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Opioid-Receptor Blockade Reduces Nose-Poke Self-Stimulation Derived From Medial Entorhinal Cortex

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REYMANN, K G , S WULCKO, T OTT AND H MATTHIES *Opioid-receptor blockade reduces nose-poke self-stimulation derived from medial entorhinal cortex* PHARMACOL BIOCHEM BEHAV 24(3) 439–443, 1986 —Rats were trained to nose-poke for intracranial self-stimulation (SS) with electrodes unilaterally implanted in the medial entorhinal cortex The acute effects of naloxone (NX, 0 1–10 mg/kg, IP) on a continuous reinforcement schedule were determined Reductions in the self-stimulation rates occurred only at moderate doses (median of individual changes = −36% at 1 and 5 mg/kg), whereas the high dose (10 mg/kg) was ineffective None of the doses influenced operant behavior These results are consistent with the hypothesis that endogenous opioid-opiate receptor mechanisms play a modulatory role in SS reward Considering that NX was administered systemically the action of the drug on reinforcement levels may be mediated by a site distinct from the locus of stimulation

Brain self-stimulation Entorhinal cortex Rat Opioid receptor blockade Reward mechanisms Naloxone

MANY neurotransmitters such as dopamine, norepinephrine, serotonin, acetylcholine and gamma aminobutyric acid have been implicated in reward mechanisms in the CNS (for review see [4, 19, 35, 43])

Since the discovery that the brain contains endogenous opiate-like peptides, a number of investigators have suggested that also opioid-ergic neurons may play a role in pleasure and reward [3, 4, 19, 20, 22, 29, 35, 36, 38, 43]

An enkephalinergic substrate of reward was first proposed by Belluzi and Stein [3] following the successful demonstration of intraventricular self-administration of leu- and met-enkephalin

One approach in the search for possible critical transmitters subserving reinforcement derived from electrical brain stimulation has been to compare self-stimulation before and after receptor blockade If intracranial self-stimulation (SS) behavior is mediated in part by the release of endogenous opioids onto the opioid receptor, then administration of naloxone (NX), the prototypic antagonist of morphine and enkephalin at opioid receptors, should attenuate this behavior Unfortunately, previous studies have not provided consistent results Whereas some studies report reduced rates of lever pressing at moderate [3, 7, 11, 20, 32, 41] or high doses [2, 37, 40, 42], others have failed to find effects of acute NX treatment on rate of SS responding over a wide range of dosages [5, 13, 17, 18, 24, 39]. Although the above-mentioned experimental differences cannot be explained solely by use of different stimulation sites, the examination of other brain regions and more "natural" operants should convey further insight into the opioid-ergic mechanisms

Recent experiments have demonstrated conclusively that the electrical stimulation of the medial entorhinal cortex yielded a reliable SS behavior [26]. Whereas SS of the lateral entorhinal cortex was related to its dopaminergic innervation [6], no dopaminergic innervation has been reported so far for the medial entorhinal cortex. Furthermore, haloperidol injection produced a clear decrease in SS if derived from the lateral, but not from the medial entorhinal cortex (Ott, unpublished data) In the light of the postulated role of endogenous opioids in reward, the existence of enkephalinergic neurons and fibre systems in this area [14,21] raises the possibility that they are responsible for medial entorhinal cortex SS For that reason, the present experiments examine the effect of several moderate doses of NX on nose-poke response rates induced by electrical stimulation of the medial entorhinal cortex

METHOD

Fourteen male Wistar rats from our own colony weighing 220–250 g at the time of surgery served as subjects The rats

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were individually housed under the conditions of a 12 hour light dark cycle (artificial illumination between 6 00 a m and 6 00 p m), with continuous access to food and water One week before the beginning of the experiment each rat was stereotaxically implanted with a single bipolar Teflon-isolated stainless-steel stimulating electrode (110 μm in diameter) The tip was aimed for the posterior part of the right medial entorhinal cortex using the stereotactic coordinates AP −7 1 mm, lateral 4 1 mm, and 3 3 mm below the skull surface [34]

