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BRIEF COMMUNICATION

The Effects of Levonantradol on Rewarding Brain Stimulation Thresholds in the Rat[1]

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KUCHARSKI, L. T., J. E. G. WILLIAMS AND C. KORNETSKY. *The effects of levonantradol on rewarding brain stimulation thresholds in the rat.* PHARMACOL BIOCHEM BEHAV 19(1) 149–151, 1983.—Rats were implanted bilaterally with electrodes aimed at the medial forebrain bundle (MFB) and trained to deliver intracranial stimulation. Reward thresholds were determined using a modification of the psychophysical method of limits. Levonantradol, a cannabinoid with reported analgesic activity, was tested at doses between 0.0125 to 0.3 mg/kg. Significant elevations of reward thresholds were observed at 0.2 and 0.3 mg/kg. Since none of the doses tested lowered the reward threshold, an effect believed to be predictive of abuse, these results suggest that levonantradol has little or no abuse liability.

Levonantradol Brain-stimulation reward Drug abuse liability

SEVERAL recent reports suggest that the cannabinoid, levonantradol, has potent analgesic activity. In animals, increased latencies with the hot plate [1,2] and tail flick [2] tests were observed at doses between 0.1 and 0.3 mg/kg. In the PBQ writhing procedure, 0.07 mg/kg produced a 50 percent maximal analgesic effect [2]. Levonantradol has also been shown to be an effective analgesic for human post-operative pain [3]. These results are encouraging although side effects may be a limiting factor [4]. The failure of naloxone to completely antagonize levonantradol's analgesic effect in animals suggests the involvement of nonopiate mechanisms [1,2] and raises the possibility that levonantradol may have low abuse liability.

While a great deal of effort has been extended in order to ascertain the analgesic activity of levonantradol, little attention has been directed toward the evaluation of its abuse liability. Young and coworkers [5] reported that rhesus monkeys trained to self administer codeine failed to continue to self administer when levonantradol was substituted for codeine. They also reported that the discriminative effects of levonantradol were not equivalent to the narcotics ethylketazocine or etorphine. These findings suggest that levonantradol may have low abuse liability.

In order to further characterize the abuse liability of this drug we determined its effects on the threshold for rewarding brain stimulation. We have previously demonstrated [6] that many abused substances including morphine, amphetamine, cocaine, pentazocine and phencyclidine lower the threshold for rewarding brain stimulation to the medial forebrain bundle at appropriate doses.

METHOD

Four male albino CDF rats (Charles River Laboratories) weighing approximately 300 g were stereotaxically implanted bilaterally with bipolar stainless steel electrodes (0.127 cm in diameter) aimed at the medial forebrain bundle-lateral hypothalamic area (coordinates 4 mm posterior to bregma, ±1.4 lateral to the midline suture, and 8.0 mm ventral from skull surface). (Although histological verification of the electrode placement was not done on these animals, we have had, in large numbers of other animals, no difficulty in placing the electrodes in this lateral hypothalamic area using the above coordinates.) Prior to surgery all animals were anesthetized with Equi-Thesin^R (0.3 ml/100 g body weight).

The animals were trained on a rate independent procedure for determining the threshold for intracranial rewarding brain stimulation. Animals were placed in a plastic chamber (20×20 cm). Mounted in an opening on one wall of the chamber was a cylindrical wheel manipulandum, which was 16 cm in length and 7.5 cm in diameter. Four equally spaced cams were positioned on one of the end plates such that they operated a microswitch when the wheel was rotated. Reinforcement was obtained only after closure of the microswitch (one-quarter of a wheel turn). A constant current stimulator (Sunrise Systems, North Scituate, MA) was used

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to deliver the stimuli which consisted of half-second trains of biphasic symmetrical pulses. Each train occurred at a frequency of 160 Hz, with a pulse width of 0.2 msec and a delay of 0.2 msec between the positive and negative pulses. Pulse amplitude was varied according to the procedural requirements for threshold determinations. Current was periodically checked with an oscilloscope to insure constancy.

Each animal was first trained to self stimulate at current intensities below 255 μA according to individual sensitivity. After establishing the wheel turning operant, the animal was trained in a discrimination task in which a noncontingent intracranial self stimulation served as the discriminative stimulus (S^D). A response within 7.5 seconds of the S^D resulted in the delivery of a contingent intracranial stimulation identical to the S^D and terminated the trial. Failure to respond had no scheduled consequences and the trial terminated after 7.5 sec. Intervals between trials varied (average 15 sec, range 7.5–22.5 sec). Responses during the intertrial interval resulted in a 15 sec postponement of the next trial. Determination of the threshold involved a discrete trial procedure. A trial began with the delivery of a noncontingent 0.5 sec pulse train at an intensity previously shown to be reinforcing. Stimulus intensities were then varied according to the classical method of limits with slight modification. Stimuli were presented in alternating descending and ascending series with a step size of 5 to 10 μA (depending upon the individual animal's discriminative capabilities), with five trials presented at each step or level. A response on at least 3 out of five trials, at each step was considered a plus at that intensity and the intensity of both the S^D and contigent stimulus was lowered by 5 or 10 μA. This procedure was continued until the animal failed to respond (less than 3 responses) on two successive intensity levels whereupon the intensity was lowered one additional step and an ascending series was begun. The ascending series continued until two successive positive steps were attained, whereupon the intensity was increased one additional step and another descending series was begun.The threshold for each series was defined as the midpoint between the positive and negative steps. The overall threshold was defined as the mean of the ascending and descending series thresholds.

