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Identification of a Novel Class of Central Reward Sites Showing a Delayed and Cumulative Response to Opiate Blockade

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KATZ, R. J. *Identification of a novel class of central reward sites showing a delayed and cumulative response to opiate blockade.* PHARMAC. BIOCHEM. BEHAV. 15(1) 131–134, 1981.—Heretofore it has been assumed that the brain's reward system shows a response to opiate receptor blockade that is (a) immediate in nature and (b) close to asymptote after the initial dose. On theoretical grounds a graded and gradual response to blockade might also be predicted. To test this, rats were implanted with subcortical electrodes for self-stimulation and trained to panel-press for contingent intracranial reinforcement. Chronic blockade of the opiate system was produced by repeated injections of naltrexone HCl. A small percentage of self-stimulation sites proved refractory to opiate blockade both acutely and chronically. Also, some sites showed an immediate decrease in response level, as previously reported. As was predicted, a number of sites originally showing no response alteration showed a graded response decrease over a 4-day test period. These findings indicate the existence of a novel class of opiate sensitive reward sites and suggest (a) some apparently insensitive sites may become sensitive to opiate blockade under appropriate testing circumstances and (b) opioid neuropeptides may control certain tonic as well as more immediate aspects of motivation.

Central reward sites Opiate blockade Naltrexone

ENDOGENOUS opioid neuropeptides are believed to be critically involved in the regulation of pleasure and pain. Evidence for such a role in the control of pleasure includes the self-administration of endogenous opioids (e.g., [9]), the conditioned reinforcing properties of the latter (e.g., [6]), and electrical reinforcement of the central nervous system (intracranial self-stimulation, ICS). Central nervous system loci supporting self-stimulation are known to covary in density with sites showing high opiate receptor binding and tissue enkephalin levels (e.g., [10]). Moreover, opiate receptor blockade has been demonstrated to produce an immediate decrease in brain self-stimulation ([2–4, 8, 10]; note, however, [12]). A number of central nervous system sites have been shown to be inhibited by doses of the opiate blockers naloxone and naltrexone at doses considerably lower than those necessary to precipitate an abstinence syndrome or to acutely reverse morphine effects [3]. This implies at least as high a degree of behavioral and neurochemical specificity for reinforcing effects within opiate systems as for other behavioral effects.

To date, ICS reductions following opioid receptor blockade have been assumed to be rapid in onset and restricted to groups of cells with a binary response character i.e., cells were assumed to be highly sensitive or insensitive to blockade. Moreover, these properties were considered stable over time. Because testing was carried out under conditions of acute blockade, other possibilities i.e., for gradual effects as seen with tolerance have not been investigated. Nonetheless, certain of the reward enhancing effects of opiates upon ICS may take several days to develop ([1]; note, however, evidence summarized in [4]) and tolerance and dependence to opiates and enkephalins show a gradual and graded course of response [13]. Since the reward enhancing effects of morphine, and tolerance and dependence may take several days to develop, it may be predicted that a class of reward neurons with a graded and gradual response to blockade also might be present in the nervous system. To investigate this we implanted rats with electrodes in a major reward pathway, the medial forebrain bundle, and examined the acute and chronic effects of opiate receptor blockade upon responding for brain stimulation.

METHOD

Subjects

Twenty-two experimentally naive, adult male Sprague-Dawley rats, 425–550 g each were individually housed with food (Teklad 4.0% fat rodent diet S-0836) and tap water continuously available, and normal 12 hr/12 hr lighting cycles automatically programmed (lights on=0700–1900 hr).

