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Stress-Produced Analgesia and Morphine-Produced Analgesia: Lack of Cross-Tolerance[1]

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BODNAR, R. J., D. D. KELLY, S. S. STEINER AND M. GLUSMAN. *Stress-produced analgesia and morphine-produced analgesia: Lack of cross-tolerance.* PHARMAC. BIOCHEM. BEHAV. 8(6) 661–666, 1978. – Animals exposed to cold-water swims, rotation, inescapable shocks, abrupt food deprivation and other stressors display temporary analgesia. Since repeated exposures result in adaptation of this analgesia in much the same way that repeated administration of opiates results in tolerance, the possibility of cross-tolerance between cold-water stress-induced and morphine-induced analgesia was investigated. Flinch-jump thresholds were determined in ten experimental groups of six rats each. Three groups showed dose-dependent analgesia following single injections of morphine at 5, 10 and 15 mg/kg, respectively. A fourth group, subjected to a single cold-water swim at 2°C for 3.5 min, displayed analgesia comparable to that produced by 10 mg/kg of morphine. Groups subjected either to 14 daily cold-water swims or to 14 daily morphine injections at 10 mg/kg showed normal thresholds on the 14th day indicating that adaptation and tolerance had developed, respectively. The cross-over groups were exposed to either 13 days of cold-water swims followed by morphine or the reverse arrangement. Both groups showed profound analgesia instead of cross-tolerance, suggesting that a non-opiate neural mechanism may mediate stress-induced analgesia.

Morphine Stress-produced analgesia Cross-tolerance Adaptation Tolerance Endorphins Pain Rats

THE EXISTENCE of an intrinsic system within the vertebrate brain whose function is to modulate pain has recently been suggested by both behavioral and physiological evidence [32]. With the dramatic discovery of the opiate receptor [33, 37, 41], and the endogenous peptides (endorphins) with opiate-like properties [16, 19, 20, 36], most biochemical characterizations of this neural pain-inhibitory system have emphasized its opiate components. Administration of various endorphin fragments produces analgesia [3, 4, 15, 22], and tolerance develops following repeated administration of these substances [42,43]. Cross-tolerance also occurs between endorphin and morphine administration [40,44].

Several behavioral studies have also 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 [18] that rats showed increased tail-flick latencies following acute exposure to inescapable foot shock, rotation or intraperitoneal injections of hypertonic saline, the list of analgesically effective stressors has rapidly lengthened to include cold-water swims [8,9], food deprivation [38] and acute administration of 2-deoxy-D-glucose, an antimetabolic glucose analogue which produces glucoprivation [5]. The analgesia induced by these stressors has been measured by a number 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 [8, 9, 18, 23]. To date, the only presumed experimental stressors that have not induced a measurable period of analgesia are the inhalation of ether vapors and horizontal oscillation [18]. Since hormonal responses to stress vary according to the nature of the stressor [29,30], it should not be unexpected that the analgesic response to stress might similarly vary across stressors. Thus, for instance, while ether does product a classic cortisol response, it is not mediated by the same medio-basal hypothalamic system as are most other environmental stressors [26, 27, 39].

Given the number and range of environmental stressors that increase nociceptive thresholds, it would seem unlikely that various non-specific peripheral factors peculiar to the

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individual stressors could account for the threshold elevations. With respect to the analgesia induced by cold-water swims, the specific stressor employed in the present experiment, this can be accounted for neither by core or skin hypothermia nor by 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 [6, 9, 10]. 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 while both severe (3 mA/30 min/day) inescapable foot shock [1,25] and severe (2°C for 3.5 min) cold-water swims [9,10] induce pain threshold elevations following initial exposures, this analgesia gradually dminishes over repeated daily exposures. Following either twelve daily inescapable foot shock sessions [1,25] or fourteen daily cold-water swims [6, 9, 10], nociceptive thresholds adapt 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. The induction of adaptation is dependent upon both stress severity and the number of repeated exposures since lower shock levels (0.8 mA/15 sec/day) induced analgesia which has been shown to persist after eight consecutive days [12].

Some investigators have linked the analgesia produced by stressful events to endogenous opiate mechanisms since the analgesia induced by foot shock stress was paralleled by alterations in the opiate binding properties of brain tissue [1, 11, 12, 25], suggesting an increase in endorphin activity. Initial exposures to severe foot shock and daily, repeated exposure to moderate foot shock stress, both of which elicited analgesia, altered opiate binding, while daily, repeated exposure to severe foot shock stress, which failed to induce analgesia, did not. If, as this correlation would suggest, stress reduces the response to pain by enhancing opiate-like neurotransmitter activity, then strong interactions should occur between opiate and stress-induced analgesic states. In line with this suggestion, β-endorphin has been found to be released concomitantly with ACTH from the pituitary during acute injury stress [17]. β–Endorphin levels in blood, but not brain, have also been found to be increased following foot shock stress [35]. On the other hand, methionine enkephalin activity in brain is apparently not altered by foot-shock stress [14]. Also, lesions of the dorsolateral funiculus of the spinal cord which effectively eliminate analgesia induced by either opiates [34] or electrical stimulation [2] do not seem to affect analgesia produced by inescapable foot shock [34]. These data suggest that stress-induced and opiate analgesia may be dissociated.