Animals were run on the above procedure until stable threshold values were obtained, whereupon vehicle injections were initiated. When the threshold value did not vary more than ± 10 μA each session and where there was no day-to-day trend in either direction, the threshold was considered stable. Subjects completed four series (i.e., two ascending and two descending) pre-injection and then eight series post-injection, with the entire session lasting 1.5–2 hr. For further details on this procedure see Esposito and Kornetsky [7]. All experimental events and data collection were collected and stored by an on-line microcomputer. After the animals had received vehicle injections for a number of days (at least five in succession), drug injections were initiated. Two vehicle injection days were always interspersed between each day of drug treatments.

Levonantradol HCl was dissolved in 1 ml ETOH and 1 ml Emulphor EL-620 and diluted to a 5% ETOH, 5% Emulphor with 90% saline solution. All drug doses were counterbalanced and were given SC at a volume of 1 ml/kg with a 30 minute waiting period between injection and testing to allow for absorption of the drug. Threshold values were calculated for both the pre-vehicle and post-vehicle session. The change from pre- to post-vehicle was calculated. The change scores for pre- to post-vehicle were transformed to standard scores

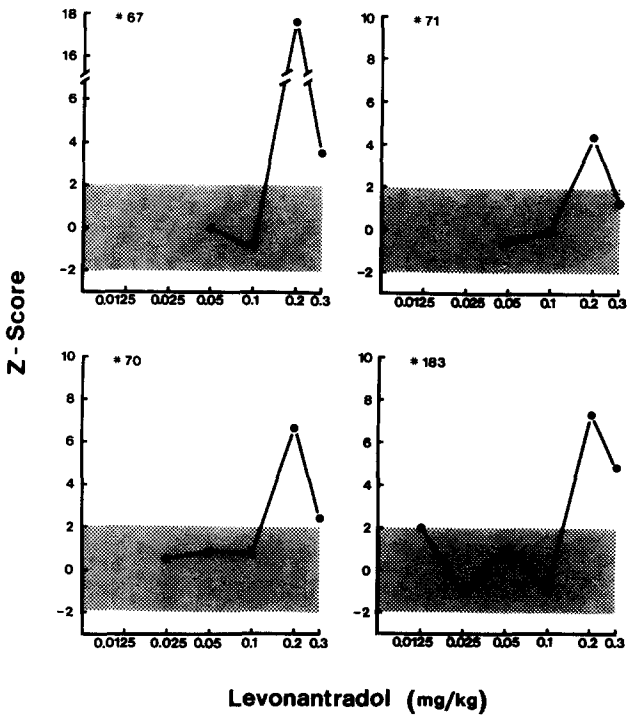

FIG. 1. Standard score (Z score) changes in threshold values from pre- to post-drug as a function of the dose of levonantradol for each of four animals. The 95 percent confidence limits for all vehicle days are indicated by the shading. Each point represents a single determination for each dose.

(Z scores). Changes in threshold from pre- to post-levonantradol were compared to the distribution of change scores seen following vehicle injections. Only data where the animal responded on all columns (series) were used in the analysis. A Z score of ± 2.0 (95% confidence limits) pre- to post-levonantradol was preselected as the level of significance.

RESULTS

The results are summarized in Fig. 1. As can be seen, levonantradol significantly elevated reward threshold at 0.2 mg/kg in all animals and at 0.3 mg/kg in 3 out of 4 animals. At no dose was the reward threshold lowered. Dosages above 0.3 mg/kg were untestable due to the induction of catalepsy severe enough to abolish all responding.

DISCUSSION

Previous findings from our laboratory have demonstrated that numerous substances with known abuse liability, lower reward threshold [6]. The only effect observed with levonantradol was threshold elevation. These results suggest that levonantradol has low abuse liability and support the self administration studies reported by Young *et al.* [5]. These animal findings are bolstered by the infrequent report of euphoria by human subjects who have participated in clinical trials of levonantradol. Caution should be observed in the interpretation of our results as Δ^9-THC and other cannabinoids, with known abuse liability, have not yet been

tested in this model. It is possible that the cannabinoids might represent a class of substances that yield false negative predictions. However, these results clearly indicate that levonantradol does not facilitate the reward system of rats as does morphine, d-amphetamine, cocaine, phencyclidine or other highly abused substances.

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