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Opiate Modification of Intracranial Self-Stimulation in the Rat[1]

STUART L. WEIBEL AND HAROLD H. WOLF

University of Utah, College of Pharmacy, Department of Biochemical Pharmacology and Toxicology Salt Lake City, UT 84112

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WEIBEL, S. L. AND H. H. WOLF. *Opiate modification of intracranial self-stimulation in the rat.* PHARMAC. BIOCHEM. BEHAV. **10**(1) 71–78, 1979.—Studies were conducted to confirm the involvement of central opiate receptors in the expression of opiate modulation of intracranial self-stimulation (ICSS). Biphasic, dose-related changes in ICSS responding are described following IP administration of morphine sulfate (1–25 mg/kg) and levorphanol tartrate (LEV, 0.5–5 mg/kg). Similar patterns of response modification are reported following intraventricular (IVt) administration of LEV (0.01–0.2 μMoles) LEV's enantiomorph, dextrorphan, was not found to elicit comparable effects after either IP or IVt administration. Both the facilitatory and the depressant phases of LEV's action were antagonized by naltrexone (10 μg, IVt), which had no apparent effect on ICSS by itself. Complete tolerance developed to the suppression of responding by 2.5 mg/kg LEV (IP) but not to the facilitatory effect of 0.5 mg/kg (IP), during a 5-day course of administration. The implications of these results for opiate reinforcement theory are discussed and possible mechanisms are advanced.

Intracranial self-stimulation Narcotic analgetics Reinforcement Narcotic antagonists Tolerance
Central administration Stereoselectivity Morphine Levorphanol Dextrorphan Naltrexone

SOLUTIONS to the biological aspects of opiate dependence require systematic illustration of the relationships between narcotic mechanisms and the behavioral patterns which promote and sustain opiate addiction. There are numerous studies supportive of the highly specific discriminable character of opiates as an internal stimulus [8, 9, 14, 15, 23, 35] and a further body of literature which demonstrates that opiates are readily self-administered by several species of laboratory animals [33, 34, 37, 40, 42, 43], as well as by man. The former investigations do not specifically address the reward value of the opiate stimulus, however, and self-administration studies suffer from an inability to distinguish conclusively between positive reinforcement and progressive physiological dependence phenomena which jointly orchestrate addiction behavior.

In recent years, intracranial self-stimulation (ICSS) behavior has been advanced as a psychophysical model of heuristic value for the investigation of motivational aspects of narcotic addiction [1]. This model adopts the assumption that reward centers exist which subserve the modulation of goal-directed activities of all types: consummatory, sexual, and general gratification behaviors. Thus, modification of the activity of such reward or pleasure centers by opiates would presumably be reflected in changes in response rates of animals bar pressing for electrical stimulation of these reward centers.

In fact, biphasic alterations of ICSS responding of both a dose-dependent and a time-dependent nature have been reported for morphine and related narcotics. Small doses of morphine (1–2.5 mg/kg, IP) have been shown to facilitate self-stimulation responding [1], while higher doses (5 mg and above) exert an early depressant influence followed by a latent facilitation which develops 3 to 5 hr post-administration [29].

Thus, three distinct manifestations of opiate activity have been demonstrated using the ICSS paradigm. Low and moderate doses of narcotics elicit a short-term increase and decrease in response rates, respectively; the development of tolerance to both effects has been reported [1, 16, 29]. The third, long-term increase in responding behavior does not decay following repeated administration but, rather, seems to be unmasked by the development of tolerance to moderate opiate doses, as evidenced by a decrease in the latency and increase in the magnitude of the effect following repeated administration of a narcotic.

It is evident, then, that the ICSS psychophysical paradigm embodies important characteristics which render it potentially valuable as a model for the investigation of opiate dependence mechanisms; in addition to the presumptive link between reward pathways and dependence phenomena, the model reflects both stimulatory and depressant opiate effects, as well as possibly nonspecific actions, all of which are prominent characteristics of opiate activity.

The present study seeks to evaluate systematically the effects of specific opiates on ICSS and determine the extent to which these actions can be ascribed to the activation of

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central opiate receptors. Acceptance of this hypothesis requires the demonstration of the following criteria: (1) effects on ICSS response rates must be dose-related, (2) the stereoselectivity of stereoisomeric pairs (such as levorphanol and dextrorphan) must be readily demonstrable, (3) observed effects must be labile to disruption by a narcotic receptor antagonist (such as naltrexone), and (4) the central locus of action must be established. The experiments described below address these criteria. Inasmuch as the development of tolerance represents a prominent characteristic of most opiate actions, it was also deemed appropriate to evaluate the degree to which tolerance developed to the facilitative and depressant effects of low and moderate doses of narcotics, respectively.

