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Stress and Morphine Analgesia: Alterations Following p-Chlorophenylalanine

RICHARD J. BODNAR,*† JEFFREY H. KORDOWER,* MARGARET M. WALLACE* AND HADDASSAH TAMIR‡

**Department of Psychology, Queens College, C.U.N.Y., Flushing, NY 11367*
and
Departments of Behavioral Physiology† and Neuroscience,‡ N.Y.S. Psychiatric Institute, 722 W. 168th St. New York, NY 10032

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BODNAR, R. J., J. H. KORDOWER, M. M. WALLACE AND H. TAMIR. *Stress and morphine analgesia: Alterations following p-chlorophenylalanine.* PHARMAC. BIOCHEM. BEHAV. **14**(5) 645–651, 1981.—Recent studies have shown that while the analgesic responses induced by certain stressors appear to be related to morphine analgesia, the analgesic responses to other stressors do not. Para-chlorophenylalanine (PCPA), a potent tryptophan-hydroxylase inhibitor has been shown to decrease both basal pain thresholds and morphine analgesia on the flinch-jump test. To assess further the relationship between morphine and stress-induced analgesia, PCPA's effects upon the analgesic responses to cold-water swims, 2-deoxy-D-glucose, inescapable foot shock and morphine were determined using the flinch-jump and tail-flick tests. PCPA, which produced an 85% depletion of brain serotonin, significantly decreased jump thresholds while significantly increasing tail-flick latencies. Similarly, while morphine analgesia was decreased by PCPA on the flinch-jump test, it was not affected on the tail-flick test. The analgesic jump thresholds induced by cold-water swims and 2-deoxy-D-glucose as well as the increased tail-flick latencies induced by foot shock were unaffected by PCPA. These results are discussed in terms of PCPA's differential effects upon basal nociception and morphine analgesia and in terms of further dissociation between morphine and stress-induced analgesia.

Pain Analgesia Parachlorphenylalanine Cold-water swims 2-Deoxy-D-glucose Inescapable foot shock Morphine

ACUTE exposure to a wide range of stressful stimuli results in a transient increase in rodent pain thresholds (see reviews [4, 7, 15]). The magnitude and duration of the anti-nociceptive effect depend upon the stressor employed. Moreover, the neural and hormonal determinants subserving these responses also depend upon the stressors employed with some, but not all, stressors displaying some opioid-like influences. Analgesic cross-tolerance with morphine has been observed with 2-deoxy-D-glucose and prolonged inescapable foot shock [33,45], but not with cold-water swims, autoanalgesia and brief foot shock [11, 17, 33]. While the opiate antagonist naloxone attenuates the anti-nociceptive responses to food deprivation, immobilization, prolonged foot shock and shock delivered to the front paws [2, 20, 32, 36], it does not alter significantly the anti-nociceptive responses to cold-water swims, 2-deoxy-D-glucose, brief foot shock, hind paw shock and autoanalgesia [8, 10, 18, 20, 32]. Indeed procedures that either potentiate or fail to affect morphine analgesia appear to alter the anti-nociceptive responses of non-opioid stressors. Hypophysectomy attenuates the analgesic effects of cold-water swims, immobilization, insulin and prolonged foot shock [3, 6, 9, 38], while potentiating morphine and 2-deoxy-D-glucose analgesia [9,30]. The analgesic response to cold-water swims, but not morphine, is also diminished in Brattleboro rats deficient in vasopressin [12]. Therefore, it appears that while some stressors interact with the opioid mechanisms subserving morphine analgesia, other stressors do not.

