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Naloxone Lowers Brain-Stimulation Escape Thresholds

STEPHEN SASSON AND CONAN KORNETSKY

Laboratory of Behavioral Pharmacology, Division of Psychiatry
Boston University School of Medicine, Boston, MA 02118

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SASSON, S. AND C. KORNETSKY. *Naloxone lowers brain-stimulation escape thresholds.* PHARMAC BIOCHEM BEHAV **18**(2) 231–233, 1983.—Rats were trained to escape from aversive electrical brain stimulation to the mesencephalic reticular formation (MRF). The threshold for this escape behavior was determined by a modification of the psychophysical method of limits. The administration of naloxone (4–16 mg/kg, IP) produced a decrease in escape threshold from MRF stimulation. These findings implicate the involvement of the MRF in the modulation of pain and suggest that threshold changes to stimulation at this level of the nociceptive neuro-axis may represent a change in a motivational-attentional dimension of pain.

Naloxone Pain Brain stimulation Reticular formation Psychophysics

EXPERIMENTAL evidence suggest the involvement of endogenous opioids in the modulation of pain [17]. Accordingly, there have been several reports of hyperalgesia following the administration of the opiate antagonist naloxone with peripherally applied nociceptive stimuli [1–4, 8–10]. Rosenfeld and Rice [15] found that naloxone produced hyperalgesia with aversive electrical stimulation to the trigeminal nuclear complex but failed to find a similar effect when the stimulation was applied to more rostral brain sites in the rat. In a parallel fashion, they found that morphine produced analgesia (raised the escape threshold) to the trigeminal stimulation but had no effect on the threshold for stimulation to the more rostral MRF [16]. These findings are explained by these investigators by a model which attributes the analgesic action of opiates to the activation of a descending inhibitory pathway which exerts effects on the transmission of nociceptive impulses at primary-to-secondary afferent synapses in the spinal cord [12]. We, [11,18] as well as Pert [13], found that morphine caused dose dependent decreases in sensitivity to aversive electrical stimulation to the MRF, which suggests that mechanisms other than the activation of descending inhibitory pathways contribute to the analgesic action of opiates. With this in mind, the present study was undertaken to determine if naloxone would have effects opposite those of morphine, that is, increased sensitivity to aversive stimulation to the MRF.

METHOD

Subjects and Surgery

Four male albino rats (CDF strain, Charles River Laboratories, Wilmington, MA), each weighing approximately 300 g, were stereotaxically implanted with a stainless steel electrode (0.13 mm) aimed at the MRF. Electrodes were insulated except at the tips. The following coordinates were employed: A-P: 7.0 mm posterior to bregma; Lateral: ±2.0 mm from the sagittal suture; D-V: 6.0 mm ventral to the skull surface. Surgery was performed under anesthesia with Equi-Thesin® (3.0 ml/kg), and lidocaine (0.5 ml) was injected intradermally around the incision. Animals were allowed at least one week of recovery prior to testing.

Testing Procedure

Animals were trained to escape from electrical stimulation to the MRF by turning a cylindrical manipulandum which was mounted in an opening in one wall of a plastic chamber (20×20 cm). Four equally spaced cams were positioned on one of the end plates of the manipulandum such that they operated a microswitch when the wheel was rotated. Termination of the aversive stimulation was obtained after two closures (within 0.75 sec of each other) of the microswitch. A constant current stimulator (Nuclear-Chicago) was used to deliver the stimuli which consisted of biphasic symmetrical square pulses and 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 requirement for threshold determination.

A trial was initiated by the onset of stimulation. If no response occurred within 7.5 sec the stimulus was automatically terminated. A new trial began on the average of every 22±7 sec. Stimulus intensities were varied according to a modification of the classical psychophysical method of limits. Stimuli were presented in an alternating ascending and descending series with a step size of 1 or 2 μA depending on the sensitivity of the animal. An ascending series was initiated at a previously determined subthreshold intensity. Three trials were given in succession at each intensity. Two or more escape responses at a particular intensity were scored as a plus while less than two responses were scored as a minus. An ascending series was conducted until plus scores were achieved in two successive steps. A descending

series was then initiated at one step size lower and current intensity continued to decrease until two successive minus scores were achieved. The threshold for a particular ascending or descending series was defined as the midpoint between those intensities which delimited the transition from plus to minus scores.

Four series, two ascending and two descending, comprised a session. A session threshold was computed as the mean of the four series thresholds. Immediately after the first session was completed animals were injected subcutaneously with either saline (0.9% NaCl) or naloxone hydrochloride (dissolved in 0.9% NaCl). A second session was started 10 min after treatment.

