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RAPID COMMUNICATION

Hyperalgesic and Analgesic Actions of Morphine, U50-488, Naltrexone, and (−)-Lobeline in the Rat Brainstem

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HAMANN, S. R. AND W. R. MARTIN. *Hyperalgesic and analgesic actions of morphine, U50-488, naltrexone, and (−)-lobeline in the rat brainstem.* PHARMACOL BIOCHEM BEHAV 47(1) 197–201, 1994.—Morphine, U50-488, and (−)-lobeline produced dose-related shortening of a low-intensity thermally evoked tail avoidance response (LITETAR) (e.g., hyperalgesia) when microinjected into the dorsal posterior mesencephalic tegmentum (DPMT) of conscious rats. The hyperalgesic potency of (−)-lobeline was greater than either morphine or U50-488. With higher doses, morphine's hyperalgesic actions diminished and prolongation of the LITETAR (e.g., analgesia) was observed. Naltrexone produced analgesia in the DPMT that diminished with increasing dose. The hyperalgesic actions of morphine, U50-488, and (−)-lobeline further suggest the presence of kappaergic opioid and nicotinic mechanisms in the DPMT of rats. The hyperalgesic actions of U50-488, a highly specific opioid κ-receptor agonist, strongly suggest the presence of a κ-opioidergic hyperalgesic mechanism in the DPMT.

Hyperalgesia Analgesia Opioid Kappaergic Nicotinic Brainstem Nociception

THE earliest reports of brainstem opioid hyperalgesic processes came from studies of the decerebrate, decerebrate and spinalized, and intact dog (16,17,33,34). Fourth-ventricle opioid and nicotinic hyperalgesia was subsequently demonstrated in anesthetized decerebrate (24) and conscious intact (11,27) rats. Ethylketazocine (EKC) and (−)-nicotine produce hyperalgesia in the rat at midline loci from the middle of the fourth ventricle to the dorsal posterior mesencephalic tegmentum (DPMT) (11). The DPMT region is sensitive to the analgesic actions of naltrexone, mecamylamine (11), dynorphin A(1-13) antiserum (10), and local anesthetics (9). Tonic hyperalgesic activity in the brainstem and DPMT has been suggested from the analgesic actions of these different pharmacological agents (9–11,34).

The present studies were conducted to determine if other opioid and nicotinic drugs produce hyperalgesia in the DPMT. To this end, the actions of morphine, U50-488, and (−)-lobeline in altering the latency of a low-intensity thermally evoked tail avoidance response (LITETAR) were studied. To extend previous studies, additional experiments were performed with the opioid antagonist naltrexone.

METHOD

Female Sprague-Dawley rats (250–300 g) were surgically implanted with stainless steel guide cannulae (22 ga) directed toward a position within the DPMT [AP, −0.2; L, 0.0; V, +3.0, interaural (28)] under ketamine (100 mg/kg)-acepromazine (1 mg/kg) anesthesia. Animals were allowed to recover 1 week before being introduced to the handling procedures during practice sessions. At 2-day intervals, graded doses of drugs were microinjected (0.5 μl) using a 28-ga chemotrode connected to a 1-μl Hamilton syringe (Hamilton Co., Reno, NV) with polyethylene tubing. A LITETAR (9–11,27) was used to assess drug-induced changes in response to nociceptive thermal stimuli. Stimulus strengths were determined using thermopile equilibrium conductance values at the beginning and throughout each experiment. The LITETAR was evoked