Extensive evidence has indicated that serotonin plays an integral role in the maintenance of morphine analgesia (see reviews [25, 35, 37, 51]). Yet the role of serotonin in stress-induced analgesia is less clear. Interruption of the descending bulbo-spinal serotonergic system by lesions placed in the dorsolateral funiculus of the spinal cord reduce the analgesic responses to morphine and electrical stimulation of the periaqueductal gray [5]. This procedure also abolishes the anti-nociceptive response to shock delivered to the forepaws [49]. By contrast, the anti-nociceptive response to shock delivered either to the hindpaws alone or all four paws is blocked by spinal transection, but not dorsolateral funiculus lesions [28, 29, 49]. Moreover, lesions placed in the dorsal raphe and surrounding caudal periaqueductal gray are effective in blocking the analgesic effects of morphine [22, 42, 43] and electrical stimulation of the rostral periaqueductal gray [39]. These same lesions diminish 2-deoxy-D-glucose analgesia yet fail to affect cold-water swim analgesia [13].

The employment of the tryptophan hydroxylase inhibitor, p-chlorophenylalanine (PCPA) [31] has also been used to determine the role of serotonin in the modulation of nociceptive and analgesic responses. Tenen found initially that 48 hr following administration of PCPA, both basal pain thresholds [47] and morphine analgesia [48] were decreased as measured by the flinch-jump technique. Vogt [50] reported similar decreases in morphine analgesia as measured by the foot pressure test 48 and 72 hr following PCPA administration. In both studies, PCPA depleted 80–85% of brain serotonin and 92% of lumbar cord serotonin 48 and 72 hr after injection.

Therefore, to assess further the relationship, if any, between morphine and stress-induced analgesia, the present study examined whether administration of PCPA altered the analgesic effects of cold-water swims (CWS), 2-deoxy-D-glucose (2-DG) and inescapable foot shock (FS). These alterations were compared with that of morphine analgesia on two pain tests, the flinch-jump [23] and the tail-flick [21]. Brain serotonin assays were carried out to correlate biochemical deficits with analgesic alterations.

METHOD

Flinch-Jump Thresholds

Thirty-six male albino Sprague-Dawley rats (250–450 g) were tested for flinch-jump thresholds using an ascending method of limits. Electric shocks were delivered through a 30 cm by 24 cm floor composed of 14 grids by a 60 Hz constant current shock generator and grid scrambler. The flinch threshold was defined in mA as the lowest intensity that elicited a withdrawal of a single paw from the grids. The jump threshold was defined as the lowest of two consecutive intensities that elicited simultaneous withdrawal of both hindpaws 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 occurred at 10 sec intervals and were increased in equal 0.05 mA steps until all nociceptive thresholds were determined. After each trial, the current intensity was reset to 0.1 mA for the next trial until 6 trials were completed. Daily flinch and jump thresholds were each computed as the mean of these 6 trials. Stable baseline flinch-jump thresholds were determined over four days.

Based on these data, rats were matched into one of six groups of six rats each. Three groups received a single intraperitoneal injection of d-l PCPA methyl ester hydrochloride (Sigma: 350 mg/ml normal saline/kg body weight), while three groups received a vehicle control (1 ml/kg). Flinch-jump thresholds were determined 48 hr later to assess effects upon basal nociception. Then, one PCPA-treated and one control group were subjected to a 2°C swim for 3.5 min followed by a second flinch-jump test 30 min later. A second PCPA and control group received a dose of 450 mg/kg of 2-DG (300 mg 2-DG/ml sterile water/kg body weight, IP) 30 min before the second flinch-jump test. A third PCPA and control group received a dose of 5 mg/kg of morphine (5 mg/ml buffer/kg body weight, SC) 30 min before the second flinch-jump test. The experimenter conducting the flinch-jump test was uninformed as to whether the rat received a PCPA or control injection.

Tail-Flick Latencies

Forty-eight additional naive male rats were tested for latency to tail-flick withdrawal in which a radiant heat source (IITC Tail Flick Analgesia Meter), mounted 8 cm above the tail of the restrained animal, was applied 4 cm proximal to the tip of the tail. When the animal flicked its tail in response to the heat, a photocell was broken automatically and the latency displayed. The intensity of the thermal stimulus was adjusted to produce a baseline tail-flick latency of between 2.5 and 4.5 sec. To avoid tissue damage in testing tail-flick latencies, the trials were automatically terminated if a withdrawal response to the heat stimulus did not occur within 6 sec. Each data point for each animal was calculated as the mean of three trials which were spaced 30 sec apart.

