The Effects of Different Monoaminergic Antidepressants on the Analgesia Induced by Spinal Cord Adrenal Medullary Transplants in the Formalin Test in Rats

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We studied the effects of chronic intraperitoneal administration of antidepressants on the antinociception induced by adrenal medullary transplants into the subarachnoid space in rats using the formalin test. Administration of drugs started 28 days after operation and the formalin test was performed on Day 56. When amitriptyline (AMT; 15 $mg \cdot kg^{-1} \cdot day^{-1}$) was administered to sham-operated rats, it decreased the licking time and increased the transplant-induced analgesia in Phase 1 when administered to transplanted rats. Chronic treatment with fluvoxamine (FVX, 10 $mg \cdot kg^{-1} \cdot day^{-1}$) had no influence on the licking response in sham rats, nor did it modify the transplant-induced analgesia when administered to transplanted rats. When desipramine (DMI; 10 $mg \cdot kg^{-1} \cdot day^{-1}$) was administered to sham rats, it significantly reduced the licking response in Phase 1, but when administered to transplanted rats it did not increase the transplant-induced analgesia. None of the drugs administered showed any effect on Phase 2 of the formalin test. These results suggest that adrenal medullary transplants into the spinal cord induce analgesia as determined by the formalin test. This effect is more pronounced when AMT (a nonselective noradrenaline-serotonin reuptake inhibitor) is chronically administered, but not when FVX or DMI are chronically administered.

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The implantation of adrenal chromaffin cells into the regions of the central nervous system responsible for pain modulation induces antinociception in acute and chronic pain animal models (1). Furthermore, the implantation of chromaffin cells into the subarachnoid space in order to alleviate cancer-related chronic pain is promising (2). Several mechanisms have been suggested to account for those analgesic effects. Transplanted adrenal medullary tissue provokes the release of both opioids and monoamines, increasing the cerebrospinal fluid concentrations of these products (3,4). Since opioids and monoamines are the main mediators of the endogenous antinociceptive system, the pharmacological manipulations directed to facilitate the action of opioids and/or monoamines should induce a facilitation of the transplant antinociceptive effect.

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Antidepressants (ADs) induce antinociception in both animals and in humans (5). In a previous study (6), we showed that chronic amitriptyline (AMT) treatment increased and prolonged the analgesia induced by adrenal medullary transplants into the subarachnoid space in rats using the tail-flick test as a nociceptive model. This suggests that AMT could be a suitable adjuvant to enhance the analgesia elicited by adrenal medullary transplants or by implants of adrenal chromaffin cells into the subarachnoid space.

ADs can either enhance or attenuate opioid analgesia. Differences in the mechanisms of action of the ADs used could account for these discrepancies (7,8). Thus, the aim of the present work was to study the effects elicited by three ADs which produce different biochemical effects on the analgesia induced by transplants of adrenal medulla into the rat spinal subarachnoid space in the formalin test. Three different ADs were used: fluvoxamine (FVX, a selective 5-hydroxytryptamine [5-HT] reuptake inhibitor) desipramine (DMI, a mainly noradrenaline [NA] reuptake inhibitor) and AMT (a mixed NA/5-HT reuptake inhibitor).

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Methods

All the experimental procedures were performed according to the ethical guidelines for investigations of experimental pain in conscious animals (9). The experimental protocol was approved by the Local Committee for Animal Experimentation of the Faculty of Medicine of the University of Cádiz (license 079604). Male Wistar rats (225–250 g at the beginning of the experiment) supplied by our University's reproduction laboratory were used. They were housed in groups of five and allowed to adapt to the animal room for at least 1 wk prior to use. Rats were either sham-operated (Sham) or autotransplanted (Trans). Then, animals from both groups were randomly allocated to four subgroups to receive either one AD or saline (SS) for 28 days. Postoperatively, animals were periodically weighed and observed for signs of illness and/or neurological deficits. Rats presenting any sign of illness (5 of 64) were excluded from the study. The formalin test was performed 24 h after the last dose of AD.

