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Potentiation of Cold-Water Swim Analgesia by Acute, but not Chronic Desipramine Administration

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BODNAR, R. J., P. E. MANN AND E. A. STONE. *Potentiation of cold-water swim analgesia by acute, but not chronic desipramine administration.* PHARMACOL BIOCHEM BEHAV **23**(5) 749–752, 1985. —Like other stress responses, cold-water swim (CWS) analgesia can be altered by changes in norepinephrine (NE) availability. While clonidine pretreatment potentiates CWS analgesia, lesions placed in the noradrenergic locus coeruleus reduce this response. Desipramine (DMI) can alter both the availability and receptor function of catecholamines, particularly NE: while both acute and chronic DMI treatments decrease NE reuptake, subsensitivity of beta-adrenergic receptors occurs only after chronic DMI treatment. The present study examined whether acute and chronic DMI treatments differentially alter CWS analgesia as measured by the jump test, CWS hypothermia and basal jump thresholds. The first experiment determined that pretreatment at either 24, 5 and 1 hr or only at 1 hr with DMI doses of 20 and 5 but not 1 mg/kg potentiated CWS analgesia. The second experiment found that chronic DMI pretreatment at a dose of 10 mg/kg administered twice daily over seven days failed to alter CWS analgesia at 1, 24, 48 or 72 hr thereafter. Neither CWS hypothermia nor basal jump thresholds were affected by the acute or chronic DMI injection regimens. The selective potentiation of CWS analgesia by acute DMI pretreatment is discussed in terms of the differential actions of acute and chronic injection regimens upon NE availability, receptor function, and adaptation processes.

Pain Analgesia Cold-water swims Desipramine Norepinephrine Rats

THE analgesic response following acute exposure to cold water swims (CWS) appears to be mediated through nonopioid/neurohormonal mechanisms [2, 3, 4, 6, 7]. The modulation of hypothalamo-hypophysial stress responses by catecholamines is well known (see review: [1]) and manipulations in catecholamine availability can affect CWS analgesia. Thus the dopamine receptor stimulant apomorphine reduced CWS analgesia [5], while dopamine receptor blockade with chlorpromazine [9] potentiates it. In addition, alpha-noradrenergic receptor stimulation with clonidine also potentiated CWS analgesia [8].

Desipramine (DMI) has been shown to alter the availability and receptor function of catecholamines, particularly norepinephrine (NE). Acute DMI treatment blocks the reuptake of NE thereby increasing its concentration at receptors. When the drug is given chronically the increased NE availability leads to a compensatory reduction in the density of postsynaptic beta-adenergic receptors and a reduction in the amount of cAMP generated in response to NE and other catecholamines [13, 16, 34, 37, 38, 39, 40, 41]. Chronic DMI also affects alpha-1 and alpha-2 adrenergic receptor function causing an increased responsiveness of the former and a decreased responsiveness of the latter to agonists [12].

Behavioral effects of DMI upon swim-induced immobility and shock-induced escape deficits provide another rationale for investigating its effects upon analgesic stress responses. Rats acutely exposed to an inescapable forced swim become immobile, an effect which is reduced by both acute and chronic DMI pretreatment [20, 25, 26, 27]. Central catecholaminergic mechanisms have been proposed to mediate this response since alpha-adrenergic receptor agonists, but not beta-adrenergic receptor agonists reduced immobility [26] and since alpha-adrenergic receptor antagonists blocked the ability of DMI to reduce immobility [19]. Furthermore, both acute and chronic pretreatment with DMI reduced escape deficits following acute exposure to inescapable shock [18, 21, 29]. Finally, morphine and methadone analgesia has been shown to be enhanced by pretreatment with DMI and other antidepressants [11, 17, 22, 24].

The first experiment investigated whether acute DMI pretreatment altered CWS analgesia on the jump test in rats. Two acute treatments were employed: (a) three injections administered 24, 5 and 1 hr prior to the swims to replicate the design used in the swim immobility studies [20, 25, 26, 27]; and (b) one injection administered 1 hr prior to the swims to find the minimal conditions for drug effectiveness. To determine the specificity of the effect, alterations in CWS hypothermia were also monitored. Further, the effects of acute DMI treatment upon jump thresholds were examined. The second experiment examined the effects of a chronic regimen of DMI injections upon CWS analgesia, CWS hypothermia and jump thresholds.

