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Antagonism of Morphine Analgesia by Intracerebroventricular Naloxonazine

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SIMONE, D. A., R. J. BODNAR, T. PORTZLINE AND G. W. PASTERNAK. *Antagonism of morphine analgesia by intracerebroventricular naloxonazine*. PHARMACOL BIOCHEM BEHAV 24(6) 1721–1727, 1986.—Intravenous pretreatment with naloxonazine, an irreversible and selective antagonist of mu-1 sites for over 24 hr, reduces analgesia induced by morphine as well as a series of opiates and enkephalins. The present study evaluated whether intracerebroventricular (ICV) administration of naloxonazine produces similar long-term (24 hr) reductions in morphine analgesia on the tail-flick and jump tests. Naloxonazine failed to alter baseline tail-flick latencies or jump thresholds, but antagonized in a dose-dependent manner morphine analgesia for 24 hr. Naloxone had no effect at 24 hr. Morphine actions in the jump test were quite sensitive to doses of naloxonazine as low as 1 μg/rat. Although tail-flick assays also revealed naloxonazine effects, far greater doses (30 μg/rat) were needed. Naloxonazine also shifted full morphine dose-response curves to the right. Again, naloxonazine antagonized morphine in the jump test more effectively than in the tail-flick assay. These data provide support for the involvement of the mu-1 opioid binding site in the central mediation of morphine analgesia and point out the differing sensitivities of two analgesiometric assay systems to naloxonazine.

Naloxonazine Morphine analgesia Tail-flick test Jump test Rats

FOLLOWING the discovery of opiate binding sites in the central nervous system [21, 24, 26], subpopulations of opiate receptors classified on the basis of their selectivity towards various opiates and opioid peptides were proposed: mu (morphine), kappa (ketocyclazocine), sigma (SKF 10,047 or N-allylnormetazocine), delta (enkephalins) and epsilon (beta-endorphin) [15, 16, 23, 28]. Recently, we proposed another class of receptor, termed mu-1, which has high affinity for opiates and opioid peptides, unlike the morphine-selective mu-2 site [27]. Although selective agonists have greatly facilitated examinations of these receptor subtypes in pharmacological and biochemical studies, few selective antagonists are available. Most behavioral and psychopharmacological studies requiring an antagonist have used naloxone, a short-acting opiate antagonist which interacts with a number of receptor subtypes ([15,19]; for review, see [22]). Recently, several long-acting antagonists have been synthesized, including naloxazone and naloxonazine [7, 19, 20]. Both compounds have similar actions, but naloxonazine is far more potent [7–9]. Both agents antagonize in a long-lasting manner the mu-1 subtype of the opiate receptor for greater than 24 hr in vivo. Administered intravenously, these two compounds antagonize for greater than 24 hr the analgesic actions of a wide variety of opiates and opioid peptides which are mediated through mu-1 sites without affecting either morphine lethality, respiratory depression or many of the signs of physical dependence [13, 14, 17–20, 25, 32]. These data suggest that the mu-1 subtype of the opiate receptor represents a common locus for the analgesic actions of different opiate and opioid peptide agonists.

The actions by which opiate receptor agonists induce analgesia and the actions by which opiate receptor antagonists attenuate or eliminate this effect, are presumed to be mediated in the central nervous system (see reviews: [1, 2, 6, 29]). Injections of opiate agonists and antagonists directly into the central nervous system has constituted one pharmacological approach to determine a central mechanism of action. Intracerebroventricular administration, like intravenous administration of naloxazone, reduces morphine analgesia although at far lower doses [11]. The present study examines whether administration of naloxonazine directly into the central nervous system would produce similar reductions in morphine analgesia as that observed systemically.

METHOD

Surgery

Adult male albino Sprague-Dawley rats (250–450 g) were

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TABLE 1

EFFECTS (MEAN, SEM) OF INTRACEREBROVENTRICULAR (ICV) NALOXONAZINE AND NALOXONE UPON MORPHINE ANALGESIA (10 mg/kg, SC) 24 HR LATER