The drugs used were AMT (Sigma Chemical Co., St. Louis, MO) (15 mg/kg), FVX (Duphar, Madrid, Spain) (10 mg/kg), and DMI (Sigma) (10 mg/kg). All drugs were dissolved in 0.9% sterile SS and injection volume was 1 mL/kg. They were daily administered intraperitoneally (IP) between 9:00 and 11:00 AM during a 28-day period. At these doses, DMI acts as a selective NA reuptake inhibitor, FVX as a selective 5-HT reuptake inhibitor while in the case of AMT, the reuptake of both monoamines is affected at a 0.6 NA/5-HT ratio (10).

The autotransplant procedure was performed as described previously (6,11). Rats were anaesthetized with chloral hydrate (420 mg/kg) administered IP. Access to the lumbar subarachnoid space was obtained via a dorsal incision and a lumbar laminectomy. The dorsal incision was also used to have access to the left adrenal gland via an incision through the left and posterior abdominal wall. The adrenal gland was dissected out and placed in a Petri dish with 0.9% sterile SS. Then, the adrenal medulla was isolated using a small pair of forceps. It was subsequently implanted into the subarachnoid lumbar space through an incision of the duramater.

The dissection of the adrenal medulla and its subarachnoid implantation was performed under microscopic guidance (Opmi-6 from Zeiss®). The duration of the procedure (around 20 min/rat) and the size of the transplanted tissue were uniform in all animals. Sham operations were performed under the same conditions but the adrenal medulla was not implanted into the subarachnoid space. No inert material was inserted in sham rats, inasmuch as in previous experiments performed in our laboratory we discovered that the insertion of inert material into the lumbar subarachnoid space does not induce significant changes when compared with unoperated control rats (unpublished results). Rats were then left to recover and were included in the experimental groups described below. To prevent infection, every procedure was performed with sterilized materials and the surgical plane was treated with disinfectant (povidone-iodine).

Fifty-nine rats were assigned to the experiment. Thirty of them were Sham and 29 Trans. Twenty-eight days after surgery they were chronically treated either with AMT, FVX, DMI, or SS. Thus, the two initial groups became eight: Sham + SS ($n = 8$), Trans + SS ($n = 8$), Sham + AMT ($n = 6$), Trans + AMT ($n = 5$), Sham + FVX ($n = 8$), Trans + FVX ($n = 8$), Sham + DMI ($n = 8$), and Trans + DMI ($n = 8$). Twenty-eight days later (i.e., 56 days after transplant) the formalin test was performed. This scheduled procedure for ADs administration (i.e., starting chronic AD treatment 28 days after surgery) was chosen in order to avoid 2 mo of daily IP administration, which could have induced excessive suffering and damage to the animal.

Experiments were performed at 21 ± 1°C in an environment-controlled quiet room. Animals were single-caged and allowed to habituate to the testing environment for 20 to 30 min the day before the test. The formalin test consisted of the following procedure: the plantar region of the right hind paw was injected with 100 μL of 5% formaldehyde (12). Each animal's behavior was recorded by video tapes for 1 h after formalin injection. The summed duration of licking behavior was calculated for the first 5 min (Phase 1 response) and for the following 45 min (Phase 2 response) by a blinded investigator (AOA and/or MMF). No restraint was applied to the rat during behavioral observations.

Results are expressed as the mean ± SE value of licking time (seconds) of number of rats. For statistical analysis, AD treatments were analyzed independent of one another using a two-factors analysis of variance (ANOVA). Factors of variation were treatment (i.e., SS or one AD) and surgery (i.e., sham or transplanted rats). For every AD, differences between groups were analyzed using the Duncan test after significant main effects by one-way ANOVA. A P value of <0.05 was considered as significant. With this statistical analysis, comparison between different AD treatments is not possible, and no assumption could be made regarding whether one antidepressant was more effective than another. Therefore, the only effect analyzed was the effect of each AD treatment on the transplant-induced analgesia.

Results

Formalin injection into the plantar region of the hind paw in naive rats produced a licking response of the

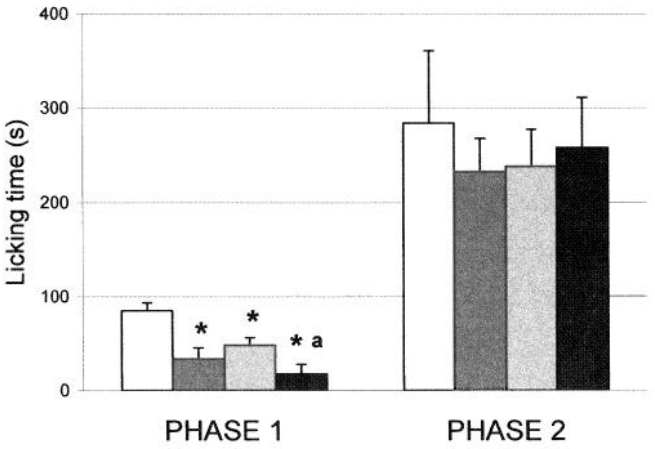

Figure 1. The effect of amitriptyline (AMT) by itself and on transplant-induced analgesia on the licking response induced by formalin at Phases 1 and 2. Sham-operated (Sham), Transplant (Trans), saline (SS). □ SHAM + SS; ▨ SHAM + AMT; ▨ TRANS + SS; ■ TRANS + AMT. * $P < 0.05$ compared with SHAM + SS; a $P < 0.05$ compared with TRANS + SS.

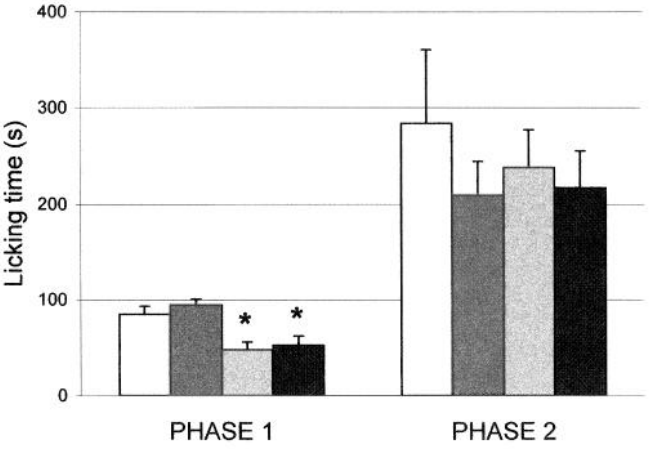

Figure 2. The effect of fluvoxamine (FVX) by itself and on transplant-induced analgesia on the licking response induced by formalin at Phases 1 and 2. Sham-operated (Sham), Transplant (Trans), saline (SS). □ SHAM + SS; ▨ SHAM + FVX; ▨ TRANS + SS; ■ TRANS + FVX. * $P < 0.05$ compared with both SHAM + SS and SHAM + FVX.

injected limb. The time course of that response consisted of two phases as previously described. The effect of both adrenal medullary transplants and chronic treatment with different ADs on the formalin-induced pain was examined measuring the licking response duration of these two phases.

The Effect of Chronic Treatment with AMT on Transplant-Induced Analgesia

In Phase 1 of the formalin test results obtained by two-factors ANOVA revealed a significant effect of the transplant ($F_{(1,26)} = 9.13$; $P < 0.01$) and the treatment with AMT ($F_{(1,26)} = 18.14$; $P < 0.001$) while both factors interaction was not significant (NS) ($F_{(1,26)} = 1.13$; NS). Subsequent one-way ANOVA showed significant differences between groups ($F_{(3,23)} = 9.33$; $P < 0.001$). The Duncan test *post hoc* comparison revealed that the transplant itself induced a significant analgesic effect when compared with sham rats receiving SS (Sham + SS versus Trans + SS = $P < 0.05$). AMT itself decreased the licking time in the first phase (Sham + SS versus Sham + AMT = $P < 0.05$). When AMT was administered to transplanted rats the analgesic effect was greater than the one observed in transplanted rats receiving SS (Trans + SS versus Trans + AMT = $P < 0.05$) (Fig. 1).

In Phase 2 of the formalin test results obtained by two-factors ANOVA revealed that neither surgery (transplant or sham) ($F_{(1,26)} = 0.08$; NS) nor treatment (SS or AMT) ($F_{(1,26)} = 0.09$; NS) induced a significant modification in licking behavior. No significant interaction was observed between both factors ($F_{(1,26)} = 0.37$; NS) (Fig. 1).

The Effect of Chronic Treatment with FVX on Transplant-Induced Analgesia

In Phase 1 of the formalin test results obtained by two-factors ANOVA revealed a significant effect of the transplant ($F_{(1,31)} = 22.92$; $P < 0.001$) but not of the treatment with FVX ($F_{(1,31)} = 0.76$; NS). In neither factor was interaction significant. ($F_{(1,31)} = 0.09$; NS). Subsequent one-way ANOVA showed significant differences between groups ($F_{(3,28)} = 7.92$; $P < 0.001$). The Duncan test *post hoc* comparison revealed that the transplant itself induced a significant analgesic effect when compared with sham rats receiving SS (Sham + SS versus Trans + SS = $P < 0.05$). FVX itself did not modify the licking time in the first phase (Sham + SS versus Sham + FVX = NS). When FVX was administered to transplanted rats the analgesic effect was not different from the one observed in transplanted rats receiving SS (Trans + SS versus Trans + FVX = NS) (Fig. 2).

In Phase 2 of the formalin test results obtained by two-factors ANOVA revealed that neither surgery (transplant or sham) ($F_{(1,31)} = 0.14$; NS) nor treatment (SS or FVX) ($F_{(1,31)} = 0.89$; NS) induced a significant modification in licking behavior. No significant interaction was observed between both factors ($F_{(1,31)} = 0.28$; NS) (Fig. 2).

The Effect of Chronic Treatment with DMI on Transplant-Induced Analgesia

In Phase 1 of the formalin test results obtained by two-factors ANOVA revealed a significant effect of the transplant ($F_{(1,31)} = 4.29$; $P < 0.05$) and the treatment with DMI ($F_{(1,31)} = 8.3$; $P < 0.01$) while both factors interaction was not significant ($F_{(1,31)} = 1.97$;

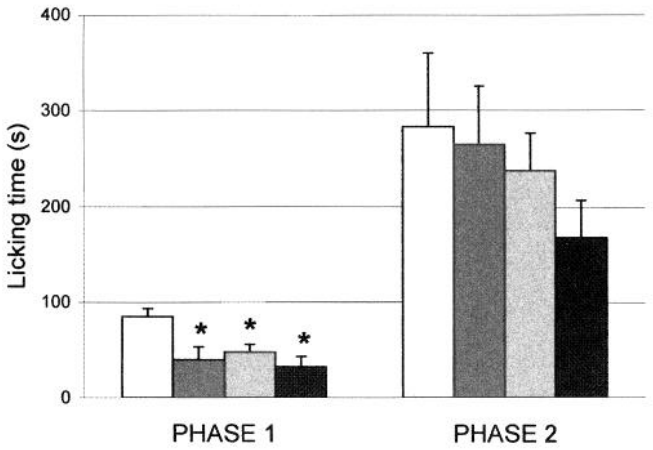

Figure 3. The effect of desipramine (DMI) by itself and on transplant-induced analgesia on the licking response induced by formalin at Phases 1 and 2. Sham-operated (Sham), Transplant (Trans), saline (SS). □ SHAM + SS; ▨ SHAM + DMI; ▩ TRANS + SS; ■ TRANS + DMI. * $P < 0.05$ compared with SHAM + SS.

NS). Subsequent one-way ANOVA showed significant differences between groups ($F_{(3,28)} = 4.85$; $P < 0.01$). The Duncan test *post hoc* comparison revealed that the transplant itself induced a significant analgesic effect when compared with sham rats receiving SS (Sham + SS versus Trans + SS = $P < 0.05$). DMI itself decreased the licking time in the first phase (Sham + SS versus Sham + DMI = $P < 0.05$). When DMI was administered to transplanted rats the analgesic effect was not different from that observed in transplanted rats receiving SS (Trans + SS versus Trans + DMI = NS) (Fig. 3).

In Phase 2 of the formalin test results obtained by two-factors ANOVA revealed that neither surgery (transplant or sham) ($F_{(1,31)} = 1.59$; NS) nor treatment (SS or DMI) ($F_{(1,31)} = 0.60$; NS) induced a significant modification in licking behavior. No significant interaction was observed between both factors ($F_{(1,31)} = 0.20$; NS) (Fig. 3).

Discussion

The effects of chronic treatment with three ADs which have different mechanisms of action (i.e., FVX, a selective 5-HT reuptake inhibitor; DMI, a mainly NA reuptake inhibitor; and AMT, a nonselective NA/5-HT reuptake inhibitor) on the antinociception induced by adrenal medullary transplants into the subarachnoid space of the rat spinal cord have been investigated. The present study confirms previously published data on animal antinociception induced by adrenomedullary transplants into the subarachnoid space (6). It also confirms these data in the case of the formalin test, which resembles chronic pain states in humans. This analgesic effect was potentiated by chronic treatment with AMT but not by chronic treatment with DMI or FVX. This chronic administration schedule was chosen because of the hypothesis that the therapeutic actions of ADs are merely caused by an acute monoamine reuptake inhibition—a hypothesis that has been questioned due to the observed effects of long-term administration of ADs. Current research is being shifted from studies on acute drug effects to studies on chronic administration (13).

The first and the second phases of the formalin test involve different nociceptive mechanisms (12). The second phase apparently implies an important inflammatory process (14), and a direct action of ADs on the mechanisms of inflammation has not yet been proved. These facts could explain the lack of effect on the second phase observed in this study. The capability of some drugs to modify one phase while the other remains unaltered has already been shown (14).

In our study, only chronic treatment with AMT or DMI, but not with FVX, induced an analgesic effect which suggests that, in accordance with Dennis and Melzack (15), there is a preferential implication of noradrenergic pathways mediating antinociception in the formalin test.

Only chronic AMT facilitated the analgesia induced by the transplant. Since DMI and FVX had influence on only one monoamine (NA in the case of DMI and 5-HT in the case of FVX), perhaps these two systems work synergistically, as in the case of AMT, to enhance and prolong posttransplant analgesia. But the inhibition of monoamine reuptake cannot be presented as the only factor responsible for the effects observed in this study. In fact, monoamine reuptake inhibition has been observed in acute administration, while after chronic administration of ADs, a wide set of biochemical effects (including changes in receptor number and changes in physiological and behavioral sensitivity to monoamine agonists and antagonists) has been observed (13).

The different interactions of the ADs used with the opioid system could also account for the differences observed in the facilitation of the transplant-induced analgesia. AMT increases opiate-induced analgesia (16), decreases morphine tolerance when chronically administered (17), and increases both opioid peptide levels and opioid receptor densities in the spinal cord (18). Furthermore, its analgesic effect can be antagonized by naloxone (19). DMI binds to opiate receptors and its acute analgesic effect can also be antagonized by naloxone (19). However, after chronic treatment, DMI attenuates morphine analgesia (8), probably due to a loss in opiate binding sites (20). FVX inhibits the binding of tritiated met-enkephalin to enriched synaptosomes (21), but there is no information about its effect on opiate analgesia.

Differences in the pharmacokinetics of the drugs used could also account for the different results obtained with these three ADs. The possibility that the dose chosen for each drug might be inadequate, and

that a single dose a day might be insufficient, cannot be ruled out.

In conclusion, this study shows that adrenal medullary transplants into the spinal cord induce analgesia in the formalin test. This effect is more pronounced when AMT is chronically administered but not when DMI or FVX are chronically administered, suggesting an implication of both NA and 5-HT systems in the enhancement of posttransplant analgesia. Nevertheless, in agreement with previous reports, the influence of opioidergic mechanisms cannot be ruled out. Therefore, AMT administration after adrenal medullary transplant might provide an alternative approach to enhance and prolong the analgesia induced by the transplant alone. In addition to that, the mental depression frequently associated with chronic pain could also be ameliorated with this combined treatment. In fact, transplants of adrenal medullary tissue into the rat frontal cortex (22), or into the spinal subarachnoid space, induce a clear antidepressive effect (23). However, further research will be necessary in order to clarify the biochemical effect directly related to the analgesia observed in this study.

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References

1. Sagen J. Transplantation of cells or tissue into the spinal fluid to alleviate chronic pain symptoms. Drug News Perspect 1994;7:325–33.
2. Winnie AP, Pappas GD, Das Gupta TK, et al. Subarachnoid adrenal medullary transplants for terminal cancer pain. A report of preliminary studies. Anesthesiology 1993;79:644–53.
3. Sagen J, Kemmler JE. Increased levels of Met-enkephalin-like immunoreactivity in the spinal cord CSF of rats with adrenal medullary transplants. Brain Res 1989;502:1–10.
4. Sagen J, Kemmler JE, Wang H. Adrenal medullary transplants increase spinal cord cerebrospinal fluid catecholamine levels and reduce pain sensitivity. J Neurochem 1991;56:623–7.
5. Eschalier A, Mestre C, Dubray C, Ardid D. Why are antidepressants effective as pain relief? CNS Drugs 1994;2:261–7.
6. Ortega-Alvaro A, Gibert-Rahola J, Chover AJ, et al. Effect of amitriptyline on the analgesia induced by adrenal medullary tissue transplanted in the rat spinal subarachnoid space as measured by an experimental model of acute pain. Exp Neurol 1994;130:9–14.
7. Malseed RT, Goldstein FJ. Enhancement of morphine analgesia by tricyclic antidepressants. Neuropharmacology 1979;18:827–9.
8. O'Neill KA, Valentino D. Chronic desipramine attenuates morphine analgesia. Pharmacol Biochem Behav 1986;24:155–8.
9. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.
10. Hyttel J. Citalopram—pharmacological profile of a specific serotonin uptake inhibitor with antidepressant activity. Prog Neuropsychopharmacol Biol Psychiatry 1982;6:277–95.
11. González-Darder JM, Ruz-Franzi JI. Acción antálgica del trasplante de médula suprarrenal al espacio subaracnoideo lumbar. Estudio experimental. Rev Esp Anestesiol Reanim 1991;38:230–3.
12. Dubuisson D, Dennis SG. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine and brain stem stimulation in rats and cats. Pain 1977;4:161–74.
13. Cooper JR, Blomm FE, Roth RH. Dopamine. In: Cooper JR, Blomm FE, Roth RH, eds. The biochemical basis of neuropharmacology. New York: Oxford University Press, 1991:285–337.
14. Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103–14.
15. Dennis SG, Melzack R. Comparison of phasic and tonic pain in animals. In: Bonica JJ, Liebeskind JC, Albe-Fessard D, eds. Advances in pain research and therapy. New York: Raven Press, 1979:747–60.
16. Botney M, Fields HL. Amitriptyline potentiates morphine analgesia by a direct action on the central nervous system. Ann Neurol 1983;13:160–4.
17. Sewell RDE. Supraspinal and spinal monoamine-modified function and the expression of opioid antinociception. J Psychopharmacol 1991;5:352–9.
18. Hamon M, Gozlan H, Bourgoin S, et al. Opioid receptors and neuropeptides in the CNS in rats treated chronically with amoxapine or amitriptyline. Neuropharmacology 1987;26:531–9.
19. Valverde O, Micó JA, Maldonado R, et al. Participation of opioid and monoaminergic mechanisms on the antinociceptive effect induced by tricyclic antidepressants in two behavioral pain tests in mice. Prog Neuropsychopharmacol Biol Psychiatry 1994;18:1073–92.
20. Reisine T, Soubrie P. Loss of rat cerebral cortical opiate receptors following chronic desimipramine treatment. Eur J Pharmacol 1982;77:39–44.
21. Somoza E, Galindo A, Bazán E, et al. Antidepressants inhibit enkephalin binding to synaptosome-enriched fractions of rat brain. Neuropsychobiology 1981;7:297–301.
22. Dougherty DD, Sortwell CE, Sagen J. Pharmacologic specificity of antidepressive activity by monoaminergic neural transplants. Psychopharmacology [Berl] 1995;118:10–8.
23. Tejedor-Real P, Micó JA, Corbalán JP, et al. Attenuation of learned helplessness in rats after transplant of adrenal medulla into the spinal cord. Eur Psychiatry 1996;11:249–53.