METHOD

Animals

Adult, male, albino, Sprague-Dawley derived rats between 300 and 500 g (Lab Supply, Indianapolis, Indiana) were employed for all studies. Following surgical manipulations, animals were maintained individually in clear acrylic cages (24×24×15 cm) at 21° ± 2°C on a 12 hr light/dark cycle. Food and water were available ad lib, except during experimental trials.

Surgical Procedures

Stimulating electrodes and microinjection guide cannulae were implanted under pentobarbital anesthesia (50 mg/kg, IP) according to standard stereotaxic procedures.

Electrodes were fashioned from twisted stainless steel bipolar electrodes (0.0254 cm dia.) insulated except at their cross-sectional tips (MS 303, Plastic Products Co., Roanoke, VA). Guide cannulae were constructed from 22 ga stainless steel hypodermic tubing.

Electrodes were aimed for the medial forebrain bundle as it courses through the lateral hypothalamus, an area of confluence of the major ascending catecholamine pathways known to support ICSS behavior [13]. Lateral (–1.5 mm) and Anterior/Posterior (+5.9) coordinates were referenced to stereotaxic zero, while the vertical coordinate (–3.5) was measured from skull surface. Orientation of the rat in the stereotaxic stage was according to the method described in the Konig and Klippel atlas [20]. Placement sites were confirmed histologically following termination of experiments; Fig. 1 illustrates typical successful electrode placements.

Indwelling guide cannulae were implanted near the intersection of the left lateral and the third ventricles in order to sustain the greatest degree of ventricular distribution from a unilateral cannula placement. The tips of the guide cannulae were placed 2 mm above the intended injection sites in order to minimize backflow of injection solutions between the injection and guide cannulae.

ICSS Apparatus

The ICSS apparatus consists of four functionally integrated components: a manipulandum-equipped environment (Skinner Box), programming circuitry, a stimulus generator (stimulator), and a data collection system.

The Skinner boxes were constructed of clear acrylic plastic (23×23×19 cm) with a standard paddle-type rat manipulandum (Lehigh Valley No. 121-05, Beltsville, Maryland)

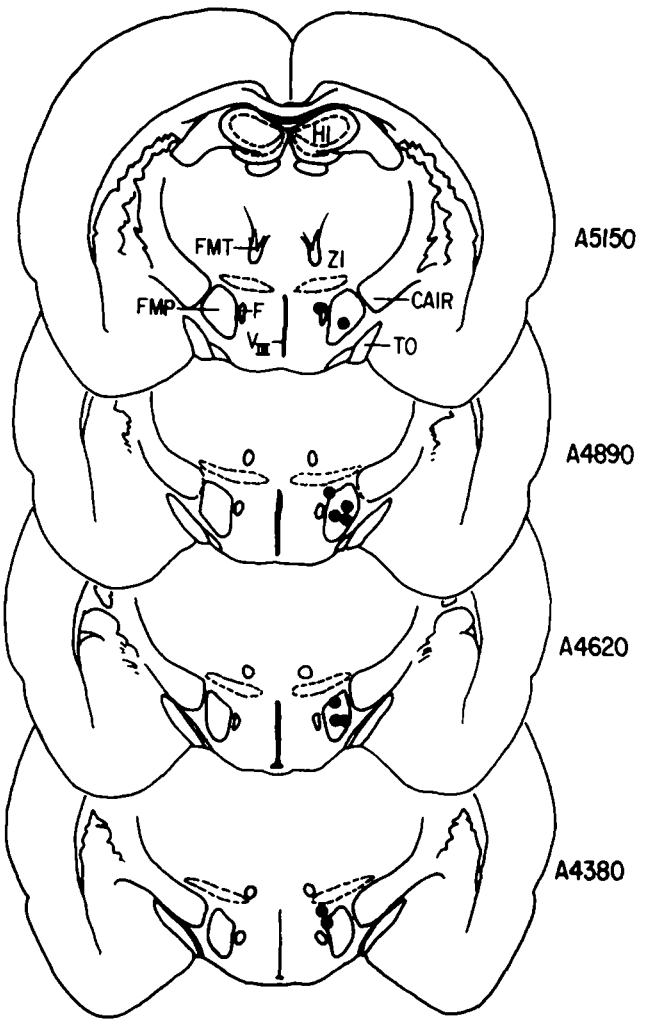

FIG. 1. Typical electrode placements, confirmed by histological examination following termination of experiments. Coronal sections are adapted from König and Klippel [20] CAIR=Capsula interna, pars retrolenticularis; F=Columna fornicis; FMP=Fasciculus medialis prosencephali; FMT=Fasciculus mamillothalamicus; HI=Hippocampus; TO=Tractus opticus; VIII=Third ventricle; ZI=Zona incerta.

