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BRIEF COMMUNICATION

Chronic Desipramine Attenuates Morphine Analgesia

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O'NEILL, K. A. AND D. VALENTINO. *Chronic desipramine attenuates morphine analgesia.* PHARMACOL BIOCHEM BEHAV **24**(1) 155–158, 1986.—Two experiments were conducted to explore the effects of chronic antidepressant treatment on endogenous opioid systems. In the first study, mice received desipramine for 21 days, a regimen which down-regulates beta-adrenergic receptors [13]. Subsequently, hotplate jump latencies were measured after acute saline, morphine or naloxone, to test for dynamic changes in endogenous opioid systems. Chronic desipramine treatment resulted in a significant attenuation of morphine analgesia, but had no effect on latencies of saline and naloxone treated mice. In the second experiment, naltrexone or propranolol were given with desipramine for 21 days, in an attempt to block the development of subsensitivity to morphine. Naltrexone had no effect on desipramine attenuation of morphine analgesia. Propranolol given with desipramine slightly lowered jump latencies of acute saline controls, resulting in a significant analgetic effect of morphine. These data suggest that attenuation of morphine analgesia by chronic desipramine treatment may be mediated by actions on noradrenergic systems, rather than direct effects on opioid receptors.

Antidepressants Analgesia Desipramine Opioid

SEVERAL reports indicate that antidepressant drugs may exert influences on endogenous opioid systems. Acute administration of antidepressants produces analgesia in a variety of pain assays [1, 8, 14]. Antidepressants also potentiate the analgesic effects of morphine given systemically [5,7] or into the periaqueductal gray [11], suggesting an interaction between antidepressants and opioid systems with the CNS.

However, the effect of chronic antidepressant treatment on opioid systems has not been fully explored. Because chronic administration of antidepressants is necessary for clinical efficacy, it is of interest to investigate opioid system responses to chronic treatment. The possibility that opioid subsensitivity may occur as a result of chronic antidepressant treatment has been suggested by the finding that ^{3}H-naloxone binding is significantly reduced in rat cortex after 21 days of desipramine treatment [13].

Therefore, the purpose of these experiments was to examine the effect of chronic desipramine treatment on pain sensitivity in mice treated acutely with morphine or saline. If subsensitivity of opioid systems develops as a function of chronic desipramine treatment, then animals receiving desipramine chronically should show attenuated responses to morphine. In order to elucidate the mechanisms of desipramine's actions on opioid systems, the effects of opiate and beta-adrenergic antagonists on desipramine induced opioid subsensitivity were also examined.

METHOD

CD-1 male mice (Charles River) weighing 14–17 g at the start of chronic drug treatment, served as subjects. Mice were housed 17 per cage, under standard laboratory conditions, with food and water available ad lib.

Analgesia was assessed using an automated hotplate apparatus which has been described in detail elsewhere [9]. Briefly, the apparatus consisted of two thermostatically controlled water baths, each of which heat a stainless steel plate on which 15 Plexiglas chambers rest. A Plexiglas top covers each chamber, preventing the animal from escaping. Jump latencies in sec were electronically recorded and printed by an AIM minicomputer. In these experiments, the plates were maintained at 50°C. Mice that did not jump within 300 sec were removed, and their latencies were recorded as 301 sec. This method was chosen since it is sensitive to endogenous opioid influences [9,10].

For 21 days, mice received desipramine (10 or 15 mg/kg) or saline, b.i.d., by gavage with 20 gauge stainless steel oral needles, at approximately 9 a.m. and 5 p.m. Twenty-six hours after the last chronic injection, mice were treated with saline, morphine (3.2 mg/kg, SC) or naloxone (2 mg/kg, SC). Thirty min after morphine and fifteen min after naloxone and saline injections, mice were tested for latency to jump on the 50°C hotplate.

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TABLE 1

EFFECT OF CHRONIC DESIPRAMINE TREATMENT ON MORPHINE ANALGESIA

	Chronic Treatment		
		Desipramine	
Acute Treatment	Saline	10 mg/kg	15 mg/kg
Saline	83.8 ± 13.08	83.7 ± 9.49	91.2 ± 21.16
Morphine	194.5 ± 24.98*	95.8 ± 16.80†	157.7 ± 25.04*
Naloxone	76.6 ± 25.30	55.8 ± 11.50	39.3 ± 8.77

Data are expressed as means and standard errors of hotplate jump latencies, in seconds. For chronic treatments, drugs were given twice daily, for 21 days. Acute treatments were given 26 hours after the final injection of the chronic treatment schedule. N=8–10 per group. Statistical significance was determined by Newman-Keuls tests.

*Significantly greater than respective acute saline treated controls, which received the same chronic treatment, $p<0.05$.

†Significantly less than chronic saline, acute morphine group and chronic desipramine (15 mg/kg), acute morphine group, $p<0.05$.

TABLE 2

EFFECT OF CHRONIC DESIPRAMINE AND ANTAGONIST TREATMENT ON MORPHINE ANALGESIA

Chronic Treatments		Acute Treatments	
Anti-depressant	Antagonist	Saline	Morphine
Saline	Saline	81.6 ± 11.59	228.1 ± 23.01*
	Naltrexone	114.7 ± 22.16	185.0 ± 28.13*
	Propranolol	93.5 ± 17.59	187.8 ± 21.73*
Desipramine (10 mg/kg)	Saline	116.0 ± 23.19	149.6 ± 11.17†
	Naltrexone	99.9 ± 15.07	113.7 ± 12.43‡
	Propranolol	57.1 ± 10.39	159.3 ± 29.45*†

Data are expressed as means and standard errors of hotplate jump latencies, in seconds. For chronic treatments, drugs were given twice daily, for 21 days. Acute treatments were given 26 hours after the final injection of the chronic treatment schedule. N=9–10 per group. Statistical significance was determined by Newman-Keuls tests.

*Significantly greater than respective acute saline control in left column, $p<0.05$.

†Significantly less than chronic saline—saline, acute morphine, $p<0.05$.

‡Significantly less than chronic saline—naltrexone, acute morphine, $p<0.05$.

In a second experiment, mice were injected orally, b.i.d., as described above, with desipramine (10 mg/kg) or saline, either alone, or in combination with naltrexone (2 mg/kg) or propranolol (10 mg/kg). Twenty-six hours after the last injection, mice from each group were injected SC with morphine (3.2 mg/kg) or saline, and tested 30 min later for latency to jump on a 50°C hotplate.

The drugs used were desipramine HCl (USV), morphine sulfate, naloxone HCl and naltrexone HCl (DuPont) and propranolol HCl (Ayerst). All drugs were dissolved in saline, and doses refer to the salts.

Data were analyzed by factorial analysis of variance. Significant main effects were further analyzed by post-hoc Newman-Keuls tests, and significant interactions of main effects were tested by simple main effects analysis of variance. Differences between individual groups were tested by Newman-Keuls tests.

Results of Experiment One

There was a significant effect of both chronic, $F(2,78)=3.49$, $p<0.05$, and acute, $F(2,78)=18.81$, $p<0.001$, treatments on hotplate jump latencies. However, there was no significant interaction between chronic and acute treatments. Thus, chronic desipramine treatment at 10 mg/kg significantly decreased jump latencies (when analyzed across all acute treatments), compared to chronic saline treated controls ($p<0.05$). Further, acute morphine treatment significantly increased jump latencies (across all chronic treatment groups), compared to acute saline ($p<0.05$) or naloxone ($p<0.01$) treated groups. Chronic desipramine administration at 15 mg/kg had no significant effect on jump latencies ($p>0.05$). There was also no significant difference between the latencies of acute saline and naloxone treated groups ($p<0.05$), indicating that under these conditions, naloxone did not have a hyperalgesic effect. (See Table 1.)

Mice given an acute injection of morphine after chronic desipramine (10 mg/kg) had significantly lower jump latencies than those treated with chronic saline ($p<0.01$) or chronic desipramine at 15 mg/kg ($p<0.05$). Chronic desipramine (10 mg/kg) had no significant effect on latencies of mice treated acutely with naloxone or saline. Thus, chronic administration of desipramine at 10 mg/kg attenuated the analgesic effect of morphine without altering the latencies of saline or naloxone treated mice.

Results of Experiment Two

Although chronic desipramine, $F(1,107)=8.66$, $p<0.005$, and acute, $F(1,107)=45.91$, $p<0.001$, treatments had significant effects on jump latencies, the chronic administration of naltrexone with desipramine had no significant effect, $F(2,107)=0.3214$, $p>0.05$. Further, there was a significant interaction between chronic desipramine treatment and acute treatments, $F(1,107)=4.83$, $p<0.05$. Analysis of this interaction revealed that (across all antagonist treatments) chronic desipramine treatment significantly reduced the jump latencies of mice which received acute morphine, $F(1,107)=4.24$, $p<0.05$, but had no effect on latencies of control animals which received saline, $F(1,107)=0.114$, $p>0.05$. Thus, chronic desipramine at this dose (10 mg/kg, b.i.d.) attenuated morphine analgesia without affecting the jump latencies of control animals, confirming the results of the first experiment.

As shown in Table 2, morphine significantly increased jump latencies of mice chronically treated with saline ($p<0.01$), saline and naltrexone ($p<0.05$) and saline and propranolol ($p<0.01$). In contrast, morphine had no significant effect on the jump latencies of groups which received

chronic desipramine, either alone or with naltrexone. Acute injections of morphine apparently increased the jump latencies of mice treated with chronic desipramine and propranolol ($p<0.01$), compared to the group which received chronic desipramine and propranolol and acute saline. However, this result may be artefactual, due to the low latencies of the control group. The latencies of this control group did not differ significantly from those of the other acute saline controls.

DISCUSSION

The results of these experiments show that chronic desipramine treatment (10 mg/kg, b.i.d.) significantly attenuated the acute analgesic effect of morphine, without affecting the nociceptive responses of acute saline or naloxone treated animals. Chronic treatment with a higher dose of desipramine (15 mg/kg, b.i.d.) also reduced the analgesic action of morphine, although this effect was not statistically significant. The reason for the ineffectiveness of the higher dose is unclear, and possible causes are discussed below.

There are a variety of mechanisms by which desipramine might influence the effect of opiates. Of particular concern is the possibility of metabolic interactions, since acutely, desipramine elevates plasma morphine levels [2] by competitive inhibition of metabolism [6], resulting in an enhancement of morphine analgesia. In the present studies, morphine analgesia was attenuated by chronic desipramine treatment. This is the opposite of the results produced by metabolic interactions. Further evidence against the influence of metabolic effects on the results reported here is provided by investigations employing central administration of morphine, which avoids the confounding influence of peripheral metabolic effects. Using this method, chronic desipramine (or clomipramine) treatment significantly attenuated the analgetic effect of morphine injected into the periaqueductal gray [4]. These data suggest that desipramine exerts influences on the CNS which result in subsensitivity to morphine.

Thus, while metabolic influences do not appear to be involved in desipramine induced subsensitivity to morphine, the results obtained with a chronic dose of 15 mg/kg b.i.d. of desipramine may be due to a metabolic interaction. After morphine treatment, mice which received this higher dose of desipramine chronically had lower jump latencies than those animals which were treated chronically with saline. This effect was not statistically significant, however. One can speculate that after the higher dose of desipramine (30 mg/kg/day), the one day washout period was insufficient, and the results may reflect an acute metabolic interaction between morphine and residual desipramine, which overcame the opiate subsensitivity observed after chronic desipramine treatment with a lower dose.

Since morphine is a direct agonist at opiate receptors, it is likely that desipramine induced attenuation of morphine analgesia is related to a change in the affinity and/or number of opiate receptors. Indeed, significant reductions in opiate receptors have been observed after chronic desipramine treatment [13]. Such changes in opiate receptors may occur in two ways. Desipramine might exert a direct action at the receptor, or may act indirectly by influencing other transmitter systems, which in turn act on opioid systems. This idea gains further support from the data presented here, which show that chronic blockade of opiate receptors by naltrexone had no effect on desipramine induced attenuation of morphine analgesia.

Desipramine is known to exert influences on several CNS neurotransmitter systems. Chronic desipramine treatment produces a significant reduction in beta-adrenergic receptors [15], and this is thought to be a therapeutically important action. In addition, since acute morphine administration increases central noradrenergic activity [12], it is conceivable that this effect of desipramine might be related to the attenuation of morphine analgesia. To evaluate this hypothesis, propranolol was given chronically with desipramine in the second experiment. Chronic treatment with saline and propranolol had no significant effect on jump latencies. In mice thus treated, acute morphine administration produced significant analgesia, while acute saline had no effect. Thus, chronic blockade of beta-adrenergic receptors alone does not appear to interfere with nociceptive processing. In contrast, when desipramine and propranolol were given together for 21 days, a nonsignificant reduction in control (acute saline) latencies was observed. When given morphine acutely, mice in this group showed significantly less analgesia than the positive controls (chronic saline—acute morphine). However, the latencies were significantly higher than the respective saline control group (chronic desipramine and propranolol—acute saline). This difference may be artefactual, and reflect the relatively low latencies of the acute saline control group (which did not differ significantly from other acute saline controls). Alternatively, this result may indicate the involvement of noradrenergic systems in desipramine induced attenuation of morphine analgesia.

Further investigation is necessary to determine the role of noradrenergic systems in nociceptive processing, and interactions with opioid systems. The interactions suggested by these and other data indicate that reciprocal actions between these systems may be involved in various CNS disorders, such as affective disorders and chronic pain syndromes.

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REFERENCES

1. Eschalier, A., J. L. Montrastruc, J. L. DeVoize, F. Rigal, G. Gaillard-Plaza and J. C. Pechardre. Influence of naloxone and methysergide on the analgesic effect of clomipramine in rats. *Eur J Pharmacol* **74:** 1–7, 1981.
2. Goldstein, F. J., P. Mojoverian, M. H. Ossipov and B. N. Swanson. Elevation in analgetic effect and plasma levels of morphine by desipramine in the rat. *Pain* **14:** 279–282, 1982.
3. Hall, H. and S. O. Ogren. Effects of antidepressant drugs on different receptors in the brain. *Eur J Pharmacol* **70:** 393–407, 1982.
4. Kellstein, D. E., R. T. Malseed and F. J. Goldstein. Contrasting effects of acute vs. chronic tricyclic antidepressant treatment on central morphine analgesia. *Pain* **20:** 323–334, 1984.

5. Lee, R. L. and P. S. J. Spencer. Effect of tricyclic antidepressants on analgesic activity in laboratory animals. *Postgrad Med J* **56:** Suppl 1, 19–24, 1980.
6. Lui, S. J. and R. I. H. Wang. Increased analgesia and alterations in distribution and metabolism of methadone by desipramine in the rat. *J Pharmacol Exp Ther* **195:** 94–104, 1975.
7. Malseed, R. T. and F. J. Goldstein. Enhancement of morphine analgesia by tricyclic antidepressants. *Neuropharmacology* **18:** 827–829, 1979.
8. Ogren, S. O. and A. C. Holm. Test specific effects of the 5-HT uptake inhibitors alaproclate and zimelidine on pain sensitivity and morphine analgesia. *J Neural Transm* **47:** 253–271, 1980.
9. O'Neill, K. A., C. Courtney, R. Rankin and A. Weissman. An automated, high capacity method for measuring jump latencies on a hotplate. *J Pharmacol Methods* **10:** 13–18, 1983.
10. O'Neill, K. A., C. W. Scott and A. Weissman. Naloxone enhances nociceptive responding. *Soc Neurosci Abstr* **9:** 274, 1983.
11. Ossipov, M. H., R. T. Malseed and F. J. Goldstein. Augmentation of central and peripheral morphine analgesia by desipramine. *Arch Int Pharmacodyn Ther* **259:** 222–229, 1982.
12. Reigle, T. G., G. S. Wilhait and M. J. Moore. Analgesia and increases in limbic and cortical MOPEG-SO_4 produced by periaqueductal gray injections of morphine. *J Pharm Pharmacol* **34:** 496–498, 1982.
13. Reisine, T. and P. Soubrie. Loss of rat cerebral cortical opiate receptors following chronic desipramine treatment. *Eur J Pharmacol* **77:** 39–44, 1982.
14. Speigel, K., R. Kalb and G. W. Pasternak. Analgesic activity of tricyclic antidepressants. *Ann Neurol* **13:** 462–465, 1983.
15. Vetulani, J., R. J. Stawarz, J. V. Dinghell and F. Sulser. A possible common mechanism of action of antidepressant treatments. Reduction in the sensitivity of the noradrenergic cyclic AMP generating system in the rat limbic forebrain. *Naunyn Schmiedeburgs Arch Pharmacol* **292:** 109–114, 1976.