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TABLE 1

CHANGES IN LITETAR FOLLOWING DRUG MICROINJECTION INTO THE DPMT OF CONSCIOUS RATS

	Change in LITETAR Latency (s)								
	Time After Microinjection (min)							Change in AUC (s × min)	
Drug Dose (μg)	5	10	15	20	30	45	60	0–30	0–60
Morphine (n = 6)									
0.01	−0.9 ± 2.6	−2.8 ± 4.1	−3.4 ± 5.4	−3.5 ± 3.7	−4.5 ± 3.7	−3.6 ± 4.3	+1.4 ± 2.2	−84.1 ± 85.8	−161.5 ± 178.9
0.1	−8.8* ± 2.1	−3.9 ± 4.2	−7.7 ± 4.0	−5.6 ± 3.3	−7.2† ± 2.9	−7.7 ± 4.8	−1.9 ± 3.3	−180.0† ± 70.5	−363.2† ± 178.5
1.0	−8.6* ± 2.0	−6.0† ± 2.8	−8.1 ± 4.9	−9.5‡ ± 3.5	−10.1‡ ± 3.3	−11.5† ± 5.1	−1.9 ± 3.3	−234.8‡ ± 78.2	−497.0‡ ± 193.5
0.5	+8.0‡ ± 2.4	+3.7 ± 3.2	−0.7 ± 5.3	−1.7 ± 4.6	−2.2 ± 2.5	−1.8 ± 4.6	+11.5* ± 2.3	+32.0 ± 81.8	+128.6 ± 174.6
10.0	+6.0 ± 3.6	+10.9‡ ± 2.9	+4.0 ± 4.6	+3.5 ± 3.8	−1.5 ± 4.7	+5.7 ± 2.7	+2.3 ± 3.2	+123.7 ± 73.7	+215.5 ± 133.4
30.0	+12.1* ± 1.1	+15.7‡ ± 4.2	+16.4‡ ± 5.0	+14.7‡ ± 4.1	+10.4† ± 5.3	+9.0 ± 6.5	+18.6* ± 4.6	+383.1‡ ± 96.0	+735.9‡ ± 255.4
U50-488 (n = 6–10)									
0.9	−3.9 ± 4.1	−4.1 ± 4.6	+1.8 ± 4.8	−3.1 ± 5.6	+0.5 ± 3.3	−3.8 ± 4.5	−1.2 ± 4.1	−52.1 ± 106.7	−120.7 ± 205.2
9.0	−8.9 ± 5.8	−9.3 ± 5.2	−5.8 ± 5.8	−13.5‡ ± 5.2	−9.1‡ ± 3.1	−11.5† ± 5.3	−7.9 ± 6.4	−266.4† ± 115.4	−577.0† ± 246.1
30.0	−1.0 ± 6.5	−3.5 ± 5.4	−4.7 ± 4.9	−9.2 ± 5.7	−6.0 ± 3.3	−11.4‡ ± 4.3	−13.0‡ ± 3.7	−145.2 ± 120.3	−458.7† ± 200.0
90.0	+0.2 ± 5.5	+3.3 ± 5.7	+1.0 ± 4.1	−6.4 ± 4.3	−5.0 ± 3.8	+1.1 ± 3.2	−3.8 ± 4.5	−0.5 ± 102.1	+35.5 ± 191.3
Naltrexone (n = 5)									
2.5	+8.1 ± 5.4	+14.8* ± 3.1	−0.4 ± 3.7	+7.2 ± 3.7	−0.4 ± 3.5	−3.1 ± 3.3	+10.2‡ ± 2.7	+164.6‡ ± 59.8	+191.6‡ ± 71.0
15	+4.8 ± 4.0	+9.5 ± 4.5	+4.2 ± 3.6	+0.4 ± 5.3	+3.4 ± 5.4	−1.6 ± 5.2	+6.1 ± 4.6	+113.0 ± 97.6	+160.7 ± 228.9
30	−0.6 ± 1.8	+0.1 ± 3.6	+1.8 ± 4.4	−9.6‡ ± 3.5	+1.4 ± 3.3	+8.2 ± 5.7	+12.2‡ ± 4.2	−58.9 ± 61.0	+165.0 ± 180.9
(−)-Lobeline (n = 6–9)									
0.00015	−2.0 ± 4.0	−2.9 ± 4.6	+6.4 ± 3.7	−1.1 ± 4.3	−1.9 ± 2.6	−1.1 ± 4.7	−1.4 ± 3.2	−9.8 ± 66.6	−58.4 ± 159.1
0.0015	−4.1 ± 2.0	−3.1 ± 3.7	−1.5 ± 1.8	−7.3 ± 4.4	+2.1 ± 1.8	−0.4 ± 3.2	−4.5 ± 2.3	−87.8 ± 39.1	−112.2 ± 72.9
0.015	−16.3* ± 3.7	−6.1 ± 4.9	−0.3 ± 2.8	−12.2‡ ± 3.2	−8.2 ± 2.9	−8.9‡ ± 3.3	−11.6† ± 4.8	−245.3‡ ± 76.4	−521.6‡ ± 182.5
0.15	−10.9‡ ± 4.7	−2.9 ± 4.4	+7.0† ± 3.1	−5.8 ± 5.1	−3.6 ± 3.6	−1.7 ± 6.7	−4.4 ± 4.8	−95.8 ± 79.2	208.4 ± 217.4
10.0	−11.3‡ ± 3.2	+3.6 ± 5.5	+3.9 ± 3.3	+1.7 ± 3.4	−2.5 ± 4.3	−1.7 ± 6.0	−3.0 ± 4.9	−17.4 ± 86.2	−89.9 ± 230.5

AUC, area under the time-action curve calculated using the trapezoidal rule. Changes in LITETAR latency and AUCs have been corrected for vehicle effects. There were no statistically significant effects of the vehicle observed in any of the microinjection experiments. Statistical analyses compared drug effects to vehicle effects using paired Student's t-test.

*$p < 0.01$.

†$p < 0.10$.

‡$p < 0.05$.

with a heat stimulus intensity sufficient to give mean control latencies of 15-20 s. The cutoff used for the LITETAR was 40 s.