centered on one wall 3 cm above a wire fabric floor. The paddle was adjusted to a tension of 15 G.

The programming circuitry and stimulus generator were incorporated into a stimulator of in-house design and construction, details of which have been published elsewhere [41]. The stimulator was programmed for a continuous reinforcement schedule such that each bar press resulted in a 200 msec train of 100 Hz, constant current, biphasic sinusoidal stimulation. Bar presses during an ongoing stimulus train were recorded as responses but had no scheduled consequences.

The stimulator output was connected to the electrode by means of a shielded two-conductor cable with an intervening ball bearing mercury swivel, application of which is described elsewhere [4]. That portion of the signal lead vulnerable to rat gnawing was sheathed in stainless steel spring

tubing. The sheathing, together with the swivel lead arrangement, allowed for adequate freedom of movement for the rat for extended periods of time without risk of severed leads.

Training Sessions

Following post-surgical recovery periods of approximately one week, animals were shaped to perform the bar pressing response for intracranial stimulation. Stimulus intensities were adjusted for each animal to a level consistent with reliable response rates of at least 2000 responses/hour. In no instance was the stimulus intensity advanced beyond 200 μA; animals not responding at or below this level were discarded as nonresponders.

Drugs, Solutions, and Experimental Procedures

Drugs employed in this study included morphine sulfate (Merck), levorphanol and dextrorphan tartrate (Hoffmann La Roche), and naltrexone hydrochloride (Endo).

Peripheral drug treatments were administered by the intraperitoneal route. Sodium Chloride for Injection, USP was employed as the vehicle for all drugs, as well as for control injections.

Intraventricular (IVt) microinjections were made via 28 ga stainless steel injection cannulae attached to a pump-driven 10 μl syringe with PE-10 polyethylene tubing. Injection procedures described in the literature [36] were employed to ensure accurate injections. Drug solutions were prepared in Sterile Water for Injection, USP and rendered isotonic with NaCl according to procedures described elsewhere [30]. Injection volumes of 5 μl, and occasionally, 2.5 or 10 μl were employed.

All experimental trials were conducted for a period of 2 hr: a 1 hr baseline control interval, followed by administration of a drug treatment or vehicle control, and a subsequent 1 hr post-treatment response period. Previous literature results [1, 16, 25, 29] and our own observations validate the inclusion of the times of peak effect of the agents employed within this time period.

Cumulative response records were analyzed to determine hourly response rates and percent change in response rates for pre- and post-administration intervals. The percent data so derived were subjected to arcsin transformation to normalize the statistical distribution prior to further statistical analysis. Single classification analysis of variance and Newman-Keuls multiple range tests [44] were performed to evaluate differences between treatment groups.

In general, six animals were used at each dose level and all animals received each treatment (including vehicle control injections) in a given experiment in order to balance the experimental design. In practice, however, loss of animals from the study or other constraints required deviation from this protocol in certain instances.

Drug administrations to any given animal were in all cases separated by at least seven days, an interval which we found precludes the development of tolerance to the effects of these agents on ICSS response rates.

RESULTS

Experiment 1

The data described in this section address the dose-dependent and stereoselective nature of the effects of morphine sulfate (MS), levorphanol tartrate (LEV), and dextrorphan tartrate (DEX) following peripheral administration. Figure 2 compares the modification of ICSS responding by each of these agents and by saline.

