Medical Journal of the
Islamic Republic of Iran

Volume 10
Number 4
Winter 1375
February 1997

Basic Science In Medicine

INVOLVEMENT OF SUPRASPINAL ALPHA-ADRENERGIC RECEPTORS IN TONIC PAIN

S. SEMNANIAN, S. HAJISAYAH*, AND M.R. ZARRINDAST**

*From the Department of Physiology, Shaheed Beheshti University of Medical Sciences, the *Department of Physiology, Tarbiat Modarres University and the **Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Islamic Republic of Iran.*

ABSTRACT

The involvement of supraspinal alpha-adrenergic receptors in tonic pain was assessed in formalin-induced pain in rats. The alpha$_2$ adrenoceptor agonist clonidine, along with yohimbine and prazosin, α_2 and α_1 receptor antagonists, were introduced intracerebroventrically (icv) and/or systemically in different doses. The data show that 1) clonidine exerts an alpha adrenergic analgesic effect, in addition to its known alpha$_2$ role in this kind of pain, 2) icv yohimbine did not change the rat's nociception, and 3) icv prazocin also failed to alleviate the animal's nociception although both the latter drugs show analgesic activity in the formalin test when injected systemically. It can be concluded that α_1 receptors contribute significantly to adrenergic analgesia in the formalin test in supraspinal structures, by undefined nature and site(s).

MJIRI, Vol. 10, No. 4, 279-284, 1997.

INTRODUCTION

In the past two decades, there has been considerable interest in the involvement of adrenergic systems in pain and analgesia. It has been shown that noradrenergic pathways originating in the brainstem and terminating in the spinal dorsal horn are involved in descending inhibitory mechanisms of nociception.[2,28] Alpha$_2$ receptor subtypes are believed to be the major adrenoceptors responsible in spinal antinociception.[13, 24, 25] Systemic administration of noradrenaline does not produce analgesia in mammals, because it cannot cross the blood-brain barrier. It was discovered that intrathecal (i.t.) injection of α_2 agonists induces antinociception, and i.t. α_2 antagonists antagonize antinociception produced by intrathecally administered noradrenaline which may be mediated through activation of the bulbospinal noradrenergic pathway.[10,30] However, there are few reports expressing the relative role of α_1 adrenoceptors in tail-flick or hot-plate induced pain[13, 18] or the Randall-Sellito paw pressure test,[6] or following electrical stimulation.[21] Kuraishi et al.[16] have found that i.t. prazocin in addition to i.t. yohimbine enhances substance P release in the spinal dorsal horn in response to peripheral mechano-receptive stimuli and inhibits the noradrenaline-mediated decrease in substance P release, suggesting a role for both α_1 and α_2 subtypes of adrenoceptors in spinal nociceptive modulation.

At present the detailed mechanisms of nociceptive modulation by supraspinal noradrenergic systems are not well understood, and both positive and negative properties of

these systems have been reported.[3,9,19] In animals, antinociceptive tests vary in their sensitivities to centrally-acting analgesic drugs. It has been proven that some mechanisms involved in the perception of pain in the tail-flick test are anatomically and pharmacologically dissociable from those involved in pain processing in the formalin test.[4,7]

Most of the studies concerning adrenergic analgesia have focused on spinal and supraspinal mechanisms of reflexive withdrawal from a phasic stimulus of short duration. The role of α_1 and α_2 adrenergic receptors in the formalin tests, a model for testing tonic pain, has been assessed by Tasker and Melzack.[27] They administered prazocin and yohimbine, showing some α_1 activity in this kind of pain. These findings confirmed the need for further studying the spinal and supraspinal structures' role in the formalin test by local injections of the above-mentioned drugs.

The present study was undertaken to investigate the nature of the alpha-adrenergic receptor subtypes involved in the supraspinal control of tonic pain. A preliminary report of these experiments has been presented in abstract form.[26]

MATERIALS AND METHODS

Animals

Male NMRI rats weighing 250 to 300g were used for all studies. Animals were housed in groups of 4 to 5 rats per each cage at room temperature (25±3°C) with a natural light-dark cycle. The animals had free access to food and water prior to the experiments. Testing took place between 9 a.m. and 3 p.m. The animals were brought to the test lab the day before testing, and were thus adapted to the testing environment for at least 18 hours. No animal was tested more than once.