Eight groups of six animals each were selected based upon four days of matched baseline tail-flick latencies. Four PCPA-treated (350 mg/ml/kg, IP) and four vehicle control (1 ml/kg) groups were tested 48 hr following injection to assess effects upon basal tail-flick latencies. Then one PCPA-treated and one control group were exposed to 20 sec of 1.0 mA inescapable foot shock followed by tail-flick tests 0 and 15 min later. One PCPA-treated and one control group received 5 mg/kg of morphine followed by a tail-flick test 30 min later. Based on the latter results, a dose-dependent and time-dependent analysis of PCPA-induced alterations upon morphine analgesia was done to control for possible ceiling effects caused by the 6 sec cutoff criterion. Thus, one PCPA-treated and one control group received 5 mg/kg of morphine followed by tail-flick tests 30, 60 and 120 min later. Finally, one PCPA-treated and one control group received 2.5 mg/kg of morphine followed by tail-flick tests 30, 60 and 120 min later.

Serotonin Determinations

Forty-eight hr after injection, five additional PCPA-treated (350 mg/ml/kg, IP) and five control rats were decapitated, their brains quickly removed and weighed without cerebellum. The brains were then homogenized in 10 ml 0.10 N HCl, centrifuged for 15 min at 10,000 rpm and the supernatent removed. The serotonin content of brain was measured according to Saavedra *et al.* [41]. The samples were incubated with serotonin-N-acetyl transferase (Acetyl-CoA arylamine N-acetyl transferase, EC 2.3.1.5) and hydroxyindole O-methyl transferase (S-adrenosyl-L-methionine: N-actyl serotonin methyl transferase, EC 2.1.1.4) in the presence of [^{3}H] methyl-S-adenosyl-methionine (Amersham-Searle Co.). The melatonin produced from serotonin was isolated and counted in toluene based scintillation fluid (6 g PPO and 0.075 g POPOP in 1 liter toluene—Packard Co.).

RESULTS

Flinch-Jump Thresholds

Figure 1 shows that PCPA decreased both basal jump thresholds and the analgesic response to morphine. By contrast, the analgesic responses to CWS and 2-DG were similar in PCPA-treated and control rats. A two-way split-plot analysis of variance revealed significant differences in jump thresholds across the six groups, $F(5,30)=3.75$, $p<0.009$, across the three time conditions, $F(2,60)=310.49$, $p<0.001$ and for the group by time interaction, $F(10,60)=8.72$, $p<0.001$. Post-hoc Scheffé comparisons revealed that while the pre-injection baseline values did not differ significantly, $F(1,34)=0.02$ between PCPA-treated and control rats, jump thresholds were significantly lower, $F=18.03$, $p<0.01$ in PCPA-treated rats 48 hr after the injection.