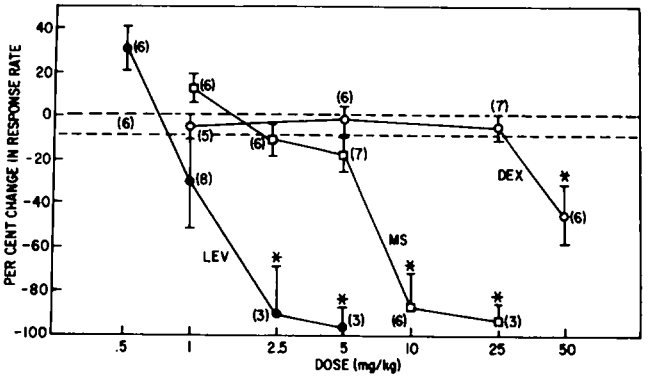

FIG. 2. Mean changes in ICSS responding following IP administration of levorphanol tartrate (LEV), morphine sulfate (MS), and dextrorphan tartrate (DEX) ± SEM. Dashed horizontal bars indicate the SEM of the saline control group. Numbers in parentheses indicate the number of animals per treatment group. Asterisks denote groups statistically different from saline controls ($p<0.05$).

An 11% increase in bar pressing rate was observed following 1 mg/kg MS, as compared with a mean depression of 7% in the saline control group. With increasing dosage, progressive degree of depression of response rate are observed, reaching 85% at 10 mg/kg; at 25 mg/kg bar pressing essentially ceases in the presence of profound catatonia. Anova statistics confirm the dose-responsiveness of the effects: $F(5,28)=12.2$, $p<0.05$.

In like manner, the effects of LEV range from a 31.5% facilitation at 0.5 mg/kg to a 97% depression of responding at 5 mg/kg, $F(3,19)=9.75$, $p<0.05$. As with MS, catatonia was observed at the higher doses of LEV (although not with equimolar doses of DEX).

DEX administration failed to enhance responding over a wide range of dosages. Further, depression of response rates (46%) was observed only at a dose of 50 mg/kg.

Experiment 2

The data generated in this experiment distinguish between central and peripheral sites of action through the use of IVt microinjection techniques. This route of administration is particularly appropriate to the study of opiates; numerous investigations have established periventricular and periaqueductal areas as loci of a variety of opiates effects [17, 19, 22, 24, 25].

These data further substantiate the stereoselectivity of the modification of ICSS by opiates through a comparison of LEV and DEX following central administration.

Figure 3 portrays the dose response characteristics of IVt administered LEV. Maximal, statistically significant facilitation (36%) was observed at a dose of 0.02 μMoles, while a 55% depression was elicited by a dose of 0.2 μMoles, $F(5,23)=12.2$, $p<0.05$. In order to confirm that these effects were not due to peripheral redistribution following central administration, these same doses were administered IP, re-

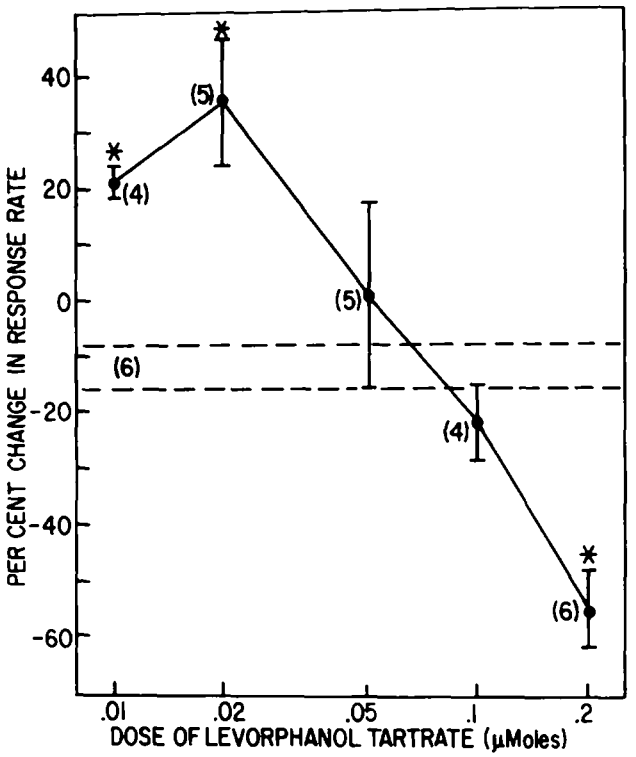

FIG. 3. Mean changes in ICSS response rates following IVt administration of levorphanol tartrate ± SEM. Saline control group (± SEM) is indicated by dashed horizontal lines. Numbers in parentheses indicate number of animals per treatment group. Asterisks denote groups statistically different from saline controls ($p<0.05$).