Cannulation

Icv infusions were performed according to the method of Popick.[23] Briefly, the system consists of a 28-gauge stylet and a 21-gauge injection cannula, cut 1 mm longer than the guide. The unilateral guide cannula was aimed stereotaxically 1mm above the lateral ventricle (AP= -0.8, L= 1.5, H= -3.3 mm from bregma)[22] of the rats while they were anesthetized with intraperitoneal (i.p.) sodium pentobarbital (65 mg/kg). The guide was attached to the skull with 3 stainless steel screws and dentate cement. A stylet was introduced in the cannula to prevent its obstruction.

Formalin test

The formalin test[8,20] was used according to the modifications described by Cohen et al.[5] The rats were observed in a formalin test box of clear plexiglas, 32×32×32 cm in size. A mirror was positioned at a 45° angle below the floor of the test box, allowing an unobstructed view of the animal's paw.

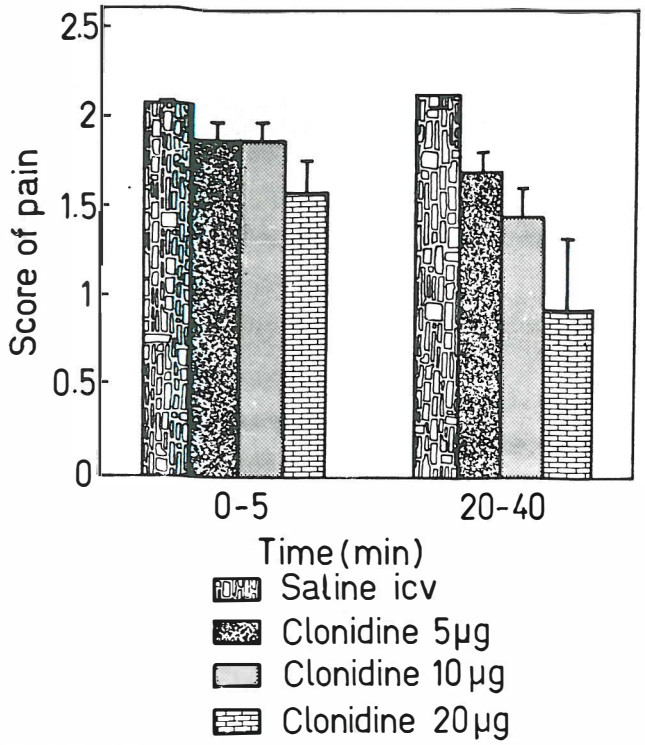

Fig. 1. The effects of different doses of clonidine (5, 10, 20 µg-icv) on formalin-induced pain. The error bars represent S.E.M. (n= 6 in each dose) (P<0.005).

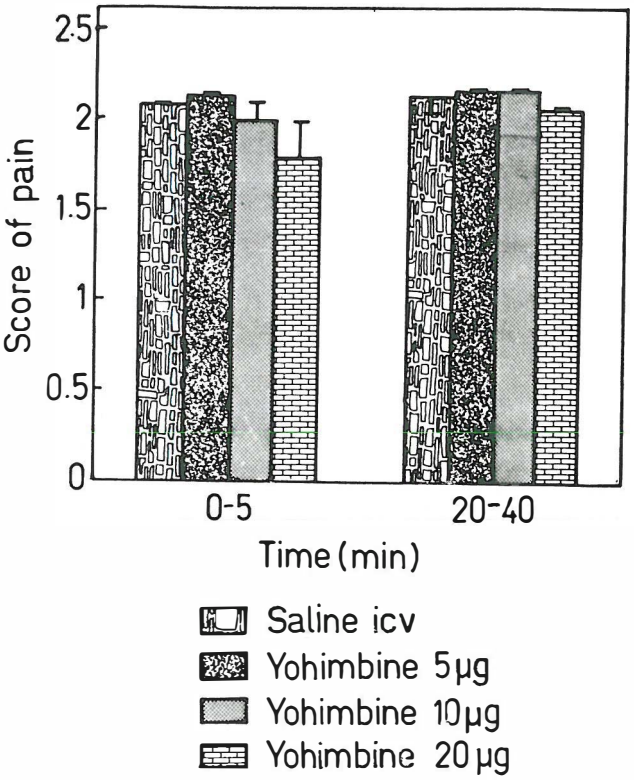

Fig. 2. The effects of different doses of yohimbine (5, 10, 20 µg-icv) on formalin-induced pain. The error bars represent S.E.M. (n= 6 in each dose).