	ICV Treatment		
	Vehicle	Naloxone 50 μg	Naloxonazine 50 μg
	Jump Thresholds (mA)		
Baseline	0.343 (0.017)	0.334 (0.017)	0.315 (0.016)
60 min	0.629 (0.031)†	0.620 (0.027)†	0.496 (0.039)†*
% Reduction in Analgesia		0%	37%*
	Tail-Flick Latencies (sec)		
Baseline	2.63 (0.24)	2.15 (0.12)	2.44 (0.20)
60 min	6.00 (0.00)†	6.00 (0.00)†	5.57 (0.19)†
% Reduction in Analgesia		0%	7%

All main and interaction effects were significant at the 0.01 level. The crosses denote where Dunnett comparisons ($p<0.01$) revealed significant increases in thresholds and latencies relative to baseline values. The asterisks denote where Dunnett comparisons ($p<0.01$) revealed significant attenuations in morphine analgesia relative to vehicle-pretreated rats. Note that values of 6.00 sec indicate that latencies equalled or exceeded the 6 sec cutoff criterion.

housed individually, maintained on a 12 hr light/dark cycle and were provided with food (Purina Rat Chow) and water ad lib. Each rat was pretreated with chlorpromazine (3 mg/ml/kg body weight, IP) and 20 min prior to anesthesia with Ketamine HCl (100 mg/kg, IM). A stainless steel guide cannula (22 gauge; Plastic Products) was stereotaxically positioned 0.3 mm above the left lateral ventricle and secured with three screws and dental acrylic. With the incisor bar set at +5 mm, the coordinates were 0.5 mm anterior to the bregma suture, 1.3 mm lateral to the mid-sagittal suture and 3.6 mm from the top of the skull.

Test Procedures

Seven days following surgery, baseline tail-flick latencies [4] and jump thresholds [5] were assessed. Tail-flick latencies were measured by mounting a radiant heat source (IITC) 8 cm above the dorsum and 4 cm proximal to the tip of the tail of a lightly restrained animal. During each test session, three trials of tail-flick latencies were determined with a 10 sec interval elapsing between trials. The intensity of the thermal stimulus was set so as to elicit stable baseline latencies between 2 and 3 sec at a tail temperature between 46 and 53°C [3]. A 6 sec cutoff latency was used to minimize tissue damage. Immediately thereafter, jump thresholds were determined by delivering electric shocks through the grid floor of a 30 cm by 24 cm chamber with a 60-Hz constant current shock generator (BRS/LVE) and grid scrambler (Campden Instruments). Using an ascending method of limits procedure, the jump threshold was defined in mA as the lowest of two consecutive intensities which elicited simultaneous removal of both hind paws from the grids. Each trial began with the animal receiving a 300 msec foot shock at a current intensity of 0.1 mA. Subsequent shocks were delivered at 5 sec intervals and increased in 0.05 mA steps until the jump threshold was determined. After each trial, the current intensity was reset to 0.1 mA and the procedure repeated until six trials were completed. Daily baseline tail-flick latencies and jump thresholds were determined for four days and were used to equate baseline thresholds among experimental conditions. The tail-flick tests were performed before the jump test to minimize carry-over effects [10].

Histology

Following completion of experimental testing, all rats were pretreated with sodium pentobarbitol (200 mg/kg, IP) and were perfused transcardially with normal saline followed by 10% buffered Formalin. The brains were removed, blocked, sliced into 40 μm coronal sections through the lateral ventricle, and stained with cresyl violet. Cannula placement was determined with light microscopy and only those animals with lateral ventricle cannulae were included in the statistical analyses.

Drug Administration

All injections of naloxone and naloxonazine were administered intracerebroventricularly (ICV) through a 28 gauge internal cannula (Plastic Products) in a volume of 10 μl over a 100 sec interval. Naloxonazine doses, synthesized as previously reported [7], are reported as the free base and were dissolved in normal saline with 0.2% acetic acid. Naloxone (Endo Laboratories) doses are expressed as the hydrochloride salt and were dissolved in normal saline. Doses of morphine sulfate (Pennick Laboratories) were dissolved in normal saline at a concentration of 1 ml/kg of body weight, and were injected subcutaneously (SC). All ICV and SC vehicle injections consisted of normal saline. The 60 min post-injection interval used in the morphine analgesia exper-

TABLE 2

EFFECTS OF INTRACEREBROVENTRICULAR (ICV) NALOXONAZINE UPON BASELINE JUMP THRESHOLDS AND TAIL-FLICK LATENCIES

	ICV Naloxonazine Dose			
	Vehicle	1 μg	5 μg	50 μg
Jump thresholds (mA)				
Baseline	0.350	0.338	0.343	0.344
30 min	0.307	0.310	0.322	0.351
60 min	0.311	0.310	0.322	0.351
120 min	0.329	0.317	0.317	0.292
24 hr	0.318	0.329	0.317	0.332
Tail-Flick Latencies (sec)				
Baseline	3.08	3.14	3.04	3.24
30 min	2.62	2.95	2.24	3.38
60 min	2.57	2.43	2.56	2.85
120 min	2.39	2.61	2.39	2.81
24 hr	2.84	2.66	2.80	2.77

No significant differences ($p>0.05$) were observed from comparisons made between vehicle and naloxonazine conditions at each post-injection interval.

TABLE 3

EFFECTS (MEAN, SEM) OF MULTIPLE INTRACEREBROVENTRICULAR (ICV) NALOXONAZINE DOSES UPON MORPHINE ANALGESIA (5 mg/kg, SC) 24 HR LATER

	ICV Naloxonazine Dose					
	Vehicle	1 μg	5 μg	15 μg	30 μg	50 μg
Jump Thresholds (mA)						
Baseline	0.389	0.372	0.352	0.338	0.345	0.322
	0.024	0.016	0.019	0.020	0.019	0.013
60 min	0.561†	0.468†*	0.489†*	0.477†*	0.388†*	0.396†*
	0.018	0.043	0.039	0.013	0.033	0.028
% Reduction in Analgesia		44%	20%	19%	75%	57%
Tail-Flick Latencies (sec)						
Baseline	2.19	2.35	2.31	2.26	2.40	2.15
	0.11	0.22	0.13	0.12	0.12	0.07
60 min	6.00†	6.00†	6.00†	6.00†	4.60†*	3.78†*
	0.00	0.00	0.00	0.00	0.68	0.42
% Reduction in Analgesia		4%	3%	2%	42%	57%

All main and interaction effects were significant at the 0.01 level. The crosses denote where Dunnett comparisons ($p<0.01$) revealed significant increases in thresholds and latencies relative to baseline values. The asterisks denote where Dunnett comparisons ($p<0.01$) revealed significant attenuations in morphine analgesia relative to vehicle-pretreated rats. Note that values of 6.00 sec indicate that latencies equalled or exceeded the 6 sec cutoff criterion.

TABLE 4

EFFECTS (MEAN, SEM) OF MULTIPLE INTRACEREBROVENTRICULAR (ICV) NALOXONAZINE DOSES UPON THE MORPHINE DOSE-RESPONSE CURVE ON THE TAIL-FLICK TEST 24 HR LATER

	ICV Naloxonazine Doses		
	Vehicle	1 μg	50 μg
Morphine: 0.5 mg/kg			
Baseline	2.51 (0.33)	2.50 (0.31)	2.25 (0.12)
60 min	2.43 (0.14)	2.59 (0.50)	2.14 (0.06)
Morphine: 2.5 mg/kg			
Baseline	2.47 (0.14)	2.74 (0.34)	2.93 (0.11)
60 min	5.67 (0.21)†	4.61 (0.51)†*	2.81 (0.15)*
% Reduction in Analgesia		42%	100%
Morphine: 5.0 mg/kg			
Baseline	2.54 (0.16)	2.17 (0.20)	2.44 (0.11)
60 min	6.00 (0.00)†	5.90 (0.67)†	5.07 (0.55)†*
% Reduction in Analgesia		0%	24%
Morphine: 10.0 mg/kg			
Baseline	2.62 (0.23)	2.09 (0.09)	2.45 (0.20)
60 min	6.00 (0.00)†	5.94 (0.62)†	5.57 (0.19)†*
% Reduction in Analgesia		0%	8%
Morphine: 15.0 mg/kg			
Baseline	2.84 (0.20)	2.66 (0.23)	2.77 (0.26)
60 min	6.00 (0.00)†	5.98 (0.03)†	6.00 (0.00)
% Reduction in Analgesia		0%	0%

All main and interaction effects were significant at the 0.01 level. The crosses denote where Dunnett comparisons ($p<0.01$) revealed significant increases in thresholds and latencies relative to baseline values. The asterisks denote where Dunnett comparisons ($p<0.01$) revealed significant attenuations in morphine analgesia relative to vehicle-pretreated rats. Note that values of 6.00 sec indicate that latencies equalled or exceeded the 6 sec cutoff criterion.

iments is within the peak effect of morphine analgesia on these analgesiometric tests [1, 2, 6, 11, 13, 14, 17–20, 25, 29, 32].