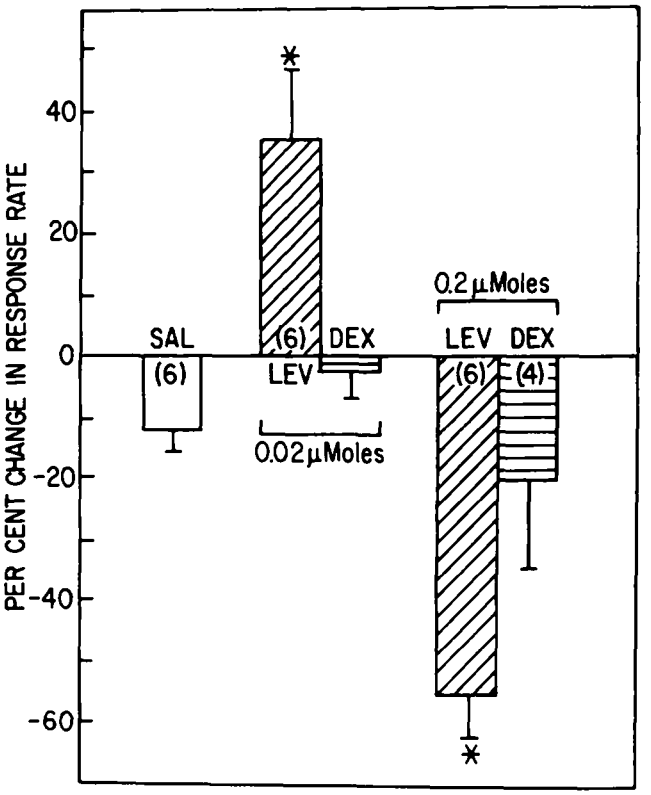

FIG. 4. Comparison of the mean changes in ICSS response rates following IVt administration of 0.02 and 0.2 μMoles of levorphanol tartrate (LEV) and dextrorphan tartrate (DEX) ± SEM. Numbers in parentheses indicate number of animals per treatment group. Asterisks indicate statistical difference from saline controls ($p<0.05$).

sulting in response changes of −16.1% (± 17.9 SEM) and −1.1% (± 11.3 SEM), respectively.

For comparison purposes, two equimolar doses of DEX were administered IVt. Figure 4 illustrates the relative effects of equimolar doses of these enantiomorphs on ICSS responding following central administration. In contrast to the LEV effects, the DEX-induced changes are not significantly different from controls.

Experiment 3

Blockade of opiate effects with a specific receptor antagonist comprises an essential element of the demonstration of receptor mediation of such an effect. Naltrexone (NTX) has been employed here to challenge both the stimulatory (low dose) and depressant (moderate dose) effects of LEV administration on ICSS.

Following a 1 hr baseline response period, each animal received one of three IP treatments: LEV (0.5 mg/kg), LEV (2.5 mg/kg), or saline. Immediately following the IP pretreatments, NTX (10 μg) or saline was administered IVt.

Figure 5 illustrates the effects of centrally administered NTX and saline on groups receiving IP doses of 0.5 mg/kg LEV, 2.5 mg/kg LEV, or saline. These data do not implicate an effect of NTX alone on ICSS; minimal change from baseline rate (−3%) was observed following the NTX/saline treatment combination.

NTX challenge of the facilitatory effect of 0.5 mg/kg LEV resulted in a statistically significant reversal of the enhancement observed in the absence of NTX, $F(2,16)=3.96$, $p<0.05$. Similarly, the depression observed following 2.5 mg/kg LEV was substantially blocked by NTX, $F(2,14)=20.1$, $p<0.05$.

Experiment 4

A final set of experiments was conducted to assess the degree to which acute tolerance develops to the facilitative and depressant effects of LEV on ICSS. Two LEV treatment groups (0.5 and 2.5 mg/kg, IP) and a saline control group were run on each of five successive days, employing the experimental protocol described for previous experiments.

Figure 6 reflects the results of the five-day administration sequence for the three treatment groups. Predictably, no significant changes from baseline response rates were observed for the saline control group, $F(4,25)=0.97$, $p<0.05$. In contrast, there is an obvious, virtually complete attenuation of the depressant effect of the 2.5 mg/kg LEV treatment: mean changes in response rate for this group progresses from an 83% depression on Day 1 to a 17% enhancement on Day 5, $F(4,17)=3.9$, $p<0.05$.

Contrary to a previous literature report [16], no evidence of significant tolerance was apparent for the facilitatory effect of the lower (0.5 mg/kg) dose of LEV, $F(4,19)=0.14$, $p<0.05$.