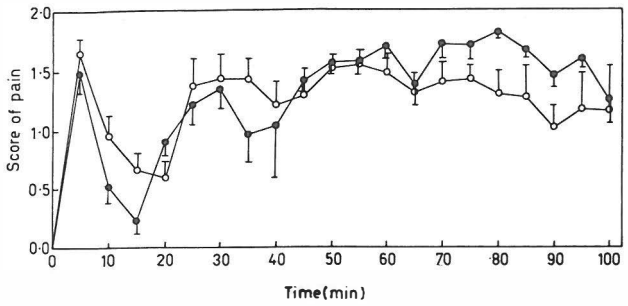

Fig. 3. The effects of clonidine (20 µg-icv) injected 30 minutes after yohimbine (0.5, 1 mg/kg-ip) on tonic pain. The error bars represent S.E.M. (n= 5 in each dose) -○- yoh(0.5 mg/kg) + clo (20 µg)
-●- yoh (1mg/kg) + clo (20 µg).

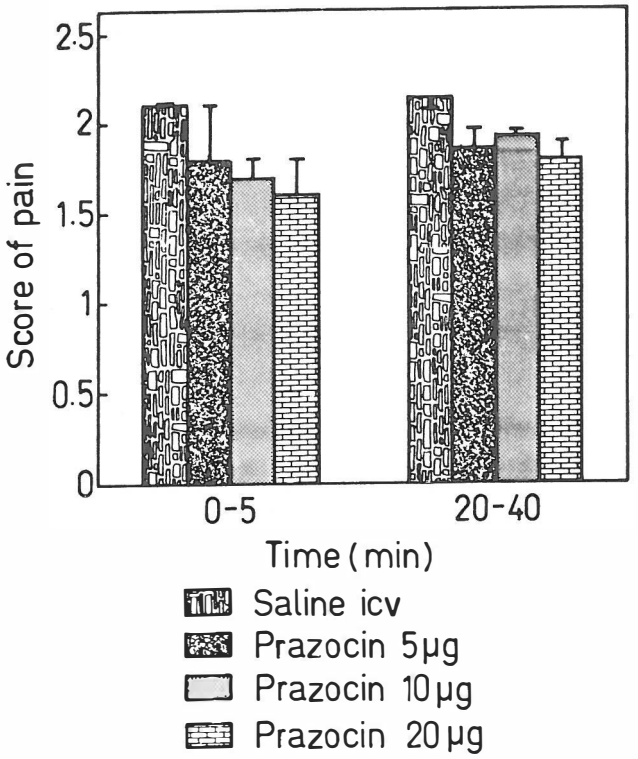

Fig. 4. The effects of different doses of prazocin (5, 10, 20 µg-icv) on formalin -induced pain. The error bars represent S.E.M. (n= 6 in each dose).

The rats were habituated to the test box for 5 min before the experiment to minimize stress. On the day of testing, 50 µL of 2.5% buffered formalin acetate was injected subcutaneously in to the planter surface of the right hind paw using a microsyringe with a 26-gauge needle. Formalin test pain was rated by an experimenter who was blind to the kind of drug being used, by recording the number of seconds that the rat engaged in each of the following behaviors: 0, the injected paw is placed normally on the floor; 1, the injected paw is favored, but still in contact with the floor; 2, injected paw is elevated and not in contact with the floor; 3, the injected paw is licked or chewed. Mean pain scores were recorded for blocks of 5 min. A weighted average of time spent in the four basic categories was taken as a measure of the primary response. Behavioral responses to formalin were recorded from 0 to 100 minutes following the formalin injection. Formalin injection induces an immediate nociceptive response consisting of shaking and licking of the injected paw. This substance produces a characteristic biphasic response. Pain behavior diminishes in 5-10 min following formalin administration and then increases after 15-20 min to a stable level which lasts an additional 40-60 min.

Drugs

Four to five days after surgery the stylets were removed while the rats were gently restrained and the drug was slowly injected through an internal cannula (28g) extending 1mm beyond the guide cannula. All drugs were injected icv in a volume of 5µL. Sixty seconds after drug infusion, the injection cannula was removed and the stylet was replaced in the guide cannula. Icv injections were given 1 min. before the actual testing (in the formalin test). Clonidine was dissolved in water and given in doses of 5, 10 and 20 µg per rat (n= 5). Yohimbine and prazosin were dissolved in water and administered in doses of 5, 10 and 20 µg per rat (n= 5). Control animals (n= 10) were injected with the same volume of saline. The experiments with clonidine, yohimbine (n= 18) and prazosin (n= 15) were performed in separate groups of animals. Control groups received saline. I.p. injections were carried out 40 min. before the formalin test.