To examine further the relationship between opiates and the analgesia induced by stressful events, the present study investigated whether cross-tolerance would develop between analgesia produced by cold-water stress and that induced by morphine.

METHOD

Sixty male albino Holtzman Sprague-Dawley rats were tested for flinch-jump thresholds using a modification of Evans' method [13]. Scrambled electric shocks were delivered through a grid floor by a shock generator. 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 which elicited simultaneous removal of both hindpaws from the grids. The jump threshold was defined as the lowest of the two consecutive intensities which 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 until each nociceptive threshold was determined. 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 tests were uninformed both of the purpose of the experiment and of the specific experimental conditions.

Ten separate groups of six rats each were all subjected to five days of flinch-jump testing. Since all experimental conditions, which differed in length, were manipulated prior to this sequence of flinch-jump tests, the first flinch-jump session was designated the experimental day and the last flinch-jump session in the 5-day sequence served as the baseline control day. The first, second and third groups were administered a single SC injection of morphine sulfate at one of three dose levels, 5, 10 or 15 mg/kg, respectively, 30 min prior to the initial flinch-jump test (acute morphine groups). The fourth group was exposed to a single, forced cold-water swim (2°C for 3.5 min) 30 min prior to the initial flinch-jump test (acute stress group). Rats in the fifth and sixth groups were subjected to either chronic morphine injections (10 mg/kg) or chronic daily cold-water swims for 14 consecutive days before threshold testing. On the fourteenth day of chronic treatment, the last injection or the last swim was followed 30 min later by the initial flinch-jump test (chronic morphine and chronic stress groups). The seventh group was subjected to chronic daily cold-water swims for 13 days; on the fourteenth day, morphine (10 mg/kg) was administered 30 min before the initial flinch-jump test (chronic stress/morphine cross treatment group). The eighth group received daily morphine injections (10 mg/kg) for 13 days; on the fourteenth day, a cold-water swim preceded initial flinch-jump testing (chronic morphine/stress cross-treatment group). The ninth group was exposed to the identical series of chronic swims as the seventh group, except that the water temperature was 28°C (chronic warm-water control/acute morphine group). The tenth group experienced an identical series of chronic injections as the eighth group, except that a buffered vehicle was injected in lieu of morphine (chronic placebo control/acute stress group).

RESULTS

Figure 1 summarizes the effects of the various experimental manipulations upon jump thresholds on the experimental day. Flinch and initial jump thresholds displayed an identical pattern of effects. As expected, acute exposure to a cold-water swim or to a single dose of morphine elevated nociceptive thresholds above baseline levels, while chronic exposure to either treatment resulted in unaltered or depressed thresholds. On the other hand, in

