Pharmacology Biochemistry & Behavior, Vol. 7, pp. 413–416.

Etorphine and Shuttle-Box Self-Stimulation in the Rat

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(Received 29 June 1977)

BALTZER, J. H., R. A. LEVITT AND J. E. FURBY. *Etorphine and shuttle-box self-stimulation in the rat.* PHARMAC. BIOCHEM. BEHAV. 7(5) 413–416, 1977. – Rats were trained to turn rewarding electrical brain stimulation on and off by crossing back and forth in a shuttle-box. Moderate doses of the narcotic analgesic, etorphine, increased mean ON times while having little effect on OFF times. Tolerance did not develop to the reward enhancement action over five consecutive days of injections. This paradigm seems valuable for exploration of the reinforcing properties of narcotic drugs.

Etorphine Reward Narcotics Shuttle-box Self-stimulation

THE self-stimulation method has been used as a means of studying the reinforcing properties of narcotic drugs [1,16]. Using this approach the interaction between the narcotics and electrical stimulation has been interpreted as reflecting a facilitation of the rewarding effects or an inhibition of the aversive effects of electrical stimulation [12,13]. Studies employing the prototype narcotic, morphine (in analgesic doses; 5–10 mg/kg), and a standard lever press paradigm have found the drug to have a biphasic action on self-stimulation. There is an initial strong inhibition of lever pressing (rates reduced to 0 to 25% of control levels) lasting about three hours, followed by an enhancement of lever pressing rates (rates increased to about 200% to 300% of control levels) lasting from about the third to sixth hour after injection. The early inhibitory action is thought to result from confounding sedation or catatonia produced by narcotics, while the later enhancement may reflect a facilitation of reward processes [1, 4, 12, 13].

With repeated daily injections, tolerance develops to the initial behavioral inhibition, leading to an augmented and progressively earlier enhancement of self-stimulation lever pressing. Tolerance, however, has been found in several studies not to develop to the enhancement [4,13]. The later reward enhancement may be related to the mood enhancing or euphoric action of narcotics which has some role in the development of human drug dependence [5, 9, 10]. However, the biphasic nature of the action of narcotics on self-stimulation, and the discrepancy between the time course of the analgesic and mood-altering actions (within a few minutes of injection) [8, 9, 10] and the time course of the reward enhancement in the rat (3 to 6 hr after injection) [4,13] have limited the utility of this system as an animal model.

Recently, doses of morphine have been found to reduce the reward threshold for electrical stimulation shortly after drug injection, and tolerance has also been found not to develop to this action [7]. Recently also, analgesic doses of morphine have been found to selectively affect the behavior of rats self-stimulating in a shuttle-box paradigm [11]. Doses of 5 or 10 mg/kg morphine increased the amount of time rats left the stimulation on at each shuttle without altering OFF times. This action began within ten minutes of injection and lasted for at least ninety minutes. The effect was also quite dependable and robust; ON times doubled. Since this paradigm appears quite promising, the current experiment investigated the actions of a second, extremely potent narcotic analgesic, etorphine [2,15], on shuttle-box self-stimulation, and also evaluated the development of tolerance.

METHOD

Animals and Surgery

Adult Long-Evans strain rats of both sexes (weighing 250–350 grams) were used. Animals were housed individually with free access to food and water. Under sodium pentobarbital anesthesia, each animal was stereotaxically implanted with a stainless steel bipolar electrode insulated except at the tip (Plastic Products Co.). The electrodes were aimed for the medial forebrain bundle as it passes through the lateral hypothalamic area, a site commonly used in self-stimulation research [4, 13, 16]. Implant coordinates were 0.4 mm posterior to bregma, 1.75 mm lateral to the midsaggital suture, and 9.5 mm below the surface of the skull [17].

Apparatus and Procedure

Testing occurred in wire mesh cages (shuttle-boxes) measuring 35 × 20 × 20 cm, set on a fulcrum at the center, and with a microswitch under one end of the cage. The

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shuttle-boxes were adjusted so that the animal's weight on one side of the cage would close the microswitch, which would open when the animal moved to the other side of the cage.

Electrical stimulation to the brain was provided by a Grass square wave stimulator (BPS–1; 60 Hz, 300 to 400 μA). Current was monitored on a Tektronix oscilloscope (5103N). A bipolar flexibile cord covered by stainless steel wire (Plastic Products Co.) connected the implanted electrode to the stimulator via a mercury commutator (Scientific Prototype Co.). Standard electromechanical devices (Lehigh Valley Co.) were used to program and time the experiment.