Threshold differences between pre- and post-injection sessions on a drug test day was expressed as a Z-score based on the standard deviation of the mean threshold difference for all saline test days. Data from three to eight saline test days were obtained before the effects of naloxone were assessed. Additional saline test days were interspersed between drug test days. Data from a total of approximately ten saline test days were collected for each animal.

The threshold effect for each dose (4, 8, 12, 16 mg/kg) was determined in each animal with the exception of one animal whose electrode platform became dislodged after only two doses of naloxone were studied. The order of administration of doses was randomly assigned. Latency of response and strength of response as measured by the number of microswitch closures per response were also recorded. At the completion of the experiment, animals were sacrificed with an overdose of anesthetic (Equi-Thesin®). After intracardial perfusion with saline, followed by 10% formalin, the brains were removed and examined histologically to verify electrode placements.

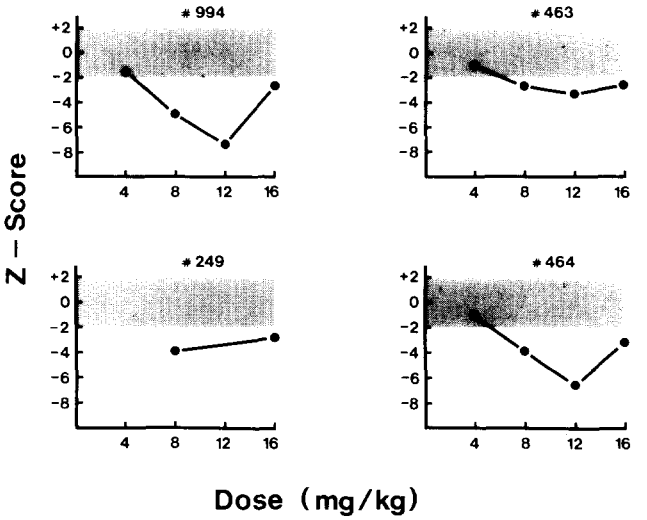

FIG. 1. Standard score (Z-score) changes in escape threshold value from pre- to post-drug as a function of dose of naloxone for each of 4 animals. The shaded area represents the 95% confidence limits for saline data.

RESULTS

Figure 1 shows the effects of each dose of naloxone on the escape threshold for each of the 4 animals. Significant threshold decreases were obtained with test doses of 8 mg/kg and greater. The mean latency to respond at threshold after saline treatment was 2.51 sec and was not significantly altered by any dose of naloxone. No intertrial responses were made on control or test days and the strength of response also was not altered by naloxone.

We were unable to histologically verify the electrode placements in two animals (no. 994 and no. 249) because of loss of electrode platform prior to being sacrificed. The electrode placements in the other two animals were located in the dorsal tegmental reticular formation just lateral to the periaquiductal gray. Along the coronal plane, placements were between the level of the brachium of the inferior colliculus and the decussation of the dorsal tegmental tract.

DISCUSSION

The findings support the hypothesis that endogenous opiate-like substances have a physiological role in pain regulation. Additionally, our data as well as data from others implicate a role for the reticular formation in pain modulation. Haigler and coworkers [5,7] have reported antagonism of nociceptively activated single units in the MRF by systemically administered as well as microiontophoretically applied morphine. Furthermore, naloxone administered intravenously or microiontophoretically has been shown to antagonize this drug-induced blockade [5,7]. In addition to the single unit studies, it has been shown that intracerebrally applied morphine to the MRF produced analgesia as measured by the tail flick test and the hemostat pinch test in the rat [6]. Similarly, Pert and Yaksh [14] found that 40 μg of morphine delivered bilaterally to the MRF of the rhesus monkey caused analgesia as measured by a shock titration procedure.

Discrepancies between our work and that of Rosenfeld and coworkers could be attributed to several factors. The latter investigators used subjective estimates of unconditioned responses to the stimulation and only employed one dose (20 mg/kg) of naloxone. In contrast, we utilized an instrumental escape paradigm and explored a range of doses, all lower than 20 mg/kg.

Opiate agonists and antagonists may influence endogenous systems which produce effects at primary somatosensory synapses but they also appear to have effects on more rostral structures such as the MRF. Recent studies by Yeung and Rudy [19,20] support the notion of multiple sites of action for morphine in the spinal cord and supraspinal structures. It is conceivable that nociceptive transmission at this more rostral level be involved with a motivational-attentional dimension of pain perception while basic neuronal mechanisms that have been studied in the spinal dorsal horn neurons are related to a sensory-discriminative dimension of pain. In fact it would be surprising to find a lack of involvement of the MRF given the critical role of the reticular formation in sensory integration.

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