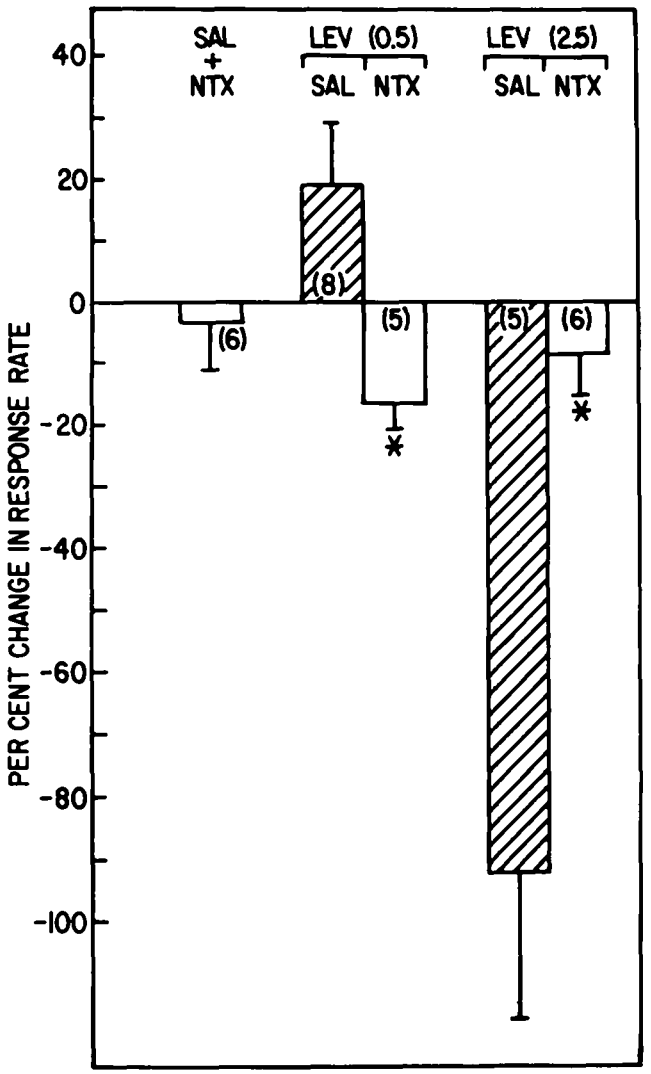

FIG. 5. Antagonism by naltrexone (NTX) of the facilitatory and depressant effects of levorphanol tartrate (LEV) on ICSS. Vertical bars indicate SEM. Numbers in parentheses represent the number of animals per treatment group. Dosages of levorphanol were 0.5 and 2.5 mg/kg administered IVt. Asterisks denote that NTX treatment significantly antagonizes the effects observed when LEV is paired with saline ($p<0.05$).