Complete crossover design was utilized where each animal received several doses of an individual drug and a vehicle. Different animals were used in studies of each drug. Predrug LITETAR determinations were made in duplicate at 10-min intervals. Mean predrug latencies were subtracted from postdrug single observations made at 5, 10, 15, 20, 30, 45, and 60 min after microinjection. For construction of dose-response curves, area under the time-action curve (AUC) was calculated from these values using the trapezoidal rule. Vehicle LITETAR latencies and AUCs were subtracted from the drug treatment effects. Two-way analysis of variance (ANOVA; rats × doses) was performed using the 0- to 30-min AUCs, partitioning the total variance into between rats, between dose, and error variances. The between-dose variance was further partitioned into linearity and deviation from linearity variances (5). Previous studies (11) have demonstrated the residual variance was less using the 0- to 30-min AUCs as compared to the 0- to 60-min AUCs or peak drug effects. Drug-induced differences from predrug latencies were determined using Student's *t*-test.

The source and doses of each drug studied were as follows: morphine sulfate (Sigma Chemical Co., St. Louis, MO), 0.01, 0.1, 1.0, 5.0, 10, and 30 μg; U50-488 (gift from Upjohn Co., Kalamazoo, MI), 0.9, 9.0, 30, and 90 μg; naltrexone HCl (du Pont, Wilmington, DE), 2.5, 15, and 30 μg; (−)-lobeline (Sigma), 0.00015, 0.0015, 0.015, 0.15, and 10.0 μg. The naltrexone doses overlapped and extended the range studied previously (11). All drugs were prepared in 0.9% NaCl. Microinjections were 0.5 μl. Drug doses are presented as the free base. At the end of the experiment, methylene blue (0.5%) was microinjected (0.5 μl) into the DPMT of two to three rats from each individual drug group to confirm location of the cannulae tips.

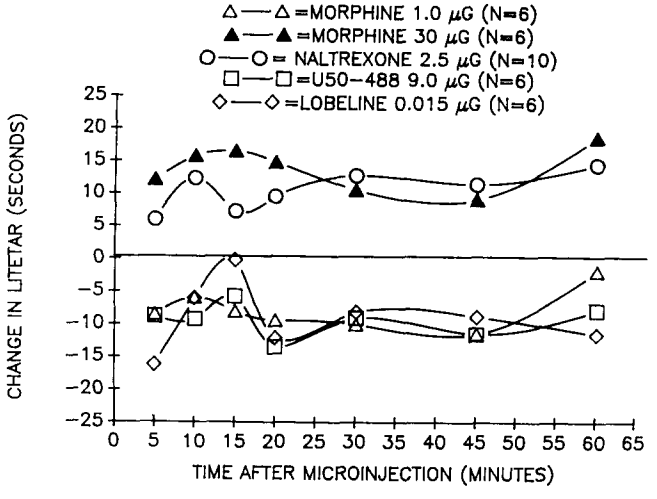

FIG. 1. Example time-action curves for the hyperalgesic or analgesic actions of morphine, naltrexone, U50-488, and (−)-lobeline after microinjection into the dorsal posterior mesencephalic tegmentum (DPMT). The doses shown produced near maximal responses observed. Data points represent mean for the number of rats indicated. Each drug shown produced significant ($p < 0.10$ to $p < <0.01$) effects within 5-20 min following microinjection. (See Table 1 for results of statistical analysis.)

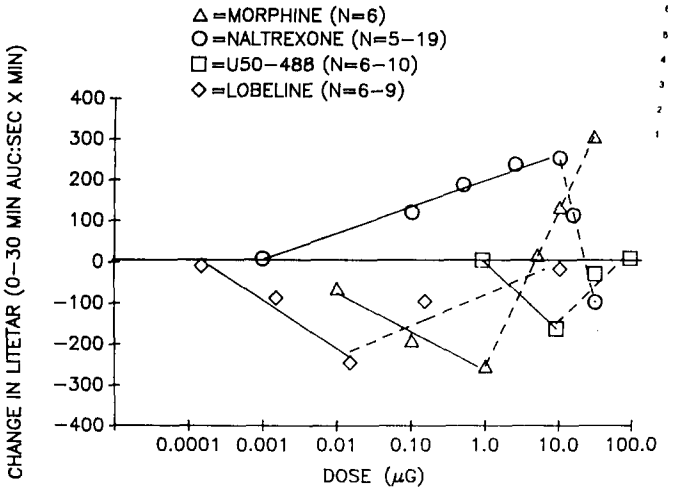

FIG. 2. Dose-response curves for morphine, naltrexone, U50-488, and (−)-lobeline when graded doses were administered into the dorsal posterior mesencephalic tegmentum (DPMT). Solid lines represent those portions of the dose-response lines that exhibited significant linearity. For comparison, data previously reported (11) for naltrexone have been included. The broken lines represent data that when included in the analysis of variance produced significant deviation from linearity. The doses of each drug microinjected in a 0.5-μl volume are listed in the Method section. Data points represent the mean change in latency for the number of rats studied. (See Table 1 for results of statistical analysis.)