One week was allowed for surgical recovery. Animals were then tested in the shuttle-box for 90 min a day on either four (1.0, 10.0, 40.0 μgm/kg groups) or ten consecutive days (20 μgm/kg and saline groups). On the first day of testing each animal was placed in the shuttle-box and connected to the stimulator set at 350 μa. Mean ON and OFF times and number of crossings were recorded for each of three consecutive 30 min periods. Current was adjusted, if necessary, after the first and second 30 min periods to obtain shuttling behavior with Mean ON times between 4 and 25 sec. Only animals that displayed such stable shuttling behavior by the third 30 min period were kept in the experiment. About 50% of the animals met this criterion. No shaping of behavior was required for these animals and stable shuttling behavior began within the first thirty minutes in the shuttle-box. The shuttle-box was also programmed so that the ON and OFF sides automatically switched every two min in order to help counteract the behavioral-inhibitory effects of etorphine.

There were five groups of eight animals each. Four groups received one of the following doses of etorphine (as the hydrochloride) dissolved in sterile water (1.0, 10.0, 20.0 or 40.0 μgm/kg intraperitoneally). A fifth group of eight animals was injected with sterile isotonic sodium chloride solution (saline). Injection volumes varied between 0.25 and 0.35 ml.

The second day was another control day; current intensity was not varied. On Day 3 all animals received their appropriate drug injection and were then immediately placed in the shuttle-box for 90 min. For the 1.0, 10.0 and 40.0 μgm/kg etorphine groups Day 4 was another control day and completed the experiment. The 20.0 μgm/kg group was selected based on pilot data to also be used in a tolerance study. The 20.0 μgm/kg group and the group of saline animals received their appropriate injections on five consecutive days (Days 3 to 7 of the experiment) and then were also tested on four consecutive postdrug days (Days 8 to 11 of the experiment). On all drug days the 90 minute test began immediately after injection.

Histology

After the completion of the experiment, animals were overdosed with pentobarbital. They were then perfused intracardially with formal saline and their brains were removed. Histological analysis was then made of thionin-stained 40 μ frozen sections to locate the electrode tips.

Results

The dose-response data are illustrated in Fig. 1. This figure shows the mean ON and OFF times per crossing for each group of eight animals during the entire ninety

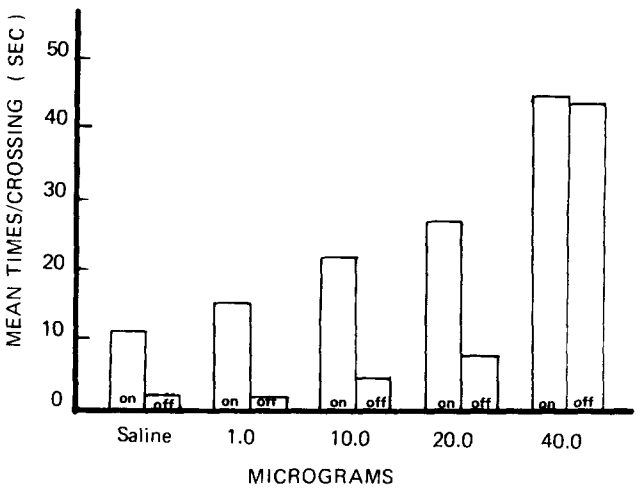

FIG. 1. The effect of etorphine on mean ON and OFF times during the first day of drug injections.

minutes of the first day of drug injection. The five groups of animals did not differ significantly in mean ON or OFF times prior to drug injections (not shown in Fig. 1). The 1.0, 10.0 and 20.0 μgm/kg doses of etorphine produced a selective and progressive increase in ON times, while the 40.0 μgm/kg dose produced an even-larger increase, but in both ON and OFF times (when compared to their control predrug scores). An analysis of variance and postanova comparisons showed that the mean ON times of the 10.0, 20.0 and 40.0 μgm/kg groups were greater than those of the saline group. The ON times of the 40.0 μgm/kg group were also greater than for each of the other drug groups. Only the OFF times of the 40 μgm/kg group were significantly greater than those of the saline group (all p's<0.05).

The tolerance data are illustrated in Figs. 2 and 3. These figures show the predrug day (Day 2 of the experiment), drug Days 1, 3 and 5, and postdrug Days 1 and 4. Analysis of variance and postanova comparisons confirmed the following results (all p's<0.05). For the etorphine group (Fig. 2) mean ON times on drug Days 1, 3 and 5 were each greater than predrug day and postdrug Days 1 and 4. ON times were not different from each other on the three drug days, nor were mean ON times on the predrug day and postdrug Days 1 and 4 different from each other. Mean OFF times for the etorphine group were elevated on drug Day 1 compared to predrug and postdrug days, but not on drug Days 3 or 5. ON and OFF times did not vary significantly over days of testing for the saline group (Fig. 3).