TABLE 1

ALTERATIONS IN JUMP THRESHOLDS (mA) FOLLOWING COLD WATER SWIMS (CWS) IN ANIMALS ACUTELY PRETREATED WITH DESIPRAMINE (DMI)

Condition (mg/kg)	Injection Number	Jump Threshold (mA: SEM) Baseline	CWS
Vehicle	3	0.355 (0.021)	0.610 (0.070)
DMI-1	3	0.368 (0.019)	0.634 (0.057)
DMI-5	1	0.341 (0.020)	1.033 (0.058)*†
DMI-5	3	0.368 (0.002)	0.724 (0.083)*
DMI-20	1	0.343 (0.015)	0.979 (0.078)*
DMI-20	3	0.352 (0.024)	1.030 (0.048)*

Note: Jump thresholds were significantly increased in all groups following CWS. The asterisk denotes a significant potentiation in the response as compared to vehicle-treated rats (Dunnett comparison, $p<0.01$). The dagger indicates that the potentiation was greater in magnitude following one as compared to three injections of the 5 mg/kg dose of DMI.

TABLE 2

ALTERATIONS IN JUMP THRESHOLDS (mA) FOLLOWING CWS IN ANIMALS CHRONICALLY PRETREATED WITH DMI (10 mg/kg) TWICE DAILY OVER 7 DAYS

		Post Injection Time (hr) Vehicle	1	24	48	72
Baseline	mean	0.371	0.367	0.373	0.367	0.364
	SEM	0.021	0.018	0.014	0.019	0.020
CWS	mean	0.747	0.752	0.768	0.812	0.790
	SEM	0.071	0.065	0.079	0.068	0.069

TABLE 3

ALTERATIONS IN JUMP THRESHOLDS (mA) IN ANIMALS CHRONICALLY PRETREATED WITH DMI

	Post Injection Time (hr) Vehicle	1	24	48	72
Mean	0.360	0.352	0.362	0.423	0.372
SEM	0.011	0.018	0.028	0.035	0.040

EXPERIMENT 1

Method

Male albino Sprague-Dawley rats (500–600 g) were housed in pairs in wire mesh cages with Purina rat chow and water available ad lib. Baseline jump thresholds were determined in a Plexiglas chamber (30×24 cm) with a floor composed of 16 metal grids. A 60-Hz constant current shock generator (BRS/LVE) and an electromechanical grid scrambler (Campden Instruments) delivered the electric shocks through the floor of the chamber. Using a modification of an ascending method of limits procedure of successively more intense shocks [15], the jump threshold was defined in mA as the lowest of two consecutive intensities that 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 with subsequent shocks increasing in 0.05-mA steps until the jump threshold was obtained. A 5-sec interval separated each step within trials and also between trials. A mean of six trials constituted the daily baseline jump threshold, and the mean baseline threshold over four days of testing was used in data analysis. Six groups of rats were matched on the basis of the baseline jump thresholds and were exposed to one and only one experimental condition. Three intraperitoneal injections of DMI HCl (Merrell Dow Research Center) were administered at 24, 5, and 1 hr before swim testing at doses of 20 (Group 1, n=8), 5 (Group 2, n=8 and 1 (Group 3, n=8) mg/kg at a volume of 2 ml distilled water/kg body weight. One injection of DMI was administered at 1 hr before swim testing at doses of 20 (Group 4, n=8) and 5 (Group 5, n=7) mg/kg. A sixth group of eight rats received three vehicle injections (2 ml distilled water/kg body weight, IP). Jump thresholds were assessed 30 min following a 3.5 min swim in a bath of 2°C water. In addition, the core body temperatures of all animals in the six groups of rats were determined immediately following the swim by inserting a probe 2 cm into the rectum for 5 sec which allowed for a stable reading on an electronic digital thermometer (Bailey Instruments). Any swim or drug effects upon hypothermia were compared with a no-swim, no-drug control group (n=8).

To examine whether the acute DMI injection regimen altered basal jump thresholds, a final group of eight rats received three vehicle injections at 24, 5, and 1 hr prior to jump threshold determinations. Then the same rats received a 20 mg/kg dose of DMI according to the same regimen 48 hr later.

A split-plot analysis of variance assessed the acute effects of DMI upon CWS analgesia with comparisons (Dunnett test) between the control (vehicle) and experimental (DMI doses) conditions. A one-way analysis of variance was used to analyze hypothermic effects, while a difference score t-test was used to analyze DMI effects upon basal jump thresholds.

Results

Table 1 indicates that significant potentiations in CWS analgesia occurred in animals pretreated with DMI. Significant differences in jump thresholds were observed among the six groups, $F(5,41)=7.16$, $p<0.001$, between baseline and swim conditions, $F(1,41)=339.67$, $p<0.001$, and for the interaction between groups and conditions, $F(5,41)=10.79$, $p<0.001$. While jump thresholds were significantly increased over baseline values following CWS in all groups, rats receiving either three injections or one injection of the 5 mg/kg and 20 mg/kg doses of DMI exhibited significant potentiations in CWS analgesia as compared to vehicle-treated rats (Dunnett test, $p<0.01$). Further, while the number of DMI injections failed to alter the potentiation of CWS analgesia following the 20 mg/kg dose, a significant increase in the magnitude of the potentiation was observed following one as compared to three injections at the 5 mg/kg dose. In contrast, CWS analgesia in rats pretreated with 1 mg/kg dose of DMI failed to differ from that observed in vehicle-treated rats. While significant differences in post-swim core body temperatures occurred relative to the no-swim, no-drug control

group, $F(6,46)=13.51$, $p<0.001$, the resultant hypothermia failed to differ as a function of DMI pretreatment, $F(5,41)=2.35$. Finally, significant differences in jump thresholds failed to occur, $t(7)=1.00$ following vehicle (mean=0.367, SEM=0.017) and DMI (20 mg/kg: mean=0.314, SEM=0.024) treatments in that group of rats tested for changes in basal nociception.

EXPERIMENT 2

Method

Forty naive male rats, matched into five groups on the basis of baseline jump thresholds, received two daily injections (0900 and 1700 hr) over seven consecutive days with the first group receiving vehicle (2 ml distilled water/kg body weight, IP) and the remainder receiving DMI (10 mg/kg). While the first two groups were exposed to the swim 1 hr after the last injection, the third, fourth and fifth groups were exposed to the swim at 24, 48 and 72 hr after the last injection respectively. The dose and injection regimens were chosen because of the effectiveness in reducing immobility [25] and cAMP synthesis (e.g., [16,41]). The time course was chosen to follow the course of behavioral changes as the drug disappeared from the brain [23]. Core body temperatures were ascertained immediately after the swim. Jump thresholds were determined 10 min before and 30 min after the swim to examine respectively the effects of chronic DMI administration upon jump thresholds per se as well as upon CWS analgesia.

Results

As summarized in Table 2, significant differences in jump thresholds were observed between baseline and swim conditions, $F(1,34)=171.33$, $p<0.001$, but not among groups, $F(4,34)=0.11$. In addition, the interaction between groups and conditions, $F(4,34)=0.18$, was not significant. Jump thresholds were significantly increased over baseline values following CWS, but the analgesic response in each DMI-treated group failed to differ from that observed in vehicle-treated rats. Significant differences in post-swim core body temperatures, $F(4,34)=1.23$, failed to occur among groups. Table 3 summarizes the failure of chronic DMI pretreatment to alter jump thresholds per se, $F(4,34)=1.38$.

Discussion

The present data indicated that acute, but not chronic, DMI pretreatment potentiated the analgesic response following CWS. This suggests that chronic pretreatment with DMI is producing tolerance to the potentiating actions of acute DMI treatment upon CWS analgesia. That one DMI injection at the 5 mg/kg dose produces greater potentiations in CWS analgesia than three injections at the same dose may imply a rapid onset of tolerance. These effects occurred in the absence of DMI-induced changes in either CWS hypothermia or basal jump thresholds. Therefore, it appears that the changes in the analgesic effect following CWS by DMI is selective, and does not represent a generalized shift in all CWS-related stress responses. Further, that acute exposure to DMI failed to alter basal jump thresholds argues against the possibility that the potentiation in CWS analgesia following acute DMI pretreatment was due to sedative properties of DMI. The selective potentiation in CWS analgesia by DMI contrasts with the observed reductions in swim-induced immobility [20, 25, 26, 27] and shock-induced escape deficits [18, 21, 29] following DMI treatment and suggest that different mechanisms may be involved in these phenomena.

The mechanisms by which DMI interacts with CWS to enhance analgesia and by which tolerance to this effect develops are not known. One possible contributing factor however is the drugs's ability to block the reuptake of brain NE. Stress is known to increase brain NE release [31] and this effect would have greater functional consequences in the presence of a NE reuptake inhibitor. Whether or not brain NE release is involved in CWS-induced analgesia however is unclear. Indirect data suggesting possible involvement include potentiations of CWS analgesia by clonidine, an agonist at alpha-2 and alpha-1 adrenergic receptors [8] and reductions of CWS analgesia by lesions placed in the locus coeruleus [10], a major source of central noradrenergic cell bodies [14]. The similar potentiations of CWS analgesia by DMI and clonidine pretreatment are presumably acting through their stimulation of post-synaptic noradrenergic receptors; however this supposition must be studied further. If the noradrenergic system is in fact involved then it is also possible that the slowly developing changes observed in adrenergic receptors accompanying chronic DMI administration are related to the mechanism(s) of tolerance [13, 16, 34, 37, 38, 39, 40, 41]. These considerations thus indicate that further studies on the role of central NE release and adrenergic receptor activation in the DMI-induced potentiation of CWS analgesia are warranted.

The present studies also bear on a hypothesis linking antidepressant drug action with adaptation to stress. It has been proposed that chronic administration of antidepressants facilitates adaptation to stress by mimicking certain of the biochemical changes that normally occur in the brain during successful adaptation to chronic stress [32, 33, 34, 35, 36]. The present results did not support this hypothesis in that repeated administration of DMI did not produce cross tolerance to CWS as would have been expected from a facilitated adaptation to stress. Chronic treatment with this antidepressant drug therefore does not appear to constitute a sufficient condition to achieve adaptation to CWS. It remains possible however that antidepressant treatment, if given concurrently with repeated stress, could enhance the rate or extent of adaptation. Furthermore, as cases of unidirectional cross tolerance have been reported for certain analgesia inducing stimuli (e.g., [30]), it would be of interest to determine if prior repeated stress (in submaximally effective doses) would produce cross tolerance to the DMI potentiation of the stress effect.

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REFERENCES

1. Axelrod, J. and T. D. Reisine. Stress hormones: their interaction and regulation. *Science* **224:** 452–459, 1984.

2. Badillo-Martinez, D., N. Nicotera, P. D. Butler, A. L. Kirchgessner and R. J. Bodnar. Impairments in analgesic, hypothermic and glucoprivic stress responses following neonatal monosodium glutamate. *Neuroendocrinology* **38:** 438–446, 1984.

3. Bodnar, R. J., G. W. Abrams, E. A. Zimmerman, D. T. Krieger, G. Nicholson and J. S. Kizer. Neonatal monosodium glutamate: effects upon analgesic responsivity and immunocytochemical ACTH/B-lipotropin. *Neuroendocrinology* **30:** 280–284, 1980.
4. Bodnar, R. J., M. Glusman, M. Brutus, A. Spiaggia and D. D. Kelly. Analgesia induced by cold-water stress: attenuation following hypophysectomy. *Physiol Behav* **23:** 53–62, 1979.
5. Bodnar, R. J., D. D. Kelly, M. Brutus, C. B. Greenman and M. Glusman. Reversal of stress-induced analgesia by apomorphine, but not by amphetamine. *Pharmacol Biochem Behav* **13:** 171–175, 1980.
6. 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. *Pharmacol Biochem Behav* **8:** 667–672, 1978.
7. Bodnar, R. J., D. D. Kelly, S. S. Steiner and M. Glusman. Stress-produced analgesia and morphine-produced analgesia: Lack of cross-tolerance. *Pharmacol Biochem Behav* **8:** 661–666, 1978.
8. Bodnar, R. J., K. P. Merrigan and E. Sperber. Potentiation of cold-water swim analgesia and hypothermia by clonidine. *Pharmacol Biochem Behav* **19:** 447–451, 1983.
9. Bodnar, R. J. and N. Nicotera. Neuroleptic and analgesic interactions upon pain and activity measures. *Pharmacol Biochem Behav* **16:** 411–416, 1982.
10. Bodnar, R. J., M. M. Wallace, J. H. Kordower, A. L. Kirchgessner, D. Simone, K. Merrigan, J. Scalisi and M. Lattner. Analgesic responses to stress and opiates following selective destruction of either catecholaminergic, serotonergic or substance P pathways. *Soc Neurosci Abstr* **6:** 56, 1980.
11. Bossa, R., S. Ferri, I. Galatulas and A. Santagostino. Effects of antimitotic agents on morphine induced analgesia. *Prog Chemother* **3:** 992–994, 1974.
12. Charney, D. C., D. B. Menkes and G. R. Heninger. Receptor sensitivity and the mechanism of action of antidepressant treatment—Implications for the etiology and therapy of depression. *Arch Gen Psychiatry* **38:** 1160–1180, 1981.
13. Clements-Jewery, S. The development of cortical B-adreno-ceptor subsensitivity in the rat by chronic treatment with trazodone, doxepin and mianserine. *Neuropharmacology* **17:** 779–781, 1978.
14. Dahlstrom, A. and K. Fuxe. Evidence for the existence of monoamine-containing neurons in the central nervous system: I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiol Scand* **62:** 1–55, 1965.
15. Evans, W. O. A new technique for the investigation of some analgesic drugs on a reflexive behavior in the rat. *Psychopharmacologia* **2:** 318–325, 1961.
16. Frazer, A., M. E. Hess, J. Mendels, B. Gable, E. Kunkel and A. Bender. Influence of acute and chronic treatment with desmethylimipramine on catecholamine effects in the rat. *J Pharmacol Exp Ther* **206:** 311–319, 1978.
17. Goldstein, F. J., P. Mojaverian, M. H. Ossipov and B. N. Swanson. Elevation in analgetic effect and plasma levels of morphine by desipramine in rats. *Pain* **14:** 279–282, 1982.
18. Kametani, H., S. Nomura and J. Shimizu. The reversal effect of antidepressants on the escape deficit induced by inescapable shock in rats. *Psychopharmacology (Berlin)* **80:** 206–208, 1983.
19. Kitada, Y., T. Miyauchi, Y. Kanazawa, H. Nakamichi and S. Satoh. Involvement of a- and b1-adrenergic mechanisms in the immobility-reducing action of desipramine in the forced swimming test. *Neuropharmacology* **22:** 1055–1060, 1983.
20. Kitada, Y., T. Miyauchi, A. Satoh and S. Satoh. Effects of antidepressants in the rat forced swimming test. *Eur J Pharmacol* **72:** 145–152, 1981
21. Leshner, A. I., H. Remler, A. Biegon and D. Samuel. Desmethylimipramine (DMI) counteracts learned helplessness in rats. *Psychopharmacology (Berlin)* **66:** 207–208, 1979.
22. Liu, S. J. and R. I. H. Wang. Increased analgesia and alterations in distribution and metabolism of methadone by desipramine in rat. *J Pharmacol Exp Ther* **195:** 94–104, 1975.
23. Maj, J., Z. Rogoz, G. Skuza and H. Sowinska. Repeated treatment with antidepressant drugs potentiates the locomotor response to (−)-amphetamine. *J Pharm Pharmacol* **36:** 127–130, 1984.
24. Malseed, R. T. and F. J. Goldstein. Enhancement of morphine analgesia by tricyclic antidepressants. *Neuropharmacology* **18:** 827–829, 1979.
25. Platt, J. E. and E. A. Stone. Chronic restraint stress elicits a positive antidepressant response on the forced swim test. *Eur J Pharmacol* **82:** 179–181, 1982.
26. Porsolt, R. D., A. Bertin, N. Blavet, M. Deliel and M. Jalfre. Immobility induced by forced swimming in rats: effects of agents which modify central catecholamine and serotonin activity. *Eur J Pharmacol* **57:** 201–210, 1979.
27. Porsolt, R. D., M. Le Pichon and M. Jalfre. Depression: a new animal model sensitive to antidepressant treatments. *Nature* **266:** 730–732, 1977.
28. Reisine, T. and P. Soubire. Loss of rat cerebral cortical opiate receptors following chronic desipramine treatment. *Eur J Pharmacol* **77:** 39–44, 1982.
29. Sherman, A. D., G. L. Allers, F. Petty and F. A. Henn. A neuropharmacologically-relevant animal model of depression. *Neuropharmacology* **18:** 891–893, 1979.
30. Spiaggia, A., R. J. Bodnar, D. D. Kelly and M. Glusman. Opiate and nonopiate mechanisms of stress-induced analgesia: Cross-tolerance between stressors. *Pharmacol Biochem Behav* **10:** 761–765, 1979.
31. Stone, E. A. Stress and catecholamines. In: *Catecholamines and Behavior*, vol 2, edited by A. J. Friedhoff. New York: Plenum, 1975, pp. 31–72.
32. Stone, E. A. Subsensitivity to norepinephrine as a link between adaptation to stress and antidepressant therapy: an hypothesis. *Res Commun Psychol Psychiatr Behav* **4:** 241–255, 1979.
33. Stone, E. A. Reduction by stress of norepinephrine-stimulated accumulation of cyclic AMP in rat cerebral cortex. *J Neurochem* **32:** 1335–1337, 1979.
34. Stone, E. A. Reduction in cortical beta-adrenergic receptor density after chronic intermittent food deprivation. *Neurosci Lett* **40:** 33–37, 1983.
35. Stone, E. A. and J. E. Platt. Brain adrenergic receptors and resistance to stress. *Brain Res* **237:** 405–414, 1982.
36. Stone, E. A., J. E. Platt, R. Trullas and A. V. Slucky. Reduction of the cAMP response to NE in rat cerebral cortex following repeated restraint stress. *Psychopharmacology (Berlin)* **82:** 403–405, 1984.
37. Sulser, F. Functional aspects of the norepinephrine receptor coupled adenylate cyclase system in the limbic forebrain and its modification by drugs which precipitate or alleviate depression: molecular approaches to an understanding of affective disorders. *Pharmakopsychiatrie* **11:** 43–52, 1978.
38. Sulser, F., D. D. Gillespie, R. Mishra and D. H. Manier. Desensitization by antidepressants of central norepinephrine receptor systems coupled to adenylate cyclase. *Ann NY Acad Sci* **430:** 91–101, 1984.
39. Vetulani, J., R. J. Stawarz, J. V. Dingell and S. Sulser. A possible common mechanism of action of antidepressant treatments. *Naunyn Schmiedebergs Arch Pharmacol* **293:** 109–114, 1976.
40. Vetulani, J., R. J. Stawarz and F. Sulser. Adaptive mechanisms of the noradrenergic cyclic AMP generating system in the limbic forebrain of the rat: adaptation to persistent changes in the availability of norepinephrine (NE). *J Neurochem* **27:** 661–666, 1976.
41. Wolfe, B. B., T. K. Harden, J. R. Sporn and P. B. Molinoff. Presynaptic modulation of beta adrenergic receptors in rat cerebral cortex after treatment with antidepressants. *J Pharmacol Exp Ther* **207:** 446–457, 1978.