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Dose-Dependent Reductions by Naloxone of Analgesia Induced by Cold-Water Stress

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BODNAR, R. J., D. D. KELLY, A. SPIAGGIA, C. EHRENBERG AND M. GLUSMAN. *Dose-dependent reductions by naloxone of analgesia induced by cold-water stress.* PHARMAC. BIOCHEM. BEHAV. 8(6) 667–672, 1978. – Animals exposed to cold-water swims, rotation, or inescapable shocks, display analgesia comparable to that of 10 mg/kg of morphine. The present study investigated whether a narcotic antagonist would eliminate analgesia induced by cold-water swims. In one group of 12 rats, naloxone at 0, 1, 5, 10 and 20 mg/kg was administered at weekly intervals immediately preceding forced cold-water swims (2°C for 3.5 min) and alterations in flinch-jump thresholds were determined 30 min thereafter. In a second group of six rats, the effects of the same dose range of naloxone were determined upon normal flinch-jump thresholds. Naloxone dose-dependently attenuated the cold-water swim-induced analgesia up to a maximal reduction of 50% at 20 mg/kg. In contrast, all doses of naloxone had no effects upon normal flinch-jump thresholds. Since low doses of naloxone completely abolish morphine-induced analgesia, the present data suggest that the analgesia induced by stress is not identical to that of opiates.

Stress Analgesia Naloxone Pain inhibition Flinch-jump test

SEVERAL behavioral studies have identified a set of severe environmental situations which can induce in the organism an analgesic response comparable to that produced by moderate doses of morphine. Following the initial observation that showed increased tail flick latencies following inescapable foot shock, rotation or intraperitoneal injections of hypertonic saline [24,25], the list of analgesically effective stressors has rapidly lengthened to include cold-water swims [9,10], food deprivation [46] and acute administration of 2-deoxy-D-glucose, an antimetabolic analogue which produces glucoprivation [7]. The analgesia induced by these stressors has been measured by a wide range of pain tests, both reflex and operant, including tail-flick withdrawal to radiant heat, hot plate, paw-pinch, flinch-jump, tail-pinch and an operant liminal escape test [9, 10, 24, 25, 33]. Given the number of environmental stressors that increase nociceptive thresholds, it would seem unlikely that various non-specific peripheral factors peculiar to the individual stressors could account for the threshold elevations. In particular, the analgesia induced by cold-water swims, the stressor employed in the present experiment, can be explained by neither core or skin hypothermia, nor hypoactivity, since both acutely-treated analgesic rats and chronically-treated non-analgesic rats have been shown to display significant core and skin hypothermia as well as similarly increased activity levels following the swims [8, 10, 11]. Rather it would seem that a temporary reduction in sensitivity to painful stimuli may be one of a shifting collection of physiological responses to challenging environmental stimuli which collectively define a stress response. Supporting this notion are the independent observations that both inescapable foot shock [1,34] and cold-water swims [10,11] induce analgesia only on initial exposure. Repeated exposures to the same stressor have been found to result in adaptation of the analgesic response in much the same way as other physiological stress responses adapt and in much the same way as repeated injections of morphine result in tolerance.

With the discovery of the opiate receptor [39, 45, 48] and the identification of the endogenous peptides with opiate-like properties [22, 26, 27, 44] most biochemical characterizations of neural pain-inhibition have emphasized its opiate components. Administration of both morphine and endorphin fragments produces analgesia [4, 6, 21, 29, 30, 31, 40, 49, 50]. The analgesia induced by stressful events was also initially linked to brain endorphin activity because acute exposure to stressors increased brain opiate receptor binding properties [1, 14, 15, 34] and because beta-endorphin and adreno-corticotrophic hormone were found to be released concomitantly from the pituitary following acute restraint stress [23]. In contrast, beta-endorphin was found in higher levels in the blood, but not in brain tissue, following foot shock stress [43] while whole brain ^{3}H-methionine enkephalin activity was unaltered following this same stressor [18]. Similarly, dorsolateral funiculus lesions in the spinal cord, which eliminate morphine-produced and stimulation-produced analgesia [3, 41, 42], have no effect upon analgesia induced by electric foot shock [41,42]. Finally, cross-tolerance fails to develop between morphine-produced and cold-water stress-produced analgesia [13].