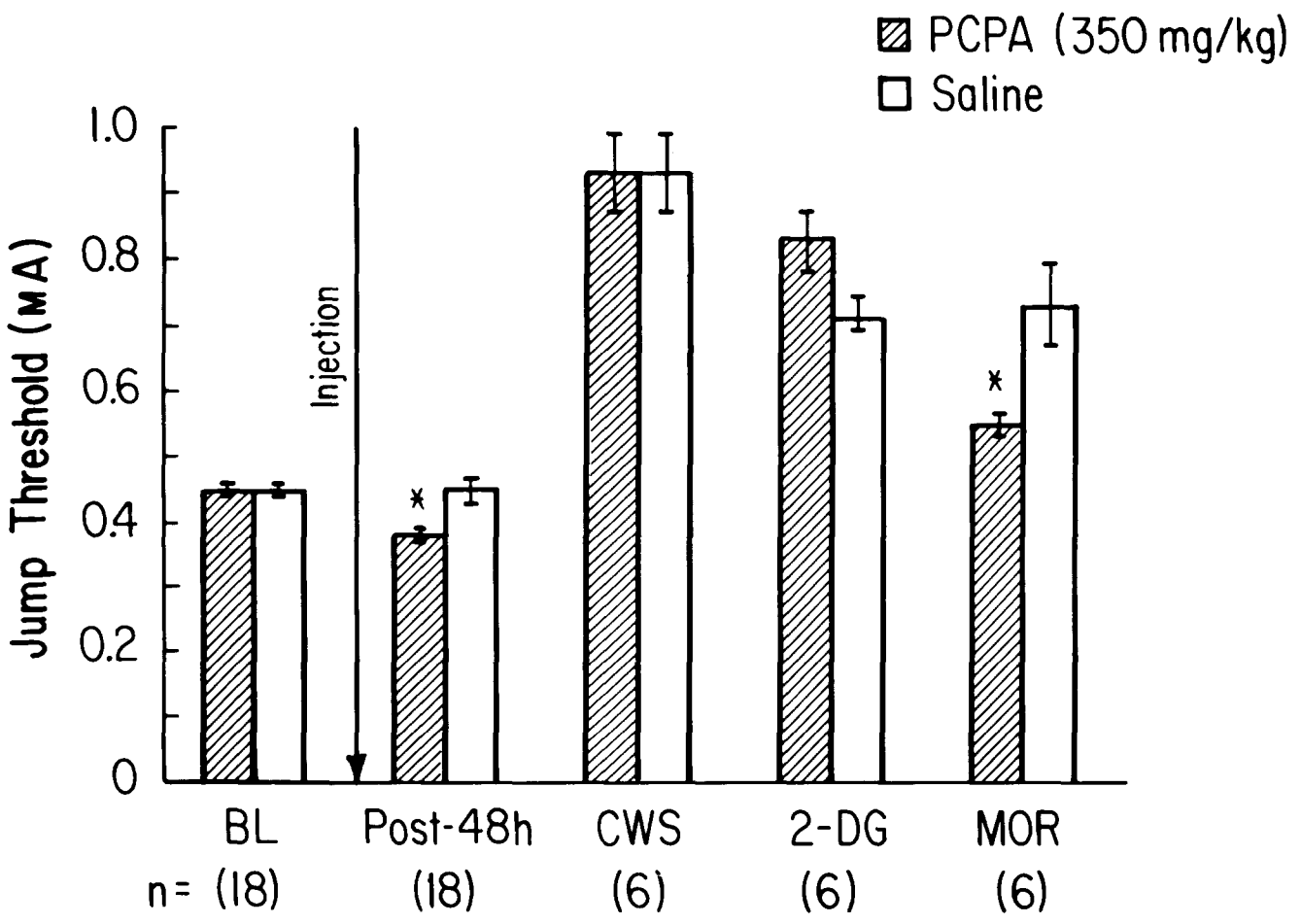

FIG. 1. Mean alterations in jump thresholds (±S.E.M.) for PCPA-treated (hatched) and saline-treated (open) rats from pre-injection baseline (BL) levels. Jump thresholds were determined 48 hr after the injection followed by acute exposure to either cold-water swims (CWS), 2-deoxy-D-glucose (2-DG; 450 mg/kg) injections or morphine (MOR: 5 mg/kg) injections. The analgesic effects of these manipulations were determined 30 min later.

The jump thresholds of PCPA-treated rats were significantly higher following morphine than thresholds determined either before, $F(1,10)=8.69$, $p<0.05$ or 48 hr after PCPA injection, $F=29.16$, $p<0.01$. Similarly, control jump thresholds following morphine were significantly higher than before, $F=13.99$, $p<0.01$ or after, $F=13.29$, $p<0.01$ vehicle administration. However, the magnitude of the analgesic response was significantly lower, $F=7.73$, $p<0.05$ in PCPA-treated than control rats. Indeed, the pre-morphine jump threshold was subtracted from the post-morphine jump threshold for both groups to partial out the basal jump threshold hyperalgesia from the diminished morphine analgesia. PCPA-treated animals still exhibited significantly less morphine analgesia, $F=6.20$, $p<0.05$.