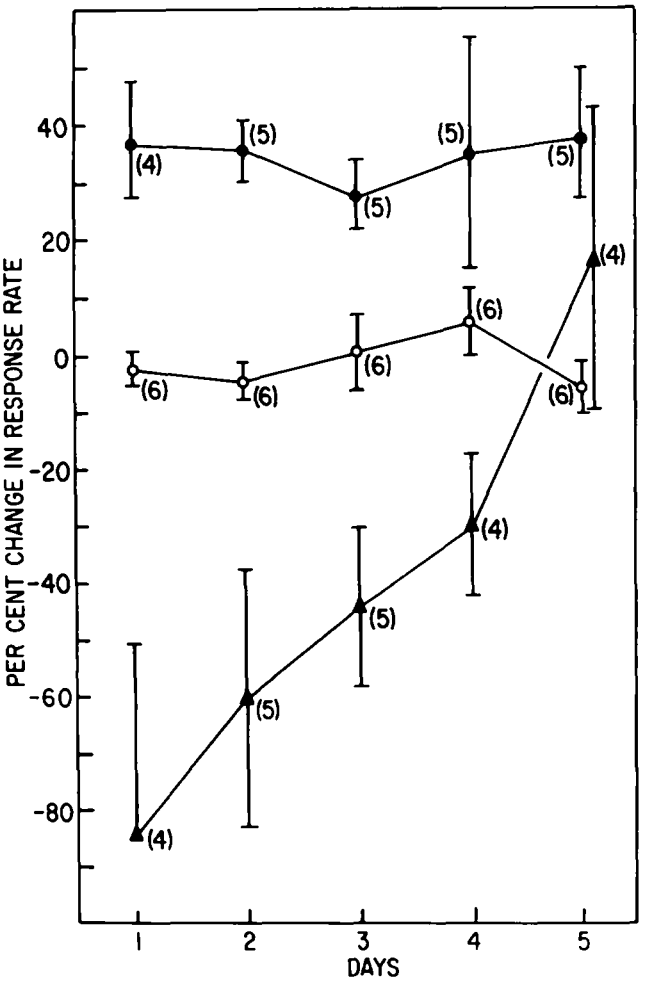

FIG. 6. Mean changes in ICSS responding produced by chronic administration of saline (○), 0.5 mg/kg levorphanol (●), and 2.5 mg/kg levorphanol (▲). Numbers in parentheses denote the number of animals per treatment group. Vertical bracketed lines indicate SEM.

DISCUSSION

The above data provide unambiguous support for the contention that modification of ICSS by opiates is mediated by specific opiate-receptor mechanisms in the CNS. The data in Figs. 2, 3, and 4 confirm the dose responsive character of these effects, as well as establishing their stereoselective nature following both peripheral and central administration. These results are consistent with several literature reports which describe the effects of morphine [1, 16, 29] in related experimental settings. The nearly 40-fold difference in doses of levorphanol and dextrorphan required to induce equivalent response suppression, coupled with the absence of any facilitative effect of dextrorphan, comprise substantial evidence for the involvement of stereoselective receptors in the expression of levorphanol's actions as described here.

The literature is less consistent, however, with respect to apparent facilitation of ICSS. Certain investigators report this enhancement [1, 16, 29] while others failed to discern consistent increases in response rates following low doses of narcotic analgesics [31,32]. In the present experiments, facilitative trends are apparent for both levorphanol and morphine following IP administration of low doses, despite the lack of statistically significant differences from saline controls. However, clear, statistically significant demonstration of facilitation following central administration of levorphanol (Fig. 3) lends credence to the trends observed after peripheral administration.

That the enhancement of responding is more easily demonstrated following central administration may be explained in several ways. Peripherally mediated depressant effects may physiologically antagonize the centrally induced

facilitatory phase of low-dose narcotic action. Alternatively, the effects of a systemically administered dose at central sites distal to the ventricular system (and hence, not influenced by ventricular administration) may counteract the facilitatory phase mediated by peri-ventricular sites of action. This interpretation is, in principle, consistent with one literature report describing the separation of inhibitory and facilitatory effects of morphine on ICSS on the basis of site of morphine microinjection in the CNS [5]. However, these authors described facilitation effects as primarily hypothalamic and inhibitory effects as being mediated by periaqueductal sites. Thus, our data are not in obvious ways reconcilable with these site studies, inasmuch as ventricular administration resulted in clearly defined facilitation in our experiments.

Observation of gross behavior is of interest to this argument. Doses of opiates which compromise ICSS after peripheral administration are accompanied by obvious behavioral depression which at higher dose levels includes catatonia, a state of waxen immobility often observed following large doses of any of several narcotic agonists [21,39]. Equipotent doses which abolish or substantially reduce ICSS after central administration, however, are associated with substantially less behavioral and motor depression. Animals which have ceased to respond for brain stimulation following IVt administration of levorphanol engage in motor activity and exploratory behavior rarely observed in their peripherally narcotized counterparts. In addition to ruling out simple motor activity effects as the basis for opiate effects on ICSS, this observation may reflect site-specific effects as suggested in the literature [5] and discussed above.

The antagonism of both the facilitatory and depressant effects of levorphanol by naltrexone (Fig. 5) contributes substantially to the current hypothesis. The evidence presented here, as well as by other investigators [18, 31, 39], clearly indicates the receptor mediated antagonism of both phases of narcotic activity on ICSS. The observation that naltrexone by itself induced no significant alteration in responding further substantiates the assertion that the observed antagonism is pharmacological rather than physiological.

Several other reports in the literature have recorded the singular lack of effects of opiate receptor antagonists on ICSS [18, 31, 38, 39]. These studies (and our own) imply that tonic activity of a putative endogenous opiate system may not be essential for the expression of ICSS behavior, and by extrapolation, is thus probably not important in reinforcement events. Some doubt is cast on this tentative conclusion, however, by a recent demonstration of the ability of naloxone to inhibit ICSS elicited in central gray regions of rat brain [3]. The discrepancy between these reports possibly reflects the different stimulation loci, a variable too often ignored in the treatment of reinforcement theories. The significance of these reports is as yet unclear; however, they may shed light on the extent to which putative endorphin transmitter systems participate in reinforcement events, thus further clarifying the relevance of opiate/ICSS interactions to theories of dependence.