Histology

Histological verification was obtained for 32 of the 40 animals. All electrode stimulation sites were located within the medial forebrain bundle-lateral hypothalamic area. Stimulation sites ranged between 0.4 and 1.4 mm posterior to bregma, 1.5 and 2.5 mm lateral to the midsaggital suture, and 8.0 to 9.5 mm below the dura [17].

DISCUSSION

The selective increase in mean ON times produced by intermediate doses of etorphine (10 to 20 μgm/kg) confirms with this extremely potent narcotic [2,15] our

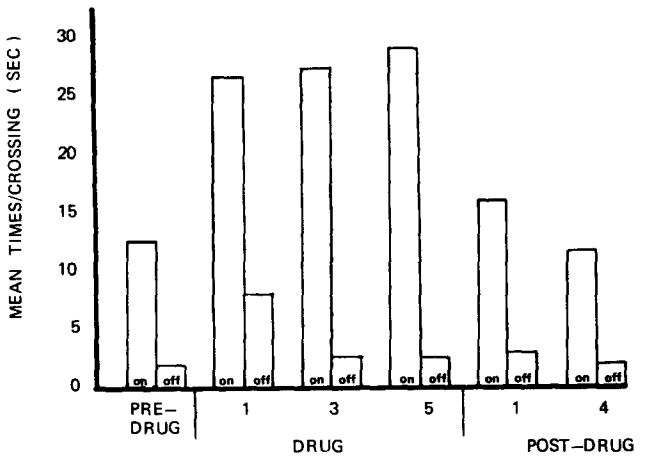

FIG. 2. The effect of etorphine on mean ON and OFF times during five daily injections.

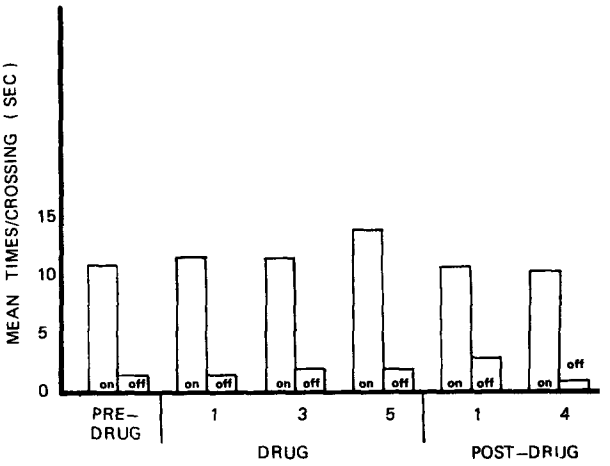

FIG. 3. The effect of saline on mean ON and OFF times during five daily injections.

previous finding employing morphine [11]. These results strengthen the conclusion that this phenomenon is a general action attributable to narcotic drugs. This action may result from either a direct facilitation of reward processes [19] or from an inhibition of an aversive component which may develop with prolonged electrical stimulation at self-stimulation sites [3, 11, 14, 18]. The fact that animals eventually turn off the stimulation they initially turn on has been taken as evidence for the development of aversion [3, 14, 18]. This phenomenon appears to be found at a wide variety of rewarding brain sites [21]. However, animals seem to find brain stimulation of varying durations about equally reinforcing in a choice situation, even when durations they normally turn off are used [6] suggesting perhaps that long duration stimulation may not become aversive. Homeostatic opponent process mechanisms such as those proposed by Solomon and Corbit [20] may be brought into play by reinforcing brain stimulation and be the basis of the repetitive turning on and off of such stimulation.

The failure of tolerance to develop over five days of drug injections suggests that the reward enhancement found in this shuttle-box self-stimulation paradigm is related to the delayed reward enhancement found in lever press studies [4,13] and to the reduction in reward threshold which has been reported [7]. Since tolerance to the analgesic action of narcotics develops rather rapidly it seems likely that the enhancement of self-stimulation reinforcement is based on a differentiable mechanism than that mediating analgesia.

The development of tolerance to the small OFF time increase found on the first drug day suggests also a relationship to the early inhibition found in lever press studies. This phenomenon may be based on the sedative and catatonia actions of narcotics. The shuttle-box self-stimulation procedure is a promising new approach to the evaluation of the effects of narcotic drugs on reinforcement processes. The stability of shuttle-box self-stimulation during the course of ten consecutive days of testing (Saline group; Fig. 3) also suggests the utility of this paradigm.

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