Whereas the narcotic antagonist, naloxone has shown

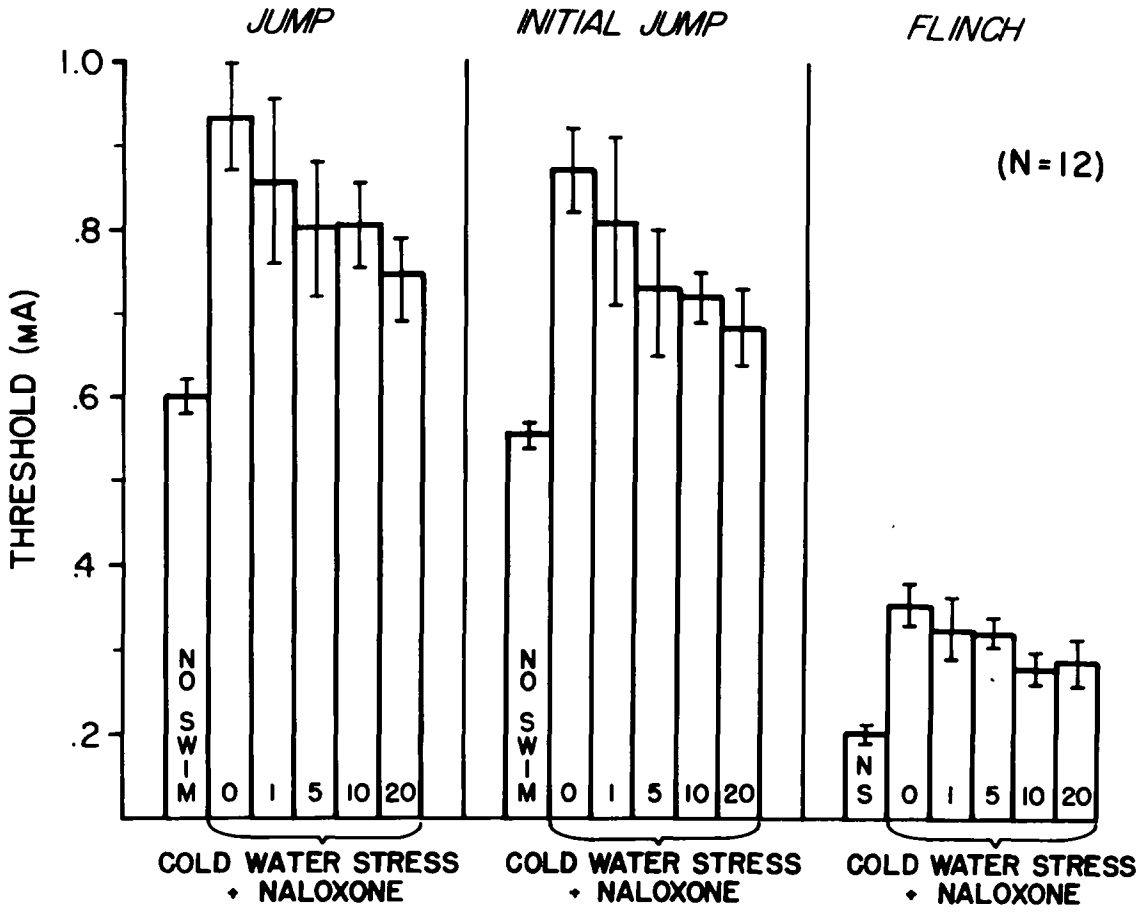

FIG. 1. Alterations in flinch-jump thresholds (±SEM) of 12 rats pretreated with either 0, 1, 5, 10 or 20 mg/kg of naloxone following acute exposure to cold-water stress. Subcutaneous naloxone injections immediately preceded the cold-water swims; flinch-jump determinations occurred 30 min post-swim. All cold-water swim/naloxone administration combinations for each pain threshold measure were significantly higher than the no swim baseline condition (Tukey repeated-measures comparisons, $p<0.05$).

only mild [5, 19, 28] or no [16,20] effects upon normal pain sensitivity, it has been able to eliminate analgesia induced by systemic or intracerebral administration of morphine [30,36] and to attenuate stimulation-produced analgesia [2,39]. Interestingly, naloxone at a dose of 10 mg/kg has been found partially effective in attenuating stress-induced analgesia [1, 10, 12], while naloxone at 1 mg/kg failed to alter foot shock-induced analgesia [24,25]. In the former studies the partial effectiveness of naloxone actually represented a statistical blend of two binary effects: marked analgesic reduction in some animals, and no effect in others. Since these studies suggested that dose might be a more critical determinant of naloxone's effect upon stress-induced as opposed to opiate-induced analgesia, the present study investigated the dose-dependent effects of naloxone upon analgesia induced by cold-water swims by testing individual animals across a wide dose range (1, 5, 10 and 20 mg/kg).

METHOD

Eighteen male albino Holtzman Sprague-Dawley rats (350–500 g) were used. Testing was carried out in a chamber with a grid floor composed of 14 grid bars (0.6-cm dia. spaced 1.8 cm apart). Electric shocks were delivered through the grids by a shock generator. The polarity of the shock was rapidly switched across grids through a shock scrambler. Each animal was tested for 10 trials daily for flinch, initial jump and jump thresholds using a modification of the Evans [17] method. Using an ascending method of limits of successively more intense shocks, the "flinch" threshold was defined in mA as the lowest intensity that elicited a withdrawal of a single paw from the grids. The "initial jump" threshold was defined as the lowest intensity that elicited simultaneous removal of both hind paws from the grids. The "jump" threshold was defined as the lowest of two consecutive intensities that elicited a jump as above. Each trial began with the animal receiving a 300-msec foot shock at a current intensity of 0.1 mA. Subsequent shocks were increased in equal 0.05-mA steps at 10-sec intervals. After each trial, the current intensity was reset to 0.1 mA for the next trial until 10 trials were completed. Daily flinch, initial jump and jump thresholds were each computed as the mean of these 10 trials. The experimenters conducting the flinch-jump test were uninformed both of the purpose of the experiment and of the specific experimental conditions.

In one group of 12 rats, each rat was subjected at one week intervals to five forced cold-water swims (2°C for 3.5

TABLE 1

TUKEY REPEATED-MEASURES COMPARISONS OF NALOXONE'S EFFECTS UPON COLD-WATER SWIM ANALGESIA

Threshold (mA)	Cold-Water Swim	Cold-Water Swim + Naloxone (MG/KG)			
		1	5	10	20
Jump					
$\bar{X}$	0.933	0.857	0.802	0.805	0.746
t		0.84	1.43	1.60	2.07
p		NS	NS	NS	$0.10>p>0.05$
Initial Jump					
$\bar{X}$	0.873	0.809	0.731	0.723	0.685
t		0.69	1.70	2.33	2.50
p		NS	NS	<0.05	<0.05
Flinch					
$\bar{X}$	0.355	0.325	0.323	.0280	0.288
t		0.64	1.06	2.73	3.95
p		NS	NS	<0.05	<0.01

min) with flinch-jump thresholds determined 30 min after each swim. Baseline control thresholds were taken on four intervening swimless weekdays. Immediately prior to each swim, each rat received a subcutaneous injection of naloxone (Naloxone Hydrochloride, Endo Labs/ 1 ml sterile water) at one of five doses: 0, 1, 5, 10 or 20 mg/kg. The order of drug administration was determined by random placement of animals in a Latin Square design, so that each rat received all four doses of naloxone and the placebo injection in conjunction with the five cold-water swims during the 5-week paradigm.

A second group of six unstressed rats was tested daily for normal flinch-jump thresholds for 17 sessions. Five min preceding every third session, each rat was administered one of the five naloxone doses outlined above. The order of drug administration occurred in an ascending order for one half of the group and in descending order for the remaining half.

RESULTS

Figure 1 displays the mean elevations over baseline thresholds produced by cold-water swims following the various levels of naloxone pretreatment. Separate one-way analyses of variance revealed significant swim-induced elevations in all nociceptive measures: jump: $F(5,66) = 2.98$, $p<0.05$, initial jump, $F = 3.31$, $p<0.01$, and flinch, $F = 4.30$, $p<0.01$. Post-hoc Tukey comparisons for all three measures further indicated that, overall, naloxone partially reduced the stress-induced analgesia in a dose-dependent manner as compared to placebo. Yet, at no dose was naloxone capable of blocking a statistically significant stress-induced elevation in any threshold measure. Still, as summarized in Table 1, when each dose of naloxone was compared to placebo, mild, yet often significant and dose-dependent, reductions in stress-induced analgesia were noted. These effects were not related to the order of dose administration. Nor did tolerance to stress-induced analgesia develop within the limited exposure to the five weekly, cold-water swims, as it has been shown to do following 8–14 daily exposures to various stressors [1, 7, 11].

There was wide individual variability across animals in naloxone's effectiveness in reducing the analgesia induced by exposure to stress. While 8 of 12 animals manifested the dose-dependent partial reductions in stress-induced analgesia which characterized the group data, the remaining four showed all-or-none dichotomous effects. Two showed full reduction of post-stress thresholds to baseline levels by all naloxone doses, and, in the other two, no naloxone dose had any effect upon analgesic thresholds. Despite individual variations in drug sensitivity, orderly downward shifts in the severity of stress-induced analgesia, as shown in Fig. 1, and in the cumulative distribution of animals displaying various degrees of analgesia were noted as the dose of naloxone was increased. For instance, Table 2 shows that, following placebo injections, two out of three animals showed analgesic response greater than 40% over baseline levels; while only one of every three animals displayed a similar degree of threshold elevation when pretreated with any naloxone dose. It is unlikely that these results could be attributed to chance factors because a significant overall relationship existed between the magnitude of stress-induced analgesia and administration of either placebo or naloxone ($\chi^2 = 5.14$, $0.05>p>0.02$).

Figure 2 shows that naloxone's alterations of analgesia induced by cold-water stress was clearly not due to naloxone's effects upon normal unstressed pain thresholds. At no dose was naloxone able to alter significantly jump,

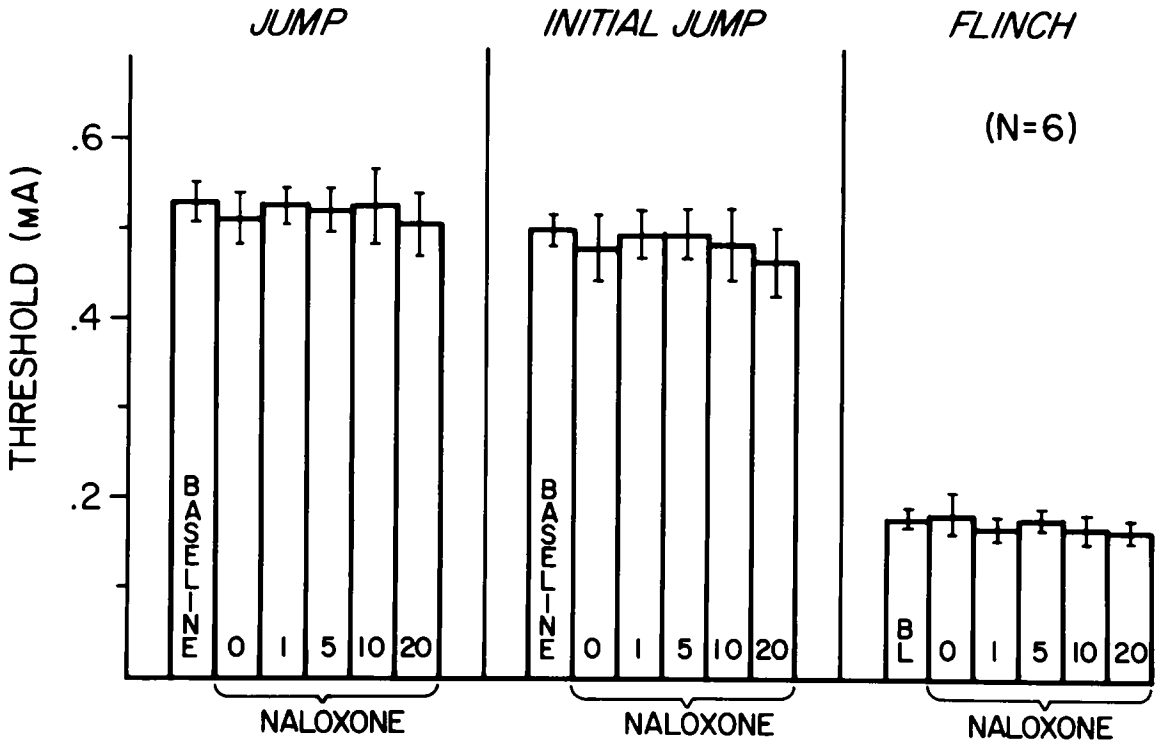

FIG. 2. Alterations in flinch-jump thresholds (±SEM) of six rats injected subcutaneously with either 0, 1, 5, 10 or 20 mg/kg of naloxone 5 min prior to nociceptive determinations. No significant changes were noted at any dose.

TABLE 2

CUMULATIVE PERCENT OF ANIMALS SHOWING VARIOUS DEGREES OF POST-STRESS ANALGESIA FOLLOWING NALOXONE PRETREATMENT

Degree of Threshold Elevation (% of baseline)	Placebo	Naloxone			
		1 MG (%)	5 MG (%)	10 MG (%)	20 MG (%)
100	0	8	17	8	17
100–120	8	50	58	42	58
120–140	33	67	67	67	75
140–160	67	83	75	83	92
160–180	83	83	92	83	92
180	100	100	100	100	100

$F(5,30) = 0.09$, initial jump, $F(5,30) = 0.17$, or flinch, $F(5,30) = 0.26$ thresholds in the six control animals.

DISCUSSION

The principal finding of the present experiment is that naloxone produces a mild, dose-dependent reduction in the analgesia induced by an acute stressor. However, even at doses normally sufficient to block the most pronounced opiate analgesia, naloxone did not fully reverse, nor limit below levels of statistical significance, the phenomenon of stress-induced analgesia. These data confirm and explain the conflicting previous reports that 10 mg/kg of naloxone reduced stress-induced analgesia following inescapable foot shock [1] and cold-water swims [10,12], while 1 mg/kg of naloxone failed to attenuate significantly similar foot shock-induced analgesia [24,25]. Naloxone's relatively mild interactions with stress-induced analgesia contrast markedly with its consistent and complete effectiveness at low doses to reverse both morphine- and endorphin-induced analgesia [4, 6, 21, 29, 30, 31, 32, 40, 49, 50]. The present data are similar in form, however, to naloxone's partial effectiveness in reducing stimulation-produced analgesia [2,39],

although sufficient dose-response data on the latter interaction is not yet available.

Naloxone's partial effectiveness in reducing stress-induced analgesia implies that the neural mechanisms mediating opiate-induced and stress-induced analgesia may not be identical, and moreover that the latter phenomenon may not be endorphin-mediated. This view is further supported by the observation that not even partial cross-tolerance develops between morphine and stress-produced analgesia [13]. This contrasts with full cross-tolerance reported between intracerebral morphine and intracerebral endorphin injections [49,54] and between the former and systemic morphine injections [31]. The lack of cross-tolerance between stress-induced and opiate-induced analgesia also contrasts with the partial development of tolerance between stimulation-induced and opiate-induced analgesia [37]. Thus, the most parismonious explanation of these results would seem to imply the possible existence of a parallel non-opiate neural route which may share access with opiate pathways to the descending serotonergic bulbospinal system that apparently serves as the final common path for pain inhibition [37].

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