There are no compelling theoretical grounds which require the demonstration of tolerance as a requisite for the assumption of receptor mediation of a narcotic effect. Nonetheless, inasmuch as tolerance is associated with many of the clinically significant aspects of opiate activity, we thought it desirable to include a tolerance assessment in these studies. Previous literature results led us to expect the development of tolerance to both the acute facilitative [16] and the depressant phase [1,29] of opiate modification of ICSS. The latter prediction was amply borne out by our data; Fig. 6 illustrates the complete tolerance that developed to the depressant effect of 2.5 mg/kg levorphanol (IP) during the five-day administration sequence, as reported in earlier literature reports [1,29]. Contrary to a previous report [16], however, no attenuation of the facilitative effect of 0.5 mg/kg levorphanol (IP) was evident. Other authors [6,31], looking at the latent facilitation evidenced 3–5 hr following depression, observed no tolerance to this phase of opiate action over an administration period of 20 days or longer, suggesting a possible relationship between the acute, low-dose facilitation and the latent facilitation that follows moderate or high doses of opiates.

The possibility that a longer period of administration would result in the development of tolerance to these effects bears further investigation; it is well known that tolerance develops more slowly at low doses than at higher doses [7]. However, the possibility that the development of tolerance requires some minimum threshold of drug-receptor interaction for expression also merits consideration. The ICSS paradigm may be particularly valuable for such studies in light of the sensitivity of the method to low doses of narcotics.

The discrepancy between our own results and the existing report of the development of tolerance to the facilitative effect of morphine during a five-day dosage regimen not unlike our own [16] is not readily accounted for. While differences in procedure and test agents (morphine vs levorphanol) do exist, these variations between reports seem insufficient to account for the disparity between the earlier report and our own. It is of interest to note, however, that no tolerance to self-stimulation threshold-lowering effects of low doses of morphine was observed in a separate report [12], suggesting a parallel between this report and our own.

Several investigators [2, 12, 26] have employed measures of opiate effects on reinforcement which are independent of bar pressing rates. In these studies the ability of opiates to lower stimulation threshold [12] and to increase the time of stimulation in a shuttle box self-stimulation technic [2,26] were not attenuated during chronic administration. The lack of tolerance to these effects suggests they may be related to the acute low-dose facilitation reported here and the latent facilitatory phase reported by several other laboratories [1, 29, 31].

In addition to dose- and time-related effects on ICSS seen following opiate administration, there are reports in the literature indicating that electrode placement may influence the pattern of responding seen after narcotic agonists [27,28], as well as narcotic antagonists [3,38]. With one exception [28], the lack of extended dose-response information in these studies prevents critical comparisons of various sites and their responsiveness to opiate modulation; however, electrode placement certainly appears to be of considerable significance in the analysis of ICSS regulation.

Finally, there is a single report of the separation of the inhibitory and stimulatory effects of morphine on ICSS as a function of intracerebral morphine microinjection sites [5]. Here again, however, interpretation is constrained by a lack of dose response information.

The significance of these studies as a group lies in the value of ICSS as a model for the investigation of opiate

mechanisms, particularly those aspects of narcotic activity relating to reinforcement or dependence. In addition to the presumptive link between the reinforcement component of opiate actions and ICSS, however, the model embodies the biphasic character often observed in narcotic mechanisms. Further, the method offers a sensitivity to low doses of opiates which are without apparent effect in more traditional in vivo narcotic bioassays such as antinociception.

The patterns of modification described above suggest possible mechanisms which merit experimental evaluation. The relationship between opiates and catecholamine (CA) dynamics is of particular interest in light of the apparent involvement of CA's in both ICSS phenomena [13] and self-administration behavior [10,11].

Inasmuch as the central actions of opiates are well known to involve a wide variety of putative neurotransmitters and because the expression of ICSS is also influenced by many of these same substances, a relatively simple, unified monoamine theory of reinforcement may be insufficient to account for the results observed. Nonetheless, the ICSS paradigm provides a powerful tool for the analysis of such phenomena, and hopefully, may provide for a deeper understanding of drug dependence phenomena in general.

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