Histology

The animals were killed after the experiments by an overdose of sodium pentobarbital. Cerebro-ventricular injection sites were marked by injection of 0.5% methylene blue solution under the same conditions as those of the experiment. The site was located by cutting frontal sections of the brain at the level of the cannula and locating the dye position by a light microscope.

Statistics

Behavioral data were analyzed by analysis of variance (ANOVA), and by Student's t-test when the analysis was restricted to two means. The difference between groups was considered statistically significant when $P<0.05$.

RESULTS

The icv injection of 5µg clonidine, an α_2 agonist, induced analgesia, especially 10 to 20 minutes after formalin injection (Fig. 1). Injection of 10 and 20 µg of clonidine also increased the analgesia in a dose-dependent manner

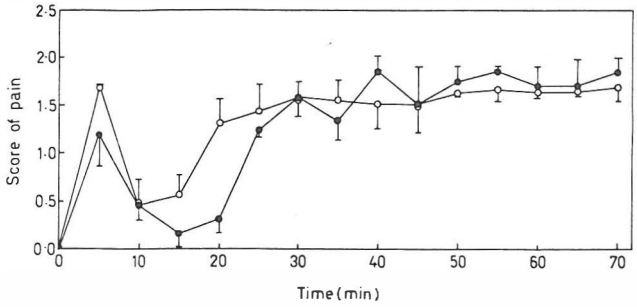

Fig. 5. The effect of clonidine (20 μg-icv) injected 30 minutes after prazocin (0.5, 1 mg/kg-ip) on tonic pain (n= 5).
-○- praz (0.5 mg/kg) + clo (20 μg)
-●- praz (1 mg/kg) + clo (20 μg)

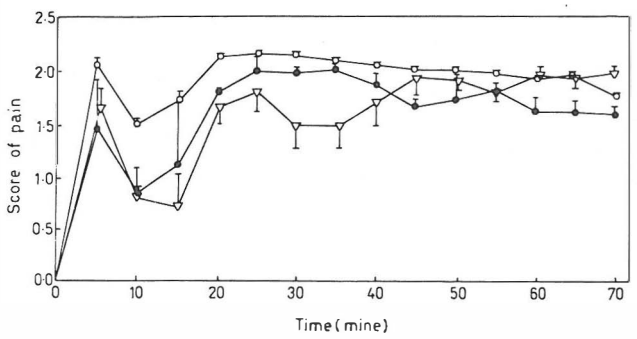

Fig. 6. Comparing the effects of 0.5 and 1 mg/kg-ip prazosin with the saline injected control group on tonic pain (n=5) (p<0.005).
-○- saline
-●- praz (1 mg/kg-ip)
-▽- praz (0.5 mg/kg-ip)

(P<0.005). The pain scoring was traced for 100 minutes after formalin injection.

As shown in Fig. 2, icv administration of yohimbine, an α_2 adrenergic antagonist, at doses of 5, 10, and 20 μg did not produce any effect on formalin -induced pain.

Because of reports concerning the probable effects of clonidine on α_1 adrenoceptors,[14,16] clonidine was used after blocking the α_2 adrenoceptors. Twenty micrograms of clonidine was injected (icv) per rat 30 minutes after injecting 0.5 and 1 mg/kg of i.p. yohimbine (Fig. 3). Yohimbine alone, when applied intraperitoneally (1 mg/kg) before the chemical stimulus, has an analgesic effect which is absent at lower doses, i.e. 0.5 mg/kg (unshown data). Comparing these two results, it is shown statistically that the combination of clonidine and yohimbine has a significant analgesic effect on tonic pain (P<0.005). This may indicate that clonidine elicits an antinociceptive response through an α_1 adrenoceptor mechanism.

At the next step, prazosin – a potent α_1 antagonist – was introduced in the cerebral ventricle at doses of 5, 10, and 20 μg (Fig. 4). No significant change was noticed in the rats' nociception at these doses of prazosin. Then, in order to further differentiate the α_1 and α_2 effects of clonidine, 20 μg (icv) of clonidine was injected 30 minutes after prazocin (0.5 and 1 mg/kg i.p.) (Fig. 5). The result was a long-lasting analgesia which still existed 70 minutes after formalin injection (P<0.005). The control group suggests that i.p. prazocin at these doses can show analgesic activity by itself on tonic pain (Fig. 6), but the group receiving clonidine in addition showed a greater degree of analgesia.