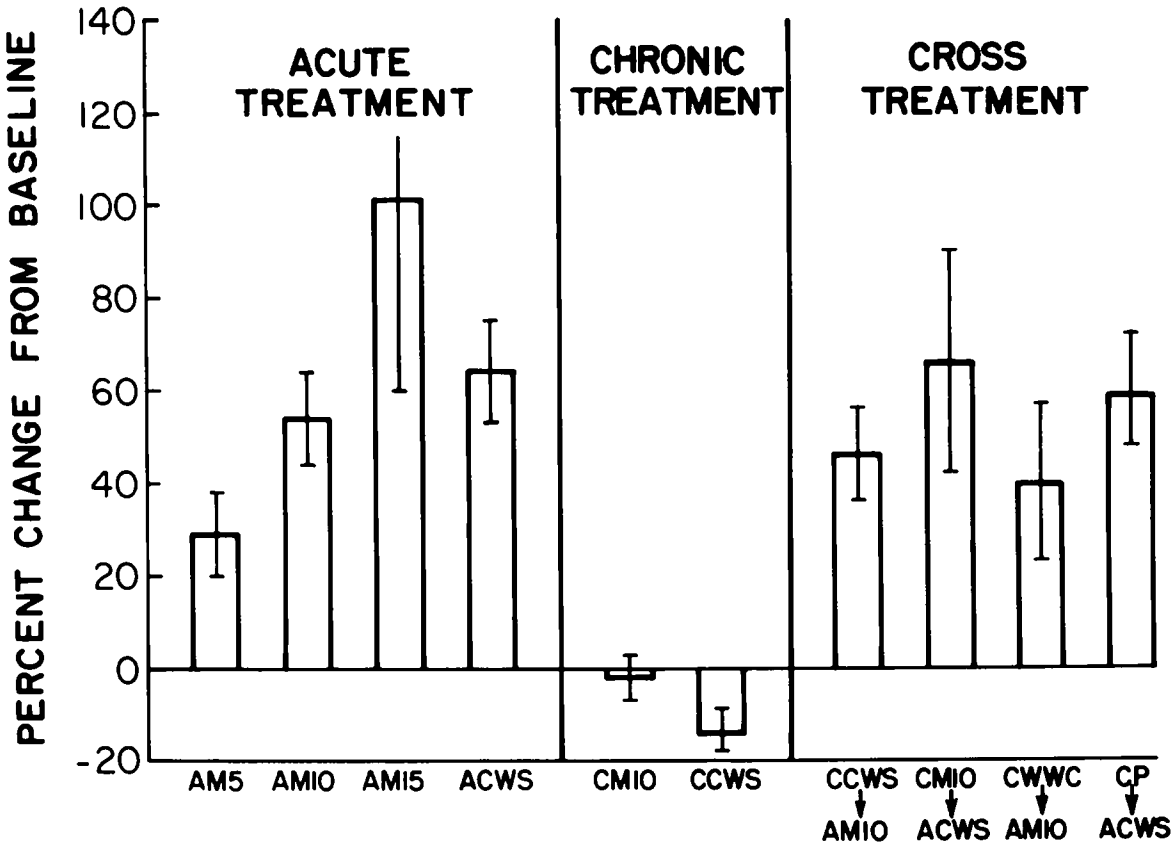

FIG. 1. Percent change (± SEM) in jump threshold from baseline values as a function of either acute, chronic or cross-treatments between morphine and cold-water stress. Abbreviations: AM5 – acute morphine (5 mg/kg); AM10 – acute morphine (10 mg/kg); AM15 – acute morphine (15 mg/kg); ACWS – acute cold-water stress; CM10 – chronic morphine (10 mg/kg); CCWS – chronic cold-water stress; CWWC – chronic warm-water control; CP – chronic placebo. There were six animals in each group.

the cross treatment groups, neither morphine-tolerant nor stress-adapted rats showed any cross-tolerance to the other analgesic treatment.

Signficiant changes in each nociceptive measure were determined by a two-way analysis of variance which included the ten experimental groups as one main effect and compared differences between experimental (first) and baseline (fifth) days within groups for the second main effect. While baseline test sessions did not differ across groups for any nociceptive measure, there were significant differences among the groups on the experimental day for all three measures: jump, $F(9,49) = 3.64$, $p<0.01$, initial jump, $F(9,49) = 3.75$, $p<0.01$, and flinch, $F(9,49) = 4.03$, $p<0.01$. *A posteriori* Tukey comparisons were made between the experimental and baseline days for each group and, where applicable, between experimental days across groups. Table 1 summarizes the effects of each experimental manipulation upon jump, initial jump and flinch thresholds.

Acute exposure to a cold-water swim or to any of the three doses of morphine significantly elevated initial jump and jump thresholds over baseline levels. Analgesia produced by the acute cold-water swim was most comparable to that produced by the acute 10 mg/kg morphine injection, jump: $t = 0.64$; initial jump: $t = 1.07$. In contrast, chronic exposure to cold-water swims significantly lowered jump and initial jump thresholds. Chronic morphine injections produced nociceptive thresholds which were similar both to baseline and to chronic stress, jump: $t = 0.86$; initial jump: $t = 0.95$, indicating that tolerance to morphine and adaptation of stress-produced analgesia occurred in the chronic groups.

In the cross-treatment groups, neither morphine-tolerant nor stress-adapted rats showed any cross-tolerance to the other analgesic treatment. Despite 13 days of morphine pretreatment, the chronic morphine-stress cross-treatment group displayed nociceptive thresholds similar to the acute cold-water stress group, jump: $t = 0.20$; initial jump: $t = 0.55$, and significantly higher than both its own baseline and the chronic cold-water stress group, jump: $t = 3.97$, $p<0.05$; initial jump: $t = 3.44$, $p<0.05$. Similarly, despite 13 days of cold-water swim pretreatment, the chronic stress-morphine cross-treatment group displayed nociceptive thresholds similar to the acute 10 mg/kg morphine group, jump: $t = 0.85$; initial jump: $t = 0.18$ and significantly higher than both its own baseline and the chronic morphine group, jump: $t = 4.56$, $p<0.01$; initial jump: $t = 5.30$, $p<0.01$. These two cross-treatment groups did not differ from one another. Both the warm-water and the placebo control groups displayed identical patterns to their

