl violet.

RESULTS

The seventeen rats stabilized at threshold levels ranging from 90–190 μA in the baseline saline trials. The average basal threshold was 139.7±7.5 μA. The mean standard deviation within the individual saline runs was ±15.2 μA, equal to a variation of one current step about the mean. As shown in Fig. 1, results after treatment with naloxone alone, or the lowest dose of pentobarbital were not significantly different from saline: $t(8)=2.05$, p=NS for naloxone vs saline; $t(5)=0.22$, p=NS for pentobarbital vs saline. At a dosage of 10 mg/kg pentobarbital, there was a significant decrease in threshold current, averaging 17% below baseline levels: $t(16)=5.06$, $p<0.001$ vs saline. This decrease ranged from 6–37% for individual rats, with only one rat failing to show a decrease. The average standard deviation for the individual test sessions was nearly identical to that seen for the same animals when given saline, indicating that the threshold decrease was not caused by disorientation or performance difficulties.

When pentobarbital (10 mg/kg) was combined with naloxone (2 mg/kg), there was a significant reversal of the threshold decrease caused by pentobarbital alone, to 4.9% below saline baseline: $t(16)=1.97$, p=NS vs saline, $t(16)=2.78$, $p<0.02$ vs pentobarbital. Thirteen rats showed some degree of reversal, while in four rats the threshold after the pentobarbital-naloxone combination was equal to or lower than in the pentobarbital test. There was no change in the average standard deviations for the individual runs during the naloxone-pentobarbital tests.

Finally, a pentobarbital dose of 20 mg/kg produced obvious sedation and ataxia, such that all eight rats tested at this dose either were unable to respond, or produced widespread and therefore meaningless reset frequency distributions.

Histological examination of 16 electrode placements indicated a clear site-specificity. As shown in Fig. 2, those placements which demonstrated a naloxone-reversible decrease in ICSS threshold after 10 mg/kg pentobarbital were primarily located within the target ventral tegmental area or else just anterior to it, in an area of the posterior hypothalamus through which the A10 axons pass. However, those rats which did not respond to pentobarbital, or did not show a reversal of pentobarbital effect by naloxone were more laterally placed, primarily in the substantia nigra, pars compacta.

DISCUSSION

In 1960, Olds and Travis described the effects of pentobarbital on self-stimulation rate in the rat [16]. Using four different placement sites, including the ventral tegmentum, they found no consistent changes in rate (25% of the rats increased their ICSS rate, 12% decreased and the rest were unchanged). The changes in rate were greatest at a dose of 10 mg/kg, but this and higher doses adversely affected the performance of the rats in a control test of motor ability (escape from aversive stimulation) using the same lever pressing task. The rate-independent threshold titration task used in the present investigation was designed to control for such confounding effects, and has proven its utility in testing the effects of other disorienting or sedative agents such as phencyclidine and morphine on ICSS sensitivity [14,15].

The present results indicate that, at a dosage which does not interfere with operant responding, pentobarbital causes a clear enhancement of reinforcement sensitivity. This increase in ICSS sensitivity takes place in the ventral tegmental area, a site at which increases of similar magnitude have been demonstrated for other agents with abuse potential [5, 14, 15] and which also supports direct local self- administration of opiates in rats [18].

In addition, the finding that the pentobarbital-induced decreases in ICSS threshold can be reversed by naloxone suggests a mechanism of enhancement involving endogenous opiate peptide systems. The electrode placements used in this study impinge upon the cell bodies of the mesolimbic dopaminergic system and self-stimulation of this area can be modulated by dopamine agonists and antagonists [7]. However, this system is also positively modulated by opiate agonists toward increased ICSS rate and decreased ICSS threshold [2,15]. Thus, the role of pentobarbital may be to cause an enhanced activity in enkephalinergic neuronal systems; in this case, in the terminal region of the mesolimbic DA system, the limbic forebrain.

Of further interest in this regard was the general trend that threshold decreases due to pentobarbital were reversed by naloxone at ventral tegmental (mesolimbic) placements, but not at placements in the substantia nigra. We have previously demonstrated a similar relationship in regard to naloxone reversal of morphine effects on ICSS current threshold [15].

Activation of enkephalinergic mechanisms has also been postulated to account for the increased ICSS rate caused by treatment with ethanol or chlordiazepoxide, on the basis of reversal by concurrent naloxone administration [12]. This theory is further supported by recent studies showing that acute treatments with ethanol or diazepam cause rapid changes in met-enkephalin levels in discrete areas of rat brain [3,19].

More relevant to the current study is the very recent demonstration that many barbiturates, including sodium pentobarbital, are potent in vitro blockers of enkephalinase, the primary degradative enzyme for endogenous opiate peptides [1]. The IC_{50} for this effect is 18 μM, while a dose of 10 mg/kg of pentobarbital will produce a plasma concentration of approximately 40 μM. Therefore, it seems likely that the increased reinforcement sensitivity seen after barbiturate treatment is due at least in part to an increase in endogenous enkephalin levels. Similar mechanisms may possibly explain the effects of other drugs of abuse on ICSS in laboratory animals, as naloxone has also been shown to block increases in ICSS rate caused by amphetamine, morphine and heroin [9,10] and decreases in ICSS threshold caused by amphetamine and phencyclidine [5,14].

ACKNOWLEDGEMENTS

This research was supported, in part, by USPHS grant DA-02089 to Dr. W. H. Bridger and USPHS grant DA-02226 to Dr. Kristin R. Carlson. Development of the ICSS paradigm was supported, in part, by the U.S. Air Force (Aeromedical Division) under Research Project 6893-02-039 to Dr. E. L. Gardner. The naloxone was generously donated by Endo Laboratories, Inc. We thank Dr. E. L. Gardner and Marie Elbert for assistance with the histological material.

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