Statistical Analyses

For each experimental protocol, a split-plot analysis of variance was performed in which the independent factor was the dose employed and the repeated measure was the baseline and post-injection test score. In all cases, the main and interaction effects exceeded a chance probability of 0.01. Dunnett comparisons were employed to discern significant differences between the vehicle control and experimental groups.

EXPERIMENT 1

Protocol

Three groups of six rats each received ICV injections of either vehicle, naloxone (50 μg) or naloxonazine (50 μg). Twenty-four hr later, morphine analgesia (10 mg/kg, SC) was assessed on the tail-flick and jump tests at 60 min following morphine administration.

RESULTS

Table 1 indicates that although morphine significantly increased tail-flick latencies and jump thresholds in all groups at 60 min following injection, ICV pretreatment with the 50 μg dose of naloxonazine significantly attenuated the analgesia on both pain tests. In contrast, the same dose of naloxone failed to produce a similar long-term antagonistic effect.

EXPERIMENT 2

Protocol

Four groups of six rats each received ICV injections of either vehicle or a 1, 5 or 50 μg dose of naloxonazine. Tail-flick latencies and jump thresholds were assessed 0.5, 1, 2 and 24 hr later.

RESULTS

Table 2 indicates that ICV administration of these doses of naloxonazine failed to alter significantly baseline tail-flick latencies or jump thresholds relative to corresponding vehicle values.

TABLE 5

EFFECTS (MEAN, SEM) OF MULTIPLE INTRACEREBROVENTRICULAR (ICV) NALOXONAZINE DOSES UPON THE MORPHINE DOSE-RESPONSE CURVE ON THE JUMP TEST 24 HR LATER

	ICV Naloxonazine Doses		
	Vehicle	1 μg	50 μg
Morphine: 0.5 mg/kg			
Baseline	0.342 (0.26)	0.314 (0.20)	0.314 (0.19)
60 min	0.312 (0.19)	0.294 (0.12)	0.304 (0.07)
Morphine: 2.5 mg/kg			
Baseline	0.352 (0.20)	0.340 (0.21)	0.333 (0.16)
60 min	0.398 (0.16)†	0.358 (0.18)	0.330 (0.16)*
% Reduction in Analgesia		62%	100%
Morphine: 5.0 mg/kg			
Baseline	0.332 (0.18)	0.338 (0.20)	0.308 (0.11)
60 min	0.540 (0.53)†	0.363 (0.25)*	0.376 (0.29)†*
% Reduction in Analgesia		88%	67%
Morphine: 10.0 mg/kg			
Baseline	0.378 (0.17)	0.358 (0.22)	0.315 (0.16)
60 min	0.640 (0.37)†	0.496 (0.11)†*	0.496 (0.39)†*
% Reduction in Analgesia		47%	31%
Morphine: 15.0 mg/kg			
Baseline	0.318 (0.15)	0.329 (0.21)	0.332 (0.51)
60 min	0.683 (0.38)†	0.586 (0.23)†*	0.561 (0.45)†*
% Reduction in Analgesia		30%	37%

All main and interaction effects were significant at the 0.01 level. The crosses denote where Dunnett comparisons ($p<0.01$) revealed significant increases in thresholds and latencies relative to baseline values. The asterisks denote where Dunnett comparisons ($p<0.01$) revealed significant attenuations in morphine analgesia relative to vehicle-pretreated rats. Note that values of 6.00 sec indicate that latencies equalled or exceeded the 6 sec cutoff criterion.

EXPERIMENT 3

Protocol

Having established an effect for an ICV injection of naloxonazine upon morphine analgesia, a naloxonazine dose-response curve upon morphine analgesia was then examined. Six groups of six rats each received ICV injections of either vehicle or a 1, 5, 15, 30 or 50 μg dose of naloxonazine. Twenty-four hr later, morphine analgesia (5 mg/kg, SC) was assessed on the tail-flick and jump tests at 60 min after morphine administration.