TABLE 1

A POSTERIORI TUKEY COMPARISONS BETWEEN BASELINE AND TEST DAYS WITHIN ALL EXPERIMENTAL GROUPS (n=6) FOR ALL THREE NOCICEPTIVE THRESHOLDS (in mA)

Group	Jump		Initial Jump		Flinch	
	Baseline	Test	Baseline	Test	Baseline	Test
ACUTE TREATMENTS						
Acute Morphine (5 mg/kg)	0.591	0.756	0.525	0.624	0.172	0.187
	t=3.54, p<0.05		t=2.57, p<0.05		t=0.52, n.s.	
Acute Morphine (10 mg/kg)	0.638	0.963	0.563	0.796	0.206	0.214
	t=7.90, p<0.01		t=4.92, p<0.01		t=0.30, n.s.	
Acute Morphine (15 mg/kg)	0.600	1.099	0.516	1.033	0.194	0.328
	t=3.51, p<0.05		t=3.32, p<0.05		t=2.47, 0.10>p>0.05	
Acute Cold-Water Stress	0.633	1.027	0.566	0.900	0.194	0.370
	t=5.36, p<0.01		t=5.26, p<0.01		t=10.78, p<0.01	
CHRONIC TREATMENTS						
Chronic Morphine (10 mg/kg)	0.774	0.739	0.734	0.642	0.226	0.211
	t=0.89, n.s.		t=1.46, n.s.		t=0.21, n.s.	
Chronic Cold-Water Stress	0.758	0.648	0.677	0.560	0.183	0.183
	t=2.58, p<0.05		t=3.40, p<0.05		t=0.00, n.s.	
CROSS TREATMENTS						
Chronic Cold-Water Stress → Acute Morphine (10 mg/kg)	0.628	0.908	0.534	0.790	0.138	0.163
	t=6.27, p<0.01		t=6.92, p<0.01		t=1.93, n.s.	
Chronic Morphine (10 mg/kg) → Acute Cold-Water Stress	0.643	1.030	0.667	0.833	0.215	0.373
	t=3.32, p<0.05		t=3.14, p<0.05		t=5.39, p<0.01	
Chronic Warm-Water Control → Acute Morphine (10 mg/kg)	0.665	0.943	0.670	0.823	0.220	0.303
	t=2.63, p<0.05		t=2.71, p<0.05		t=1.87, n.s.	
Chronic Placebo → Acute Cold-Water Stress	0.760	1.202	0.695	1.070	0.282	0.440
	t=4.77, p<0.01		t=4.15, p<0.01		t=3.69, p<0.05	

respective chronic stress/morphine and chronic morphine/stress cross-treatment groups.

DISCUSSION

The lack of even a slight cross-tolerance between morphine-produced and cold-water stress-produced analgesia contrasts with the observations of virtual complete cross-tolerance between intracerebral morphine microinjections and endorphin fragments [40,44], and between the former and systemic morphine injections [21]. It also contrasts with partial development of cross-tolerance between stimulation-produced and morphine-produced analgesia [31]. The present data would seem to suggest that cold-water stress-produced and morphine-produced analgesia are dissociable phenomena and that they must be mediated by at least partially independent neural systems. Further support for this premise is found in the in-

consistent effects of opiate receptor blockade upon stress-induced analgesia. Whereas naloxone consistently reverses opiate analgesia at low doses (1 mg/kg) [28], it only partially reverses stress-produced analgesia at doses ranging from 1 to 20 mg/kg [1, 7, 9, 18, 25].

The premise has recently been advanced that one part of an organism's innate emergency response to stress-provoking environmental stimuli is a lowered sensitivity to pain [8, 9, 18]. In such circumstances, an organism's normal reactions to painful stimuli, prompting withdrawal, rest and other recuperative behaviors, could be seriously disadvantageous. This would seem to apply particularly to such survival threatening situations as predation, defense, intra-specific confrontations for dominance and acute adaptation to extreme environmental demands, although only the latter has been experimentally verified. Acute exposure to severe stress produces analgesia; chronic exposure to severe stress does not [1, 8, 9, 10, 18, 25]. In this regard, the dampened response to pain resembles that of most other stress-induced physiological responses including pituitary-adrenal activation. The question of how stress-related analgesia is mediated neurally remains open in that the availability of functional opiate-like neurons appears not to be necessary. Coupled with the earlier experimental evidence mentioned above [34], the complete lack of cross-tolerance between cold-water stress-produced and morphine-produced analgesia in the present experiment supports the contention [18, 32, 34] that perhaps two or more intrinsic routes might exist for normal pain inhibition, at least one of which is non-opiate. The only realistic alternative at present to this interpretation would involve the participation in stress-produced analgesia of the recently described delta-type opiate receptor for which enkephalin but not morphine, possess high affinity [24].

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