By contrast, the analgesic responses of the two groups to CWS did not differ significantly from each other. Both PCPA-treated and control rats displayed significantly elevated jump thresholds following CWS than before, PCPA: $F=46.77$, $p<0.01$; control: $F=68.94$, $p<0.01$ or after, PCPA: $F=63.89$, $p<0.01$; control: $F=116.79$, $p<0.01$ the respective injections. The magnitude of the CWS response failed to differ significantly between groups, $F=0.18$ even when the basal hyperalgesia was partialled, $F=0.71$.

Similarly, the analgesic responses of the two groups to 2-DG failed to differ significantly, $F=2.46$ from each other even when the basal hyperphagia was partialled, $F=0.30$. Both groups displayed significantly elevated jump thresholds following 2-DG than either before, $F=30.85$, $p<0.01$ and after, $F=45.30$, $p<0.01$ the PCPA injection or before, $F=68.94$, $p<0.01$ and after, $F=61.46$, $p<0.01$ the control injection.

Tail-Flick Latencies

In contrast to the observed hyperalgesia following PCPA on the flinch-jump test, a correlated difference score t-test revealed that PCPA significantly increased, $t(23)=3.57$, $p<0.01$ basal tail-flick latencies 48 hr following injection. Control rats failed to show any differences, $t(23)=1.00$ over the same time period.

Table 1 summarizes the pooled effects of morphine within each group. A two-way split-plot analysis of variance revealed significant differences among the PCPA and control groups across the two morphine doses, $F(3,32)=12.41$, $p<0.001$, across the various pre-injection and post-injection time courses, $F(4,124)=10.29$, $p<0.001$ and for the group by

TABLE 1

A POSTERIORI SCHEFFÉ COMPARISONS FOR ALTERATIONS IN TAIL-FLICK LATENCIES FOLLOWING ACUTE EXPOSURE TO MORPHINE IN PCPA AND SALINE-TREATED RATS

Group	Morphine Dose (mg/kg)		Tail-Flick Latency (sec) BL	Post Injection 48 hr	Post Morphine 30 min	Post Morphine 60 min	Post Morphine 120 min
PCPA	5.0	Mean	3.06	3.86	5.97	5.25	3.88
		S.E.M.	0.21	0.39	0.00	0.49	0.96
		F Score vs BL			176.36†	35.58†	4.67
		F Score vs Post-Injection			26.11†	18.75†	1.82
Saline	5.0	Mean	3.04	3.38	4.32	3.11	2.68
		S.E.M.	0.14	0.47	0.85	0.82	0.56
		F Score vs BL			12.18†	0.89	0.36
		F Score vs Post-Injection			5.15*	0.87	0.16
PCPA	2.5	Mean	2.44	3.70	3.98	3.58	3.83
		S.E.M.	0.24	1.10	0.77	0.78	0.77
		F Score vs BL			4.00	4.20	4.54
		F Score vs Post-Injection			1.23	0.20	0.30
Saline	2.5	Mean	2.43	2.07	2.08	2.28	2.00
		S.E.M.	0.17	0.00	0.05	0.26	0.07
		F Score vs BL			2.59	0.37	0.25
		F Score vs Post-Injection			0.11	0.79	0.65

*$p<0.05$.
†$p<0.01$.

TABLE 2

A POSTERIORI SCHEFFÉ COMPARISONS FOR ALTERATIONS IN TAIL-FLICK LATENCIES FOLLOWING ACUTE EXPOSURE TO INESCAPABLE FOOT SHOCK IN PCPA AND SALINE-TREATED RATS

Group		Tail-Flick Latency (sec) BL	Post Injection 48 hr	Post Foot Shock 0 min	Post Foot Shock 15 min
PCPA	Mean	3.54	4.24	5.27	5.31
	S.E.M.	0.18	0.39	0.30	0.53
	F Score vs BL			18.15†	13.99†
	F Score vs Post-Injection			13.96*	15.53†
Saline	Mean	3.60	3.41	4.87	4.32
	S.E.M.	0.10	0.45	0.58	0.60
	F Score vs BL			10.69†	3.80
	F Score vs Post-Injection			19.80†	7.78*