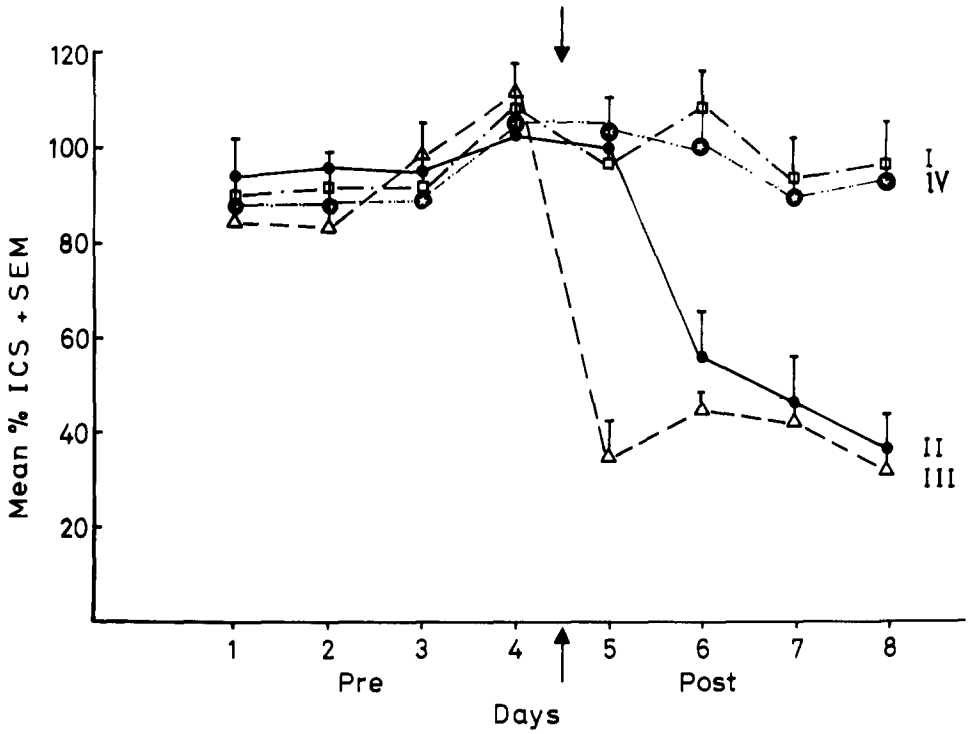

FIG. 1. Effects of opiate blockade by chronic naltrexone HCl (10 mg/kg; twice daily for 4 days) upon intracranial self-stimulation in the rat. All findings are presented as percent transformed scores (mean + standard error of the mean). Three weeks of habituation preceded the administration of drug or vehicle. The final four days of habituation and four days of experiment are presented. Groups: I=naltrexone, non-responsive groups (n=6); II=naltrexone, delayed response (n=5); III=naltrexone, immediate response (n=5); IV=vehicle (0.9% saline) (n=6).

Apparatus

Detailed description of both apparatus and surgery have been published elsewhere (e.g., [5]). All testing was carried out in the subject's home cages. Normal 25 × 18 × 17 cm rack mounted stainless steel cages were modified to house overhead mounted 14 × 16 cm stainless steel plates, which served as both manipulanda and contacts for stimulation. Contact of the plate by the rat's head-mounted brush displaced the plate upwards, closing a microswitch with a 25 g operating requirement. This allowed circuit completion and delivery of a 0.3 sec train of 60 Hz sinusoidal current through the plate to the brush and to an attached intracranial electrode. Normally a fully extended rearing response was necessary to move the plate sufficiently to complete the circuit. Circuit completion was achieved with the floor acting as the return circuit for stimulation. A series of capacitors and resistances, and a zero-crossing relay were used to assure constant current stimulation within and across stimulation trains.

Surgical details also are essentially similar to previous descriptions (e.g., [5]). Rats were anesthetized with 35 mg/kg of sodium pentobarbital and stereotaxically implanted with a single 0.025 cm diameter nichrome wire electrode insulated to the tip. Electrodes were aimed at the anterior aspect of the medial forebrain bundle (anterior-posterior, medial-lateral, and dorsal-ventral coordinates in mm from Bregma, using a level skull=0.0, 2.0, −8.0) and were attached to a tufted brass contact taken from a commercially available suede brush, as previously described [5]. The implantation site was chosen both because of extensive parametric studies which were carried out in our laboratory using this site, and because the particular area included several sites showing variable responses to acute blockade [3]. The brush-electrode assembly was attached to the skull with five stainless steel screws and acrylic dental cement [5]. Histological examination at the close of testing used wet cryostatically sectioned tissue at 40 μ as a photomicrographic negative.

Rats were allowed one week to recover from surgery prior to being exposed to any current. Current was then uniformly activated at an intensity of 50 μA, and levels were adjusted over the next seven days to maintain rates of stimulation between 1200 and 4500 responses over a 24 hr recording interval. Normal rates of response without current were consistently less than 50 responses per night. Final current values ranged between 20 and 80 μA. For all subjects greater than 95% of responses occurred during the dark phase of the lighting cycle. Stimulation parameters remained unchanged for individual subjects for the remainder of testing. All but three rats acquired the panel press response spontaneously during normal exploration of the cages. All data were acquired using BRS/LVE 12 V logic modules interfaced with a Burpee paper tape punch.

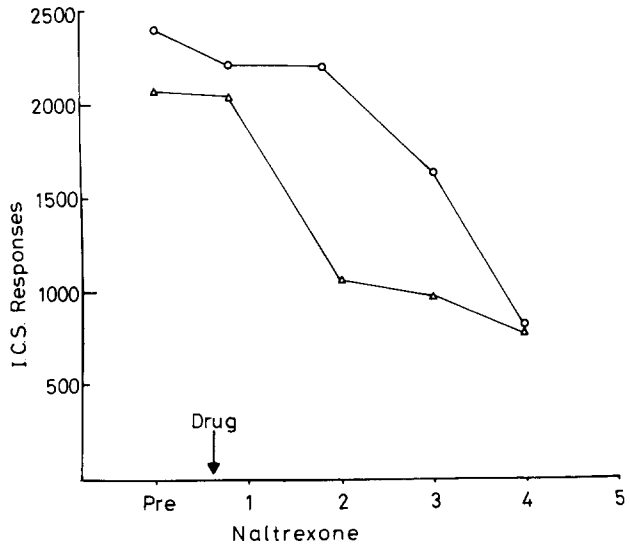

FIG. 2. Representative individual records of gradual responses to opiate blockade across days.

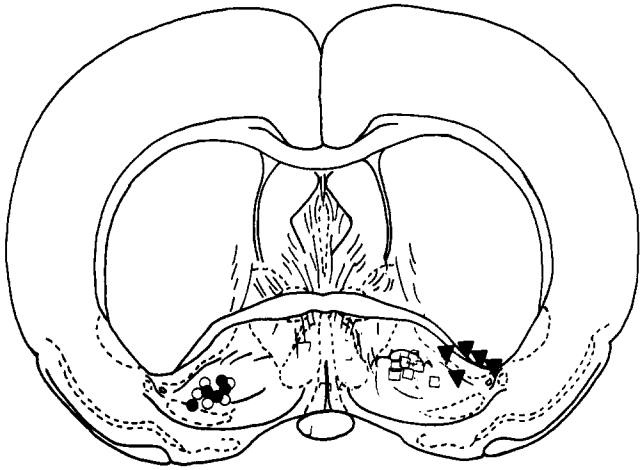

FIG. 3. Composite histology of naltrexone sensitive and resistant sites within the anterior medial forebrain bundles. The non-responsive group (n=6) is represented by open squares (right-hand side). The immediately responsive group (n=5) is represented by dark triangles and tended to be localized in proximity of basal nucleus accumbens. The control (vehicle) rats (n=6) are shown in dark circles (left-hand side); the gradually responsive rats (n=6) are shown by open circles. The only obvious histological trend was for immediately responsive rats to have sites near nucleus accumbens.

Three weeks of uninterrupted responding were allowed for response stabilization. Rats were undisturbed during this period except for normal cage maintenance (changing of food, water, and cage bedding) and daily injections of 0.9% sodium chloride to allow habituation in handling. Week four served as the experimental baseline period. A group of six control rats received twice daily injections of 0.9% sodium chloride vehicle solution. Remaining rats received daily naltrexone injections for five days. Drug treated rats were initially divided into responsive (at least 50% initial rate reduction, n=5) and non-responsive groups (n=11) the last of which was further divided into rats showing a gradual decline of overall rate (n=5) and those which remained non-responsive to treatment (n=6).

Naltrexone HCl (Endo) was administered in a standard dose of 10 mg/kg, twice daily with injections at 0800 and 1800 hr. A high dose was used to achieve chronic receptor blockade. This dose and a closely related administration schedule are known to produce chronic receptor blockade in two related designs [9,11]. Drug and vehicle injections were administered intraperitoneally 1 ml/kg.

Analysis was by 2-factor analysis of variance with repeated measures upon days. To equate animals with initially different levels of response all scores were initially percent transformed, with 100% individual activity defined as the average rate of response of a given rat for the final four days preceding the start of the experiment.

RESULTS

The pre-drug baseline for all rats was essentially stable across days (Fig. 1). Vehicle injections had no remarkable effects upon rates. Naltrexone injections produced 3 distinctive responses. One subgroup of rats was unaffected. A second group showed an immediate behavioral response. In addition and as predicted, a third group showed a gradual and graded response across days. These findings were confirmed statistically. Analysis of variance indicated significant effects of conditions, $F(3,21)=7.9$, $p<0.005$, and of conditions by days interaction, $F(9,54)=10.3$, $p<0.0001$, although a main effect of days was not present, $F(3,54)=1.4$, $p<0.2$. Based upon Sheffé analyses of post hoc differences, group three was statistically different from all other groups on the first day of drug administration (individual Fs ranged between 8.3 and 11.5). To allow further comparison curves of two individual rats showing the graded response are included in Fig. 2. An histology of the four groups is presented in Fig. 3. More dorsal sites and sites within ventral nucleus accumbens proved more acutely responsive to blockade. Other sites tended to vary throughout the target area.

DISCUSSION

These findings particularly of a conditions by days interaction empirically confirm the postulated existence of a novel subgroup of opiate sensitive sites showing a graded and cumulative response to the drug. This suggests the existence of a process similar to tolerance following agonists, but in an oppositely signed direction. It should, however, be noted that the major effects upon rate occur at 48–72 hours, which is earlier than might be predicted by normal tolerance. These findings are of interest since they suggest a novel function of opiates—the tonic control of motivational states. Although such an effect may imply that a novel subgroup of receptors exist, it may also represent differential drug accessibility or regional kinetic differences. Several questions about these effects remain as yet unanswered. Since there is variability across rates it might be questioned whether the various classes of response in fact represent cases of rate dependency. Although this cannot be ruled out, and indeed

certain CNS sites do display rate dependency [3], it should be noted that this varies across sites. Moreover, rats with wide ranges in baseline performance were used for each experimental cell in the present study. Doses were chosen in the present study to produce a fairly complete blockade (see [9,11]), the possibility of dose related effects also merits additional inquiry. It might be speculated that the gradual rate decline is a product of several experimental factors, and might be related not only to site, but also to response rate and dosage. Finally these findings suggest that ascription of opiate sensitivity or insensitivity should be carried out with an appreciation of the possible cumulative effects of chronic drug dosages. It is possible some earlier negative data (e.g., [12]) should be reinterpreted in light of the finding that apparently negative (i.e., non-responsive) sites may eventually respond to naltrexone.

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