RESULTS

Table 3 indicates that ICV administration of naloxonazine significantly attenuated morphine analgesia in a dose-dependent manner on both pain tests. On the jump test, all naloxonazine doses were effective in significantly reducing morphine analgesia at this dose, with the magnitude of reductions increasing as the naloxonazine dose increased. On the tail-flick test, the 30 and 50 μg doses of ICV naloxonazine were capable of significantly attenuating morphine analgesia. However, naloxonazine appeared less sensitive as measured by the tail-flick test, since the lower (1, 5 and 15 μg) doses of naloxonazine failed to alter morphine analgesia on this measure.

EXPERIMENT 4

Protocol

Having established a dose-response curve for naloxonazine for a single dose of morphine analgesia on the tail-flick and jump tests, the effects of the low (1 μg) and high (50 μg) doses of ICV naloxonazine were evaluated across a range of morphine doses 24 hr following naloxonazine administration. Fifteen groups of six rats received an ICV injection of either vehicle (5 groups) or a 1 μg (5 groups) or 50 μg (5 groups) dose of naloxonazine. Twenty-four hr later, analgesia induced by five doses of morphine (0.5, 2.5, 5, 10 and 15 mg/kg, SC) was assessed in separate groups of rats at 60 min after morphine administration on the tail-flick and jump tests.

RESULTS

Table 4 indicates that ICV pretreatment with

naloxonazine produced significant, dose-dependent reductions in morphine analgesia on the tail-flick test across the morphine dose-response curve. While neither dose of naloxonazine significantly altered analgesia following the 15 mg/kg dose of morphine, the 50 μg dose of naloxonazine significantly attenuated analgesia following the 5 and 10 mg/kg morphine doses, and eliminated the analgesia following the 2.5 mg/kg dose of morphine. In contrast, the 1 μg dose of naloxonazine significantly attenuated analgesia only following the 2.5 mg/kg dose of morphine.

Table 5 indicates that ICV pretreatment with naloxonazine produced similar, but larger, shifts in the morphine dose-response curve on the jump test. Both the 1 and 50 μg doses of naloxonazine produced significant, dose-dependent attenuations in analgesia following the 5, 10 and 15 mg/kg morphine doses, and eliminated the analgesia following the 2.5 mg/kg dose of morphine.

GENERAL DISCUSSION

Naloxonazine is a unique opiate whose irreversible actions are relatively selective for mu-1 sites [7,9]. Previous studies using systemically administered naloxonazine indicate that mu-1 sites play a major role in opiate analgesia [8, 13, 14, 17–20]. The effects of naloxonazine administered intracerebroventricularly were studied to establish a central mechanism of action. Further, two distinct analgesiometric assay systems were evaluated: the tail-flick response to radiant heat [4] and the jump response to electric shock [5].

Administered systemically, naloxonazine at doses between 10 and 50 mg/kg (IV) antagonizes a number of morphine's actions, including analgesia for greater than 24 hr [13,25]. Like naloxazone [11], naloxonazine is also active when administered intracerebroventricularly. The low doses (under 50 μg/animal) of naloxonazine capable of attenuating morphine analgesia contrast sharply with the systemic doses (typically greater than 2.5 mg/animal) needed to elicit similar effects. These differences support a central site of action for the compound. Similar conclusions regarding a central site of action have also been reported for naloxone [29,30]. However, naloxone's actions are quite brief, lasting only several hours. The demonstrated inability of naloxone to reduce morphine analgesia 24 hr later supports this contention.

The magnitude of naloxonazine's actions following intracerebroventricular administration was dependent upon the dose of naloxonazine and morphine as well as the pain test employed. The increased effect with larger doses of naloxonazine was expected. Similarly, the role of the morphine dose appears consistent with previous observations using systemically administered naloxonazine [13, 14, 17–20], and may reflect the activation of non-mu-1 opiate receptor mechanisms at higher morphine doses ([12]; Ling, Simantov, Clark and Pasternak, manuscript in preparation).

Although not surprising, the different sensitivities of naloxonazine effects upon morphine analgesia as measured by the jump and tail-flick tests were not anticipated. The tail-flick assay can be modulated segmentally as well as by activation of descending systems [30,31]. However, some evidence suggests that mu-1 actions are limited to supraspinal regions and do not play a major role at the level of the spinal cord [12]. Therefore, blockade of mu-1 sites may only affect the activation of descending systems, and not the segmental modulation. The greater effect of naloxonazine on the supraspinally-mediated jump test might be due to its greater reliance upon mu-1 analgesic mechanisms.

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