*$p<0.05$.
†$p<0.01$.

time interaction, $F(12,124)=4.40$, $p<0.001$. While morphine analgesia was significantly reduced by PCPA as measured by the flinch-jump test, comparisons between groups revealed that the morphine-induced increases in the tail-flick latencies of PCPA rats were significantly higher than those of control rats 30, $F(1,22)=8.07$, $p<0.01$ and 60 min, $F(1,10)=6.76$, $p<0.05$ following the 5 mg/kg morphine dose. Similarly, the lower 2.5 mg/kg dose of morphine elicited significantly longer tail-flick latencies in PCPA-treated rats 30, $F(1,10)=62.41$, $p<0.01$, 60, $F=9.24$, $p<0.05$ and 120, $F=19.10$, $p<0.01$ min following the injection. However, when the basal tail-flick analgesia is partialled out, the increases in the morphine response noted in the PCPA group over the control group approach, but do not reach, statistical significance: 5 mg/kg dose after 30, $F(1,22)=3.20$, 60, $F(1,10)=3.45$ and 120, $F=1.06$ min; 2.5 mg/kg dose after 30, $F=0.23$, 60, $F=0.54$ and 120, $F=0.38$ min.

Table 2 displays the comparisons within each group between the pre- and post-injection baselines and the FS-induced alterations. A two-way split plot analysis of variance revealed significant differences across the injection and shock time course, $F(3,30)=28.89$, $p<0.001$, but neither between groups, $F(1,10)=1.96$ nor for the group by time interaction, $F(3,30)=2.54$. Comparisons between groups showed that while both groups displayed similar FS analgesia immediately following stress, $F(1,10)=0.86$, increased tail-flick

latencies in PCPA-treated rats approached, but did not reach, statistical significance, $F=3.84$, $0.10>p>0.05$ over controls. This latter effect could be attributed to the analgesic post-injection latencies of the PCPA rats for when this factor was partialled, both groups displayed similar FS effects 0, $F=1.49$ and 15, $F=0.34$ min after shock.

Serotonin Determinations

One PCPA-treated rat died in the interval between injection and sacrifice and was not used in the assay. The mean brain serotonin content of the fice control rats (0.9304 μg/g wet weight; S.E.M.: 0.046) was significantly higher, $t(7)=10.60$, $p<0.01$ than the mean brain serotonin content of the four remaining PCPA-treated rats (0.1425 μg/g wet weight; S.E.M.: 0.004), indicating that PCPA at this dose and treatment depleted brain serotonin by 85%. It should be noted that these effects are well within the range of what has previously been reported [41,46] with the slightly higher control values accounted for by removal of the serotonin-poor cerebellum.

DISCUSSION

The present study has shown that PCPA, the potent tryptophan hydroxylase inhibitor, produced differential effects upon basal and analgesic pain thresholds. First, while PCPA lowered significantly the threshold necessary to elicit a jump response, it increased significantly the latency to withdraw a rat's tail from radiant heat. Second, PCPA reduced significantly the analgesic efficacy of morphine on the flinch-jump test, but not the tail-flick test. Third, in contrast to the PCPA-induced reversal of morphine analgesia on the flinch-jump test, PCPA failed to affect the analgesic responses to CWS and 2-DG. However, given the failure of PCPA to alter morphine and FS analgesia on the tail-flick test, little can be said conclusively about the similarities or differences of these two analgesic manipulations on this parameter. These three issues will be addressed separately.

Tenen [47] reported initially that PCPA decreased basal pain thresholds as measured by the flinch-jump technique. However, the pain test employed appears to be an important determinant in this effect. While others [26,52], including the present study, have replicated PCPA-induced decreases in flinch-jump thresholds, these effects have been attributed to alterations in the aversiveness, but not sensitivity, of the shock [24]. In this vein, PCPA administration has been shown to increase the severity of headaches in humans [44]. Yet, the hyperalgesic effects of PCPA are not typically found on the hotplate test [19] unless the animals are tested during the light cycle 72 hr following the injection [27]. Moreover, Akil and Mayer [1] found that PCPA produced non-significant increases in tail-flick latencies 48 hr after injection. That significant increases in basal tail-flick latencies were achieved in the present study may be attributed to the larger number of rats tested. Therefore, it appears that the failure of PCPA to decrease basal tail-flick latencies in the present study is not reflective of an inability to replicate prior results, but rather a selective effect of PCPA upon various nociceptive modalities.

The selective effect of PCPA in decreasing morphine analgesia is also dependent on several factors including the pain test, species, extent of serotonin depletion and interval between injection and test. In the initial study of Tenen [48], morphine analgesia was decreased significantly on the flinch-jump test only when at least 48 hr elapsed between PCPA and morphine injection and when brain serotonin was decreased by 85%, effects replicated in the present study. Indeed, Tenen found that PCPA administration increased the analgesic effects of morphine 3 hr after injection but reduced brain serotonin by only 20%. This relationship was confirmed by Vogt [50] who found that PCPA decreased morphine analgesia as measured by the foot pressure test 72 hr following PCPA administration with concomitant decreases in brain and lumbar cord serotonin of 80% and 92% respectively. By contrast, 24 hr following PCPA administration, brain serotonin was decreased by 67% and morphine analgesia was increased on the foot-pressure test. The necessity of degree of serotonin depletion is demonstrated by the observation of increased morphine analgesia following PCPA in rabbits, an effect that produces only 60% serotonin depletion [40]. Yet even when the experimental protocols adhere to the 48–72 hr interval between PCPA and morphine injections and obtain high serotonin depletion, the expected decreases in morphine analgesia do not occur when the hot-plate test is used in mice [19] and rats [14]. The present study's failure to observe decreases in morphine analgesia on the tail-flick test following PCPA administration capable of depleting brain serotonin by 85% is in line with the latter results. It appears unlikely that this lack of effect could be attributed to such experimental factors as the dose of the drug, the time course of the morphine effect or the maximal analgesic effect as defined by the cut-off criterion since both PCPA-treated and control rats behaved similarly across doses and post-injection test times. Moreover, the latencies achieved, particularly following the 2.5 mg/kg dose of morphine, but also at the longer test intervals at the 5 mg/kg dose, do not approach the 6 sec limit set to avoid tissue damage. Therefore, it is apparent that PCPA is selective in its ability to decrease morphine analgesia, an effect that appears to be dependent upon the nociceptive modality.

The observation that morphine, but not CWS, analgesia is reduced following PCPA on the flinch-jump test provides further support for the previously-formulated hypotheses that these two analgesic manipulations are dissociable, and the multiple pain-inhibitory systems exist. Manipulations that decrease the analgesic response to morphine, including development of tolerance, administration of naloxone, or lesions placed in and around the periaqueductal gray [22, 34, 42, 43], fail to alter CWS analgesia [10, 11, 13]. By contrast, manipulations that decrease the analgesic response to CWS, including adaptation, hypophysectomy, or genetic selection of Brattleboro rats deficient in vasopressin [8, 11, 12] either fail to alter [11,12] or even increase [9,30] morphine analgesia.

Moreover, the observation that morphine, but not 2-DG, analgesia is reduced following PCPA on the flinch-jump test adds further insights into the nature of the latter's analgesic effect. Though 2-DG exhibits full and reciprocal analgesic cross-tolerance with CWS [45], it also develops cross-tolerance [45] and synergy [8] effects with morphine analgesia. Like morphine, the analgesic effects of 2-DG are potentiated in hypophysectomized rats [9] and reduced significantly in rats with lesions placed in and around the periaqueductal gray [13]. The site of interaction between these two analgesic manipulations appears not to be at the opiate receptor since naloxone fails to affect 2-DG analgesia [8]. The present study suggests that the serotonergic system is also not the site of interaction given PCPA's differential effects. However, this latter supposition together with the role of serotonin in FS analgesia awaits further experimental analysis.

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