DISCUSSION

Adrenergic sedation and antinociception are complex phenomena, involving both spinal and supraspinal sites, particularly the locus coeruleus[15] and other midbrain and medullary areas. Most animal studies on adrenergic analgesia have concentrated on spinal and supraspinal mechanisms of reflexive withdrawal from a phasic stimulus, but many research works emphasize that different neural mechanisms underlie tonic versus phasic pain.[1,7] One report compared the effects of 8 irritants, namely acetic acid, carrageenan, formalin, kaolin, platelet-activating factor, mustard oil, serotonin and yeast.[29] This study strongly supports the use of formalin as a noxious stimulus in tonic pain research.

Because of the above mentioned information and the fact that the detailed mechanisms of the role of the supraspinal adrenergic system in tonic pain is still not well understood, we tried to evaluate the central alpha-adrenergic receptors involved in this kind of pain in the current study.

Icv administration of norepinephrine has been reported to produce analgesia, using phasic pain measurements.[11] Therefore at first we introduced clonidine, a potent α_2-receptor agonist, at different doses in the lateral cerebroventricular area. Clonidine produces a wide variety of effects. These include an antihypertensive action, alleviation of the opiate-withdrawal syndrome, antinociception and sedation. Clonidine is extremely potent as an antinociceptive agent. Compared to morphine, clonidine often shows greater antinociceptive potency, somtimes by a factor of more than 50. Clonidine has been shown to exhibit antinociceptive activity against a wide variety of noxious stimuli such as chemical irritants (e.g., formalin, acetylcholine, acetic acid), heat, pressure and electrical stimuli, but its exact site of action is unknown. In the present study, the icv administration of different doses of clonidine was employed. The dose-related attenuation in nociception seen in our work following icv injection of this drug, supports earlier observations of its peripheral and central α_2-adrenergic effect on phasic pain. This raises the possibility that its central analgesic effect may be mediated through an α_2-receptor subtype. However, a number of recent reports have shown that clonidine produces behavioral effects, including antinociception, *in vivo*, also by an α_1-receptor mechanism.[12] Therefore, blockade of α_2-receptors with systemic yohimbine was used to detect clonidine's effect on α_1-

receptors. Interestingly, it was shown that in spite of the blocked α_2-receptors, clonidine could still exert its antinociceptive effect in the formalin test, but the degree of analgesia was less than that of the same dose of the drug applied alone, We conclude that clonidine has α_1-adrenergic effects in addition to α_2 in this type of pain. The question of the anatomical location of the appropriate α_1-receptors has not been answered to date. Furthermore, these results are consistent with the classical model of adrenergic subtypes in which α_1-receptors are located postsynaptically on effector organs and α_2-receptors are located on noradrenaline pathway terminals and regulate noradrenaline release.[17]

REFERENCES

1. Abbot FV, Melzack R, Samuel C: Morphine analgesia in the tail-flick and formalin pain tests is medicated by different neural systems. Exp Neurology 75: 644-651, 1982.
2. Basbaum AI, Fields HL: Endogenous pain control mechanisms: review and hypothesis. Annals Neurol 4(5): 451-462, 1978.
3. Behbehani MM, Pomeroy SL, Mack CE: Interaction between central gray and nucleus raphe magnus: role of norepinephrine. Brain Res Bull 6: 361-364, 1981.
4. Coderre TJ, Abbot FV, Melzack R: Effects of peripheral antisympathetic treatments in the tail-flick, formalin and anatomy tests. Pain 18: 13-23, 1984.
5. Cohen SR, Abbot FR, Melzack R: Unilateral analgesia produced by intraventricular morphine. Brain Res 303: 277-287, 1984.
6. Colville KI, Chaplin E: Sympathomimetics as analgesics: effects of methoxamine, methamphetamine, metaraminol and norepinephrine. Life Sci 3: 315-322, 1964.
7. Dennis SG, Melzack R, Gutman S, Boucher F: Pain modulation by adrenergic agents and morphine as measured by three pain tests. Life Sci 26: 1247-1259, 1980.
8. Dubuisson D, Denis SG: The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4: 161-174, 1977.
9. Fields HL, Heinricher MM, Mason P: Neurotransmitters in nociceptive circuits. Annu Rev Neurosci 14: 219-245, 1991.
10. