*Korean J Physiol Pharmacol*
*Vol 2: 307-312, June, 1998*

# Intracisternal Antidepressants Suppressed the Nociceptive Jaw Opening Reflex in Freely Moving Rats

**Dong-Kuk Ahn and Yun-Sook Kim**

Department of Oral Physiology, School of Dentistry, Kyungpook National University, Taegu 700-422, Korea

This study was performed to investigate the mechanism of central analgesic effects of antidepressants. Thirty four male rats were anesthetized with pentobarbital sodium (40 mg/kg, ip). A stainless steel guide cannula and a PE tube (PE10) were implanted into the lateral ventricle and cisterna magna area. Stimulating and recording electrodes were implanted into the incisor pulp and anterior digastric muscle. Electrodes were led subcutaneously to the miniature cranial connector sealed on the top of the skull with acrylic resin. The jaw opening reflex was used in freely moving rats, and antidepressants were administered intracisternally in order to eliminate the effects of anesthetic agents on the pain assessment and evaluate the importance of the central action site of antidepressants. After 48 hours of recovery from surgery, digastric electromyogram (dEMG) of freely moving rats was recorded. Electrical shocks (200 μsec duration, 0.5-2 mA intensity) were delivered at 0.5 Hz to the dental pulp every 2 minute. Intracisternal administration of 15 μg imipramine suppressed dEMG elicited by noxious electrical stimulation in the tooth pulp to 76±6% of the control. Intracisternal administration of 30 μg desipramine, nortriptyline, or imipramine suppressed dEMG remarkably to 48±2, 27±8, or 25±5% of the control, respectively. Naloxone, methysergide, and phentolamine blocked the suppression of dEMG produced by intracisternal antidepressants from 23±2 to 69±4%, from 32±5 to 80±9%, and from 24±6 to 77±5% of the control, respectively. These results indicate that antidepressants produce antinociception through central mechanisms in the orofacial area. Antinociception of intracisternal antidepressants seems to be mediated by an augmentation of descending pain inhibitory influences on nociceptive pathways.

Key Words: Antidepressants, Jaw opening reflex, Antinociception, Freely moving rats

## INTRODUCTION

Antidepressants are known to be effective in the controlling pain of various origins in the clinic (Walsh, 1983), but the mechanism of their analgesic action has not been clearly established. In pharmacological studies with laboratory animals, systemic administration of antidepressants enhanced antinociception produced by opioid agonists, although antidepressants alone are frequently not effective against experimental nociception (Larson & Takemori, 1977; Malseed & Goldstein, 1979). Systemic antidepressants also produced analgesia in human study (Ardid & Guilbaud, 1992; Usaha Rani et al, 1996). These results indicate that antidepressants seem to be more effective in the treatment of chronic pain than in the treatment of depression. Antidepressants, however, have an analgesic effect in clinic, but there are several exceptions. Studies of the antinociceptive effect of antidepressants in animal experiments have also yielded conflicting results. Some reported antinociceptive effects of antidepressants (Butler et al, 1985; De Felipe et al, 1986), while others found no antinociceptive effects (Godefroy et al, 1986). These results imply that although antidepressants are used to relieve pain, whether pain is relieved by an analgesic effect, or by the treatment of a secondary,

Corresponding to: Dong-Kuk Ahn, Department of Oral Physiology, School of Dentistry, Kyungpook National University 101, Tong-In 2 ga, Chung-gu, Taegu 700-422, Korea. (Tel) 053-420-6995 (Fax) 053-421-4077, Email: dkahn@kyungpook.ac.kr

or masked depression seems to be unclear.

There are several evidences for the central antinociceptive effects of the antidepressants (Hwang & Wilcox, 1987; Lund et al, 1990). Sawynok and Reid (1992) have found that antidepressants administered intrathecally potentiated the antinociception induced by morphine, in a dose that alone showed no antinociceptive effect. These results suggest that antinociceptive action of antidepressants has central analgesic effects but no peripheral analgesic effects.

Antidepressants are also known to be effective in controlling postoperative pain after dental surgery. Levine et al (1986) reported that antidepressants suppressed pain for 6 hours immediately after dental surgery. Much work, however, has been done to elucidate the mechanism of the central analgesic action of antidepressants, but limited data is available concerning the central mechanisms of antidepressants on pain control in the orofacial area.

The aim of the present study was to examine the central effects of antidepressants on nociceptive jaw opening reflex after intracisternal injection of desipramine, nortriptyline, or imipramine in freely moving rats. On the other hand, we also investigated the mechanism of central antinociceptive action of intracisternal antidepressants.

## METHODS

Experiments were carried out in 34 male Sprague-Dawley rats (400~450 gm) which had stimulating electrodes implanted in the dental pulp, recording electrodes inserted into the anterior digastric muscle, and indwelling cannula implanted in the lateral ventricle and the cisterna magna area (Fig. 1). Surgical procedures were performed under pentobarbital sodium (40 mg/kg, ip). Anesthetized rats were mounted on a stereotaxic frame (1404, David Kopf Instruments). A stainless steel guide cannula (27 gauge) was implanted in the lateral ventricle. Coordinates were as follows, with flat skull position and bregma as the reference: A/P 0.8 to 1.0 mm, M/L 1.5 mm, and D/V 3.8 mm. A polyethylene tube (PE10) was inserted in the cisterna magna area after the exposure of the atlantooccipital membrane. A stainless steel guide cannula and PE 10 tube were secured in place by means of stainless steel screws and acrylic resin. A 48h recovery period from surgery was allowed before starting recording sessions.

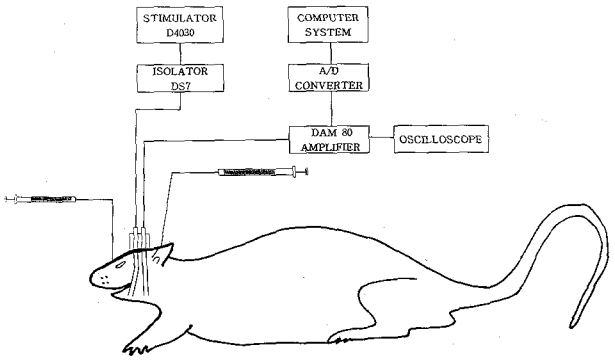


**Fig. 1.** Schematic of the basic experimental set up for recording the jaw opening reflex in freely moving rats. Stimulating electrodes implanted in the dental pulp, recording electrodes inserted into the anterior digastric muscle, and indwelling cannula implanted in the lateral ventricle and the cisterna magna area.

A pair of stimulating electrodes (150 μm in diameter) was inserted bilaterally into the lower incisor pulp. The electrodes were secured in place with dental acrylic resin. Electrical shocks (200 μsec, 0.5-2 mA intensity) were delivered at 0.5 Hz to the dental pulp. Intensity of stimulation was adjusted at 2~3 times thresholds for evoked digastric electromyogram (dEMG). At this range of stimulation and frequency, no consistent behavior responses apart from a jaw opening reflex arise. dEMGs were recorded from the anterior digastric muscle using a pair of recording electrodes inserted into the anterior belly muscle. Stimulating and recording electrodes were led to subcutaneously to a miniature cranial connector sealed on the top of the skull with acrylic dental resin. Electromyographic reflex responses were amplified (DAM 80, WPI) and fed to a computerized system for on-line digitization (CED 1401, CED). This procedure allowed reflex responses to be expressed as percentage of the control values. The amplitude of the reflex response following drug injection was expressed as percentage of the control values. For each trial, control responses were determined throughout the 3 min preceding the test period (60 stimulation). The mean control value was then calculated, and each individual reflex response was expressed as a percentage of this mean.

Antidepressants (desipramine, nortriptyline, or imipramine, 15 or 30 μg) was administered intracisternally in an attempt to examine central antinociceptive action of antidepressants. Drugs were microinjected

for 1 minute using a 30 gauge stainless steel injector that extended 1 mm beyond the end of the guide cannula. The drugs (30 μg/ 7μl) employed were naloxone, an opioid receptor antagonist, methysergide, a serotonin receptor antagonist, and phentolamine, an nonselective α-adrenergic receptor antagonist, and each of these was followed by an 8 μl flush of artificial cerebrospinal fluid (aCSF). Fifteen minutes later, the nociceptive jaw opening reflex was redetermined to assess whether these agents had altered the nociceptive response. Drugs dissolved in an aCSF were employed. The aCSF solution contained (in mM) NaCl 128, KCl 3, $CaCl_2$ 1.2, $MgCl_2$ 0.8, $NaH_2PO_4$ 0.25, $NaHCO_3$ 20, and glucose 3.4 (pH 7.4). At the end of each experiment, infusion sites were verified by conventional methods. Only data from rats with injection sites clearly within the lateral ventricle were used for final analysis.

## RESULTS

The results of the present study showed that antidepressants suppressed dEMG elicited by noxious electrical stimulation in the incisor (Fig. 2, 3). aCSF had no effects on the dEMG. After intracisternal injection of 15 μg imipramine, dEMG was decreased to 76±6% of the control. Intracisternal administra-

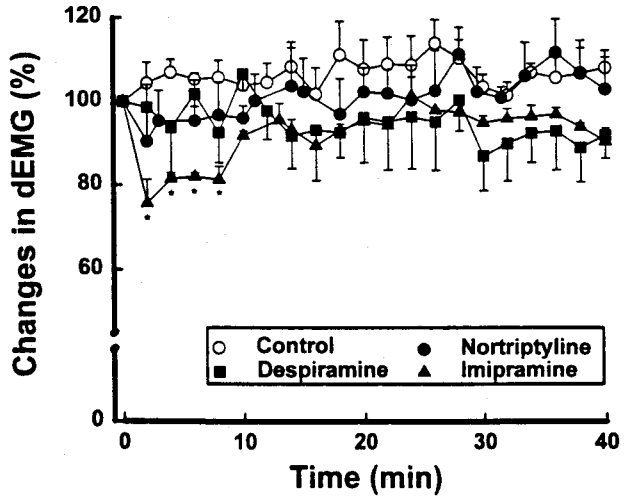


**Fig. 2.** The responses of digastric electromyogram (dEMG) produced by noxious electrical stimulation in the incisor to intracisternal injection (15 μg) of desipramine, nortriptyline, or imipramine. Number of experiments were 12. *p<0.05, vs. rest value.

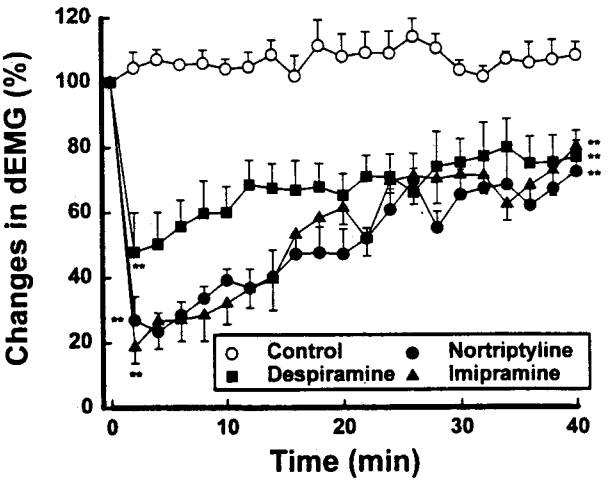


**Fig. 3.** The responses of digastric electromyogram (dEMG) produced by noxious electrical stimulation in the incisor to intracisternal injection (30 μg) of desipramine, nortriptyline, or imipramine. Number of experiments were 12. Stars represent a significant inhibition of dEMG as compared to rest values. **p<0.01. All points between two identical symbols have the same level of significance.

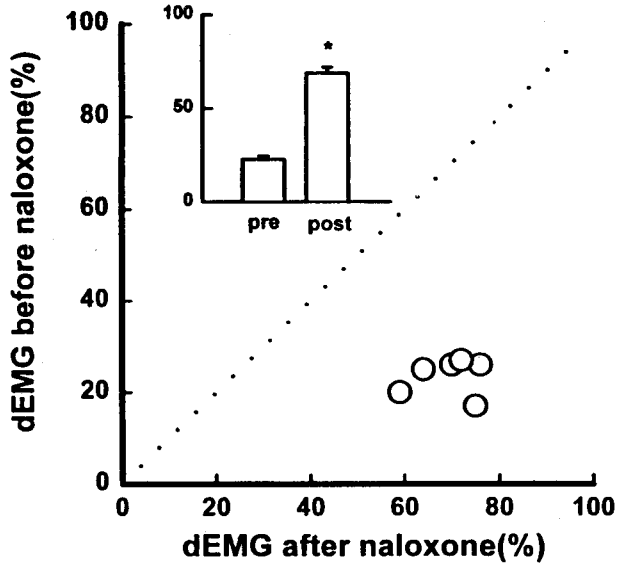


**Fig. 4.** Maximum effects of naloxone (30μg, ic) on the suppression of dEMG in response to intracisternal injection of imipramine (30 μg). *p<0.05, vs. before naloxone. Scatter diagram illustrates responses of dEMG (expressed as a percentage of the control) to injection of imipramine before and after naloxone. Dashed diagonal indicates equal inhibition. The inset in scatter diagram portrays the mean data before (pre) and after (post) the administration of naloxone.

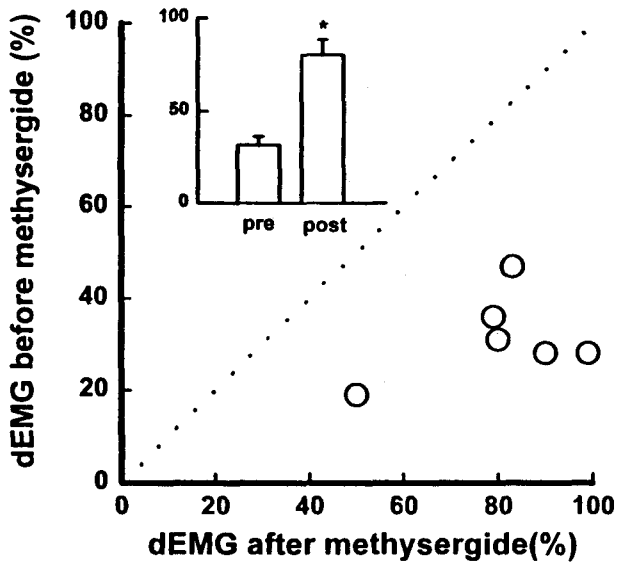


**Fig. 5.** Maximum effects of methysergide (30μg, ic) on the suppression of dEMG in response to intracisternal injection of imipramine (30 μg). *$p<0.05$, vs. before methysergide. Scatter diagram illustrates responses of dEMG (expressed as a percentage of the control) to injection of imipramine before and after methysergide. Dashed diagonal indicates equal inhibition. The inset in scatter diagram portrays the mean data before (pre) and after (post) the administration of methysergide.

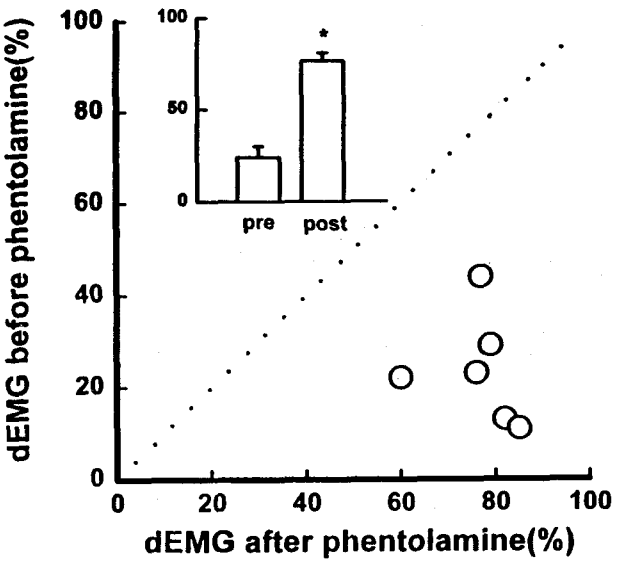


**Fig. 6.** Maximum effects of phentolamine (30 μg, ic) on the suppression of dEMG in response to intracisternal injection of imipramine (30 μg). * $p<0.05$, vs. before phentolamine. Scatter diagram illustrates responses of dEMG (expressed as a percentage of the control) to injection of imipramine before and after phentolamine. Dashed diagonal indicates equal inhibition. The inset in scatter diagram portrays the mean data before (pre) and after (post) the administration of phentolamine.

tion of 30 μg desipramine, nortriptyline, or imipramine suppressed dEMG remarkably to 48±2, 27±8, or 25±5% of the control, respectively. The suppression of dEMG was maintained for 40 minutes. We also examined the central mechanism of antidepressants after intracerebroventricular injection of naloxone, methysergide, or phentolamine (Fig. 4, 5, 6). Eighteen animals divided into three groups. Each point in scatter diagram represents one experiment, and the mean effect is summarized in the inset of the scatter diagram. We observed dEMG activity for 15 minutes after intracerebroventricular injection of naloxone, methysergide, or phentolamine. All drugs did not affect the resting dEMG activity at our dose. Naloxone, an opioid receptor antagonist, blocked the suppression of dEMG in response to intracisternal antidepressants from 23±2 to 69±4% of the control. Methysergide, a serotonin receptor antagonist and phentolamine, an α-adrenergic receptor antagonist, also blocked the suppression of dEMG from 32±5 to 80±9% and from 24±6 to 77±5% of the control, respectively.

## DISCUSSION

Jaw opening reflex, one of the withdrawal reflex in response to noxious stimuli, is used as a pain assessment method. Generally, jaw opening reflex is elicited by noxious stimulation in the orofacial area and is quantified by the magnitude of electromyogram from anterior belly of digastric or lateral ptyerigoid muscles in anesthetized animals (Ahn, 1995; Ahn et al, 1996; Ahn et al, 1991). Anesthetic agents influence on the results of pain assessment experiments. Therefore, jaw opening reflex have to be evaluated in conscious or freely moving rats to eliminate effects of anesthetic agents on pain assessment. Another significant problem with some previous studies has been the route of drug administration. Systemic administration made it difficult to determine the location of function in the central nervous system. In the present study, we examined central antinociceptive effects of antidepressants in freely moving rats. Intracisternal antidepressants suppressed dEMG remarkably under conditions of our experiments. These results indicate that antidepressants produced

antinociception through the central mechanisms in the orofacial area. This result is also supported by a previous study in which intrathecally administered antidepressants produced analgesic action in the rats (Hwang & Wilcox, 1987). On the other hand, others reported that systemic desipramine (2~25 mg/kg) had no effects in the tail flick test (Lund et al, 1989; Ossipov et al, 1982). These results may be caused by the differences between a route of administration or pain assessment methods.

It has been well documented that antidepressants block receptors of catecholamines and serotonin, or inhibit reuptake of catecholamines and serotonin (Frazer, 1997). There are several evidences for antidepressants to modulate the adrenergic and serotonergic pathways. Intravenous administration of antidepressants produced dose-dependent increase in the spontaneous firing rate of noradrenergic locus coeruleus neurons (VanderMaelen & Braselton, 1990). Interestingly, acute administration of antidepressants also produced the same effects (Hauser et al, 1985). Not only do antidepressants enhance noradrenergic transmission, but also they facilitate serotonergic transmission as evidenced by its dose-dependent increase in firing rate of serotonergic soma in the dorsal raphe nucleus (Haddjeri et al, 1996). These data indicate that antidepressants may facilitate noradrenergic and serotonergic transmission, even though the underlying mechanism by which they do so is unknown.

It has been well established that a number of brain stem nuclei, including the periaqueductal gray matter (PAG), nucleus raphe magnus (NRM), nucleus paragigantocellularis, and locus coeruleus (LC), play an important role in stimulation- produced analgesia (Basbaum and Fields, 1984; Carstens, 1987; Jensen and Gebhart, 1984). Focal electrical stimulation in these structures inhibited the responses of spinal dorsal horn neurons to noxious stimuli (Gerhart et al, 1984; Gray and Dostrovsky, 1983). Monoamines and opiates are also involved in the modulation of spinal nociceptive transmission. Intrathecal administration of norepinephrine, serotonin, or enkephalin at the level of the lumbar spinal cord produced profound antinociception (Reddy et al, 1980; Yaksh and Wilson, 1979; Yaksh et al, 1977). On the other hand, intrathecal administration of noradrenergic, serotonin, and opioid receptor antagonists attenuated stimulation produced descending inhibition (Hammond and Yaksh, 1984). Interestingly, these results indicate that there is an intimate interrelationship between the action sites of antidepressants and descending pain inhibitory nucleus. In the present study, methysergide, a serotonin receptor antagonist, and phentolamine, an adrenergic receptor antagonist, blocked the central antinociceptive action of antidepressants. Moreover, naloxone also inhibited the central antinociception of antidepressants. The present results strongly indicate that the analgesic effect of intracisternal antidepressants is mediated by an augmentation of descending pain inhibitory influences including nucleus raphe magnus and locus coeruleus that have serotonergic and adrenergic pathways.

In summary, antidepressants produced antinociception through the central mechanisms in the orofacial area. The central analgesic effects of antidepressants were attributed to an augmentation of descending inhibitory influences on nociceptive pathways, via multiple pathways such as opioid, serotonergic, and adrenergic pathways.

## ACKNOWLEDGEMENT

This paper was supported by the academic research fund of the Ministry of Education, Republic of Korea (97-227), 1997

## REFERENCES

Ahn DK, Kim KH, Yang EK, Kim HJ, Park JS, Lee WJ. Nucleus tractus solitarius-induced suppression of the jaw opening reflex and a possible interrelationship between antinociceptive and cardiovascular responses. *J Oral Biol* 20: 57−63, 1996

Ahn DK, Sung JH, Park JS, Lee WJ, Choo YE. Modulation of the nociceptive jaw opening reflex by injection of enkephalin, glutamate and GABA in PAG. *J Oral Biol* 15: 54−61, 1991

Ahn DK. Antinociceptive and cardiovascular response to chemical stimulation in the periaqueductal gray matter. *J Oral Biol* 19: 13−18, 1995

Ardid D, Guilbaud G. Antinociceptive effects of acute and chronic injection of tricyclic antidepressant drug in a new model of mononeuropathy in rats. *Pain* 49: 279−287, 1992

Basbaum AI, Fields HL. Endogenous pain control systems: brainstem spinal pathways and endorphin circuit. *Ann Rev Neurosci* 7: 309−338, 1984.

Butler SH, Weil-Fugazza J, Godefroy F, Besson JM. Reduction of arthritis and pain behavior following chronic administration of amitriptyline and imipramine

in rats with adjuvant-induced arthritis. *Pain* 23: 159–175, 1985

Carstens EE. Endogenous pain suppression mechanisms. *JAVMA* 191: 1203–1206, 1987.

De Felipe MC, De Ceballos ML, Fuentes JA. Hypoalgesia induced by antidepressants in mice: a case for opioids and serotonin. *Eup J Pharmacol* 125: 193–199, 1986

Frazer A. Pharmacology of antidepressants. *J Clinic Psychopaharmacol* 17: 2S–18S, 1997

Gerhart KD, Yeziersk, RP, Wilcox TK, Willis WD. Inhibition of primate spinothalamic tract neurons by stimulation in periaqueductal gray or adjacent midbrain reticular formation. *J Neurophysiol* 51: 450–466, 1984.

Godefroy F, Butler SH, Weil-Fugazza J, Besson JM. Do acute or chronic tricyclic antidepressants modify morphine antinociception in arthritic rat? *Pain* 25: 233–244, 1986

Gray BG, Dostrovsky JO. Descending inhibitory influences from periaqueductal gray, nucleus raphe magnus, and adjacent reticular formation. I effects on lumbar spinal cord nociceptive and nonnociceptive neurons. *J Neurophysiol* 49: 932–947, 1983

Haddjeri N, Blier P, de Montingy C. Effect of the α 2-adrenoceptor antagonist mirtazapine on the 5-hydroxytryptamine system in the rat brain. *J Pharmacol Exp Ther* 277: 852–860 1996

Hammond DL, Yaksh TL. Antagonism of stimulation-produced antinociception by intrathecal administration of methysergide or phentolamine. *Brain Res* 298: 329–337, 1984.

Hauser K, Olpe HR, Jones RSG. Trimipramine, a tricyclic antidepressants exerting atypical actions on the central noradrenergic system. *Eur J Pharmacol* 111: 23–30, 1985

Hwang AS, Wilcox GL. Analgesic properties of intrathecally administered heterocyclic antidepressants. *Pain* 28: 343–355, 1987

Jensen TS, Gebhart GF. General anatomy of antinociceptive systems. In: J Olesen & L Edvinsson eds. *Basic mechanisms of headache,* pp189–200, Elsevier Science Publishers, 1984.

Larson AA, Takemori AE. Effect of fluoxetine hydrochloride, a specific inhibitor of serotonin uptake, on morphine analgesia and development of tolerance. *Life Sci* 21: 1807–1812, 1977

Levine JD, Gordon NC, Smith R, Mcbryde R. Desipramine enhances opiate postoperative analgesia. *Pain* 27: 45–49, 1986

Lund A, Tjolsen A, Hole K. Antinociceptive effect of intrathecally-administered desipramine and zimelidine in rats. *Neuropharmacol* 29: 819–823, 1990

Lund A, Tjolsen A, Hole K. The apparent antinociceptive effect of desipramine and zimelidine in the tail flick test in the rat is mainly caused by changes in tail skin temperature. *Pain* 38: 65–69, 1989

Malseed RT, Goldstein FJ. Enhancement of morphine analgesia by tricyclic antidepressants. Neuropharmacol 18: 827–829, 1979

Ossipov MH, Malseed RT, Goldstein FJ. Augmentation of central and peripheral morphine analgesia by desipramine. *Arch Int Pharmacodyn Ther* 259: 222–229, 1982

Reddy SVR, Maderdrut JL, Yaksh TL. Spinal cord pharmacology of adrenergic-mediated antinociception. *J Pharmacol Exp Ther* 213: 525–533, 1980.

Sawynok J, Reid A. Desipramine potentiates spinal antinociception by 5-hydroxytryptamine, morphine and adenosine. *Pain* 50: 113–118, 1992

Usha Rani P, Naidu MUR, Prasad VBN, Ramesh Kumar Rao T, Shobha JC. An evaluation of antidepressants in rheumatic pain conditions. *Anesth Analg* 83: 371-375, 1996

VanderMaelen CP, Braselton JP. Acute administration of antidepressants trazodone increases noradrenergic locus coeruleus neuronal firing in the rats. *Arch Int Pharmacodyn Ther* 308: 13–20, 1990

Walsh TD. Antidepressants in chronic pain. *Clin Neuropharma* 6: 271–295, 1983

Yaksh TL, Wilson PR. Spinal serotonin terminal system mediates antinociception. *J Pharmacol Exp Ther* 208: 446–453, 1979.

Yaksh TL, Huang SP, Ruddy TA. The direct and specific opiate like effect of met-enkephalin and analogues on the spinal cord. *Neuroscience* 2: 593–598, 1977.