Animals were sacrificed by an overdose of pentobarbital (65 mg/kg). Brains were removed following decapitations and fixed in formalin (10%). Brains were examined 2-3 days later to determine the extent of tissue staining.

RESULTS

Lengthening and shortening of the LITETAR will be referred to as analgesia and hyperalgesia, respectively. This terminology is not meant to imply that changes in the LITETAR have direct relation to changes in pain perception. Table 1 presents mean ± SE for change in LITETAR latencies and AUCs for experiments performed with each drug. Figure 1 shows example time-action curves for near maximal hyperalgesic or analgesic doses of morphine, naltrexone, U50-488, and (−)-lobeline when microinjected into the DPMT. The hyperalgesic effects of morphine, U50-488, and (−)-lobeline were evident within 5 min and persisted over 1 h after microinjection into the DPMT and at no time studied was analgesia observed. After a higher dose of morphine, persisting analgesia was also evident within 5 min after microinjection. Naltrexone produced a rapid onset analgesic response at the lowest dose (2.5 μg) studied.

Figure 2 shows the dose-response relationships for the hyperalgesic and analgesic actions of morphine, U50-488, and (−)-lobeline. ANOVA performed using data from all doses detected significant deviation from linearity with each drug. However, if the dose-response line were resolved into the indicated linear components the deviation from regression was not significant. (−)-Lobeline was the most potent hyperalgesic agent. While the hyperalgesic actions diminished with larger doses of U50-488 (30 and 90 μg) and (−)-lobeline (15 and 10.0 μg), no overt analgesia was observed. In contrast, morphine produced obvious analgesia with larger doses (15

and 30 μg). Figure 2 also shows the dose–response curves for the actions of naltrexone on the LITETAR. The smallest dose of naltrexone studied (2.5 μg) produced a degree of analgesia similar to that previously reported (7). With larger doses (15 and 30 μg), the analgesic actions of naltrexone diminished and modest hyperalgesia was observed.

The methylene blue dye was distributed within 1–1.5 mm surrounding the intended site of microinjection. The darkest staining occurred within approximately a 1-mm radius near the central and pericentral dorsal tegmental nuclei within the dorsal tegmental area. There was slight staining observed along the tract of the guide cannulae, probably resulting from reflux when the guide cannulae were removed during dissection.

DISCUSSION

The hyperalgesic actions of morphine and U50-488 at the DPMT further suggest the involvement of μ- and κ-opioidergic mechanisms in brainstem hyperalgesia. Morphine, U50-488, and naltrexone exhibit complex time–action and dose–response curves, suggesting involvement of more than one type of opioid mechanism in altering the latency of the LITETAR. These complexities may be due in part to diffusion of drug to surrounding brainstem loci with differing hyperalgesic and analgesic tonic activity (11). In this regard, the approximate 1-mm radius diffusion observed for the methylene blue dye would encompass several anatomically distinct brainstem nuclei (28). Multiple brainstem opioid hyperalgesic and analgesic mechanisms have been suggested from studies in the rat fourth ventricle (11). Morphine's analgesic action with higher doses suggests the presence of a lower-affinity opioid analgesic mechanism in the DPMT. The finding that naltrexone's analgesic actions diminish and hyperalgesia appears with larger doses also supports the presence of tonically active opioid analgesic mechanisms in the DPMT. The analgesic actions of morphine after administration at several brainstem sites have been reported (15), as have its hyperalgesic actions in animals (14,18,32,35) and man (1,7). Hyperalgesic and analgesic actions, as well as biphasic dose–response relationships, have been demonstrated in animals (3,4,6,12,13,19,25,26,29–31) and humans (2,8,20–23) following administration of opioid antagonists.

(−)-Lobeline produced dose-related hyperalgesia but also exhibited nonlinear dose–response relationships. Nicotinic hyperalgesic processes have been previously demonstrated in studies in decerebrate (24) and intact rats (11,27). Further, (−)-lobeline's hyperalgesic potency appears similar to that exhibited by (−)-nicotine in the DPMT (11). Biphasic dose–response for (−)-lobeline and (−)-nicotine was previously observed using the hindlimb withdrawal reflex of the acutely decerebrated rat except that analgesia was observed with the lower doses (24). This difference from the present results is probably due to the microinjection site. These data confirm the presence of brainstem opioid and nicotinic hyperalgesic processes. The hyperalgesic actions of U50-488, a highly specific opioid κ-receptor agonist, strongly suggest the presence of a κ-opioidergic hyperalgesic mechanism in the DPMT.

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