The electrodes were attached to subminiature connectors and rigidly fixed to the skull with stainless steel screws and dental cement Surgery was carried out under sodium pentobarbital anaesthesia (50 mg/kg IP) The correct placement of the electrodes was verified by histological examination of paraffinized brain sections after completion of the experiments

The SS behavior was tested using a plastic cylinder (20 cm in diameter, 40 cm in height), with two photoelectric cells mounted into two holes (distance 14 cm) in the cylinder wall By interrupting one of the photobeams with the nose, the animal initiated a stimulus train with a frequency of 100 cps

The square wave pulses were generated by a constant-current stimulator which was connected with the electrodes by a commutator and a cable Train duration and pulse duration were fixed at 200 msec and 0 1 msec, respectively The other photobeam (non-effective hole) served as a reference for operant behavior Each rat was tested only once daily throughout stabilization and treatment, between 1 00 p m and 3 00 p m for 30 min Response rates were recorded automatically at 6 min intervals The rats were trained to self-stimulate intracerebrally until a relatively constant rate and a ratio 3 1 between response rates at the effective and noneffective hole were established For each animal a subconvulsive current intensity which elicited approximately 75% of the maximal response rate (between 80–240 μA) was selected individually

Thereafter the animals were treated with an intraperitoneal injection of isotonic saline (0 156 M) for five days Naloxone hydrochloride (Endo Laboratories) was dissolved in isotonic saline to concentrations of 0 01, 0 1, 0 5 and 1 0 mg/ml and administered in a volume of 10 ml/kg of body weight IP The different doses of the drug were tested in random sequence Data collection began 10 min after injection The total number of nose-pokes made during the 30 min test session provided the data to compare the differences in response rates between a given dose of drug and the preceding saline days

RESULTS

The histological evaluation showed that the tips of the stimulating electrodes were consistently located in the medial entorhinal cortex, mainly in the area of the fibres of the perforant path, i e , between the entorhinal cell layers and the alveus (Fig 1) A 2 to 3 week training period was necessary to obtain a reliable and stable SS of the medial entorhinal cortex (cf [7]) At the time of NX testing the rats were 12–13 weeks old Evaluation of the data indicated that there was no reliable difference between the scores on the last two preceding saline days (Fig 2) For statistical analysis, the data were reduced by averaging across the two pretest saline scores to get a single index of responding under placebo

NX treatment in moderate doses lowered the response rate on the effective hole (Fig 2) The Wilcoxon matched-pairs signed-rank test indicates that the 1 mg/kg scores (n=14) and the 5 mg/kg scores (n=10) are reliably different from the saline scores (two-tailed $p<0$ 05, Fig 2) This effect was evident throughout the whole test session (Fig 3) Although the medians of absolute values were different (Fig 2) the median of individual changes revealed the same 36 percent decrease for both 1 and 5 mg/kg doses

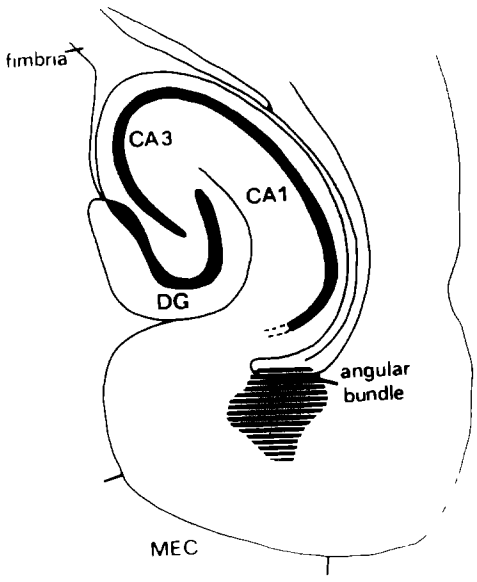

FIG 1 Diagram of electrode sites from horizontal sections (MEC=medial entorhinal cortex)

NX 0 1 mg/kg slightly reduced response rates in 3 of 4 animals (median of individual changes −12%) Interestingly enough, the highest dose (10 mg/kg, n=14) used in this study produced no change in the SS behavior (Fig 2)

The level of operant behavior indicated by the number of responses at the noneffective hole was not influenced by either dose of NX Pilot experiments with lateral entorhinal cortex implants (n=6) did not reveal such a clear NX depression of SS rates

DISCUSSION

The results of these experiments support the idea that positive reinforcement is mediated, in part, by the release of endogenous endorphins [3] In agreement with some earlier experiments [2, 3, 7, 11, 20, 32, 37, 40–42], a reduction of SS was obtained with acute NX treatment Other data suggested that NX is also reducing the reinforcing value of food and water (for review see [19, 29, 35]) Additionally, it is well established that opiate agonists facilitate intracranial SS at doses that have been shown to be self-administered (for review see [15, 22, 29]) An alternative interpretation that NX suppresses SS primarily by antagonizing endorphin-mediated analgesia and thereby increases the aversive properties of the brain stimulation was excluded at least for the central gray stimulation sites [20] Moreover, recent data support the assumption that SS and analgesia are mediated not only by different brain systems [20], but also by different opioid-receptor subtypes [2,28]

The present findings supply first evidence that SS (1) de-

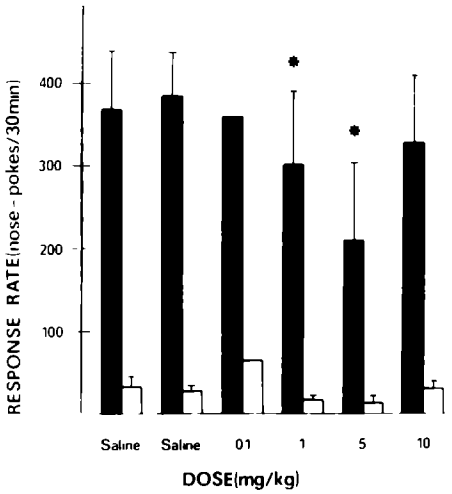

FIG 2 Effects of intraperitoneally injected naloxone on response rate per 30 min daily session One mg/kg and 5 mg/kg NX significantly (*$p<0.05$) reduced the self-stimulation (shaded columns), whereas the operant behavior (noneffective hole=open columns) is not influenced Median+standard error of the median (n=14 animals with the following exceptions 0 1 mg/kg n=4, 5 mg/kg n=10) Note that the different NX doses were given randomly after the preceding saline days

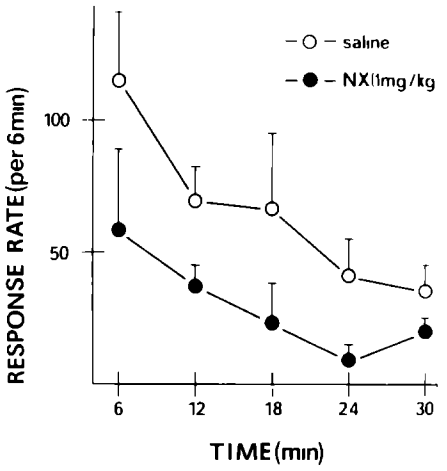

FIG 3 Time course of SS responding (counts per 6 minutes) after saline or NX (1 mg/kg) Median+standard error of the median

rived from a periarchicortical structure and (2) performed by nose-poking depends on endogenous opioids, too Therefore, the available data seem to suggest that—even if neuronal elements subserving various SS operants differ to some extent—opioid dependence is not restricted to SS by lever pressing only Although in some SS models [32, 42] the use of partial reinforcement schedules was necessary to demonstrate an effect of NX, this is not the case in nose-poke SS of the medial entorhinal cortex as well as in other models [2, 3, 11, 20, 41]

If the suppression of SS was due to a non-specific performance deficit in this experiment then this effect also should have been evident at the noneffective hole However, the response rate was not affected by NX at the noneffective hole It could be argued that the rate of non-reinforced nose-poking was too low for the demonstration of a non-specific performance deficit The lack of an effect at the noneffective hole, however, is consistent with previous findings where acute administration of moderate doses of NX failed to produce significant changes in rats' spontaneous locomotor activity [1, 9, 10]

There is evidence that NX has more influence on social behavior and exploration of novel environments than on general activity [10, 26, 31]

In contrast to most previous studies describing the effects of NX [2, 3, 11, 32, 37, 42], the highest dose of NX used in this study (10 mg/kg) was ineffective Although a simple dose-response relationship is more common for NX, there are some reports on a decreasing efficacy with increasing doses of NX in other behavioral tests (see [31,44]) One possible explanation would be that, at high doses, a second type of the opioid-receptor or another non-specific mechanism with opposing or blocking effects is involved which, in the case of our model, could cancel the effect at the more sensitive site At present, the basis of the discrepancy between the NX data in the literature is unclear The fact that NX failed to affect SS behavior in some studies can perhaps be due to the following reasons (1) Insufficient activation of the critical opioid-ergic neural substrate (i e , differences in electrode sites and stimulation parameters) (2) Testing of too high doses (e g , [17,40]). (3) Depressive effects of NX on other parameters as for example self-selected current duration [13] (4) Long-term effects of repeated administration Chronic NX treatment usually failed to alter SS (for review, see [27])

The fact that various neuronal structures were exposed to NX precludes identification of the primary site of action of NX being responsible for the suppression of the SS rate in our experiments. Since we could not find effects of NX on SS of the lateral entorhinal cortex in pilot experiments, its enkephalinergic projections [14] seem not to be involved. If NX antagonizes the action of cholecystokinin-containing projections of the medial entorhinal cortex [12] could be a matter of speculation (cf [33]) Interestingly, SS behavior obtained by electrodes in the dentate gyrus, an important target of the entorhinal projections, is NX-sensitive [7]

However, in our previous experiments it was shown by electrophysiological methods that at least the activation of the classical (glutamatergic) medial perforant path to the dentate area is not critically involved in the mediation of this type of intracranial SS [26] Little is known about the projections of the relatively small number of enkephalinergic neurons of the rats' medial entorhinal cortex [14,21] and about the connections of the entorhinal cortex with other SS substrates as the prefrontal cortex and the ventral tegmental area There is an increasing evidence, that dopaminergic neurons of forebrain represent the final common pathway for the expression of motivated behavior and that reward from opiates may involve a dopaminergic mechanism [4,43]. Some recent studies suggest that the reward-relevant opioid-receptors are localized in the ventral tegmental area (for review see [4,43]) However, considering the lack of pronounced attenuation of response rates after dopamine receptor blockage by haloperidol (Ott, unpublished data), a

separate reward system could be responsible as well for the NX effects in our model It was hypothesized that SS from cortical sites is caused by a common neural system [8] It is noteworthy that SS of another cortical area, the prefrontal cortex, is likewise depressed by NX [11]

In conclusion, our results suggest that an enkephalin and/or endorphin release may be involved in the neural mechanism of a nose-poke SS of the rat medial entorhinal cortex However, further experiments including rate-intensity functions and "rate-free" measurements [23] are appropriate to confirm the present interpretation The further identification of opioid-ergic systems as participant of the reward function of the entorhinal cortex has interesting implications for studying the relationship between reward and memory Evidence indicates that electrical stimulation of the same neuroanatomical system can be used as a conditioned stimulus in a learning paradigm and is followed by a long-term potentiation of synaptic transmission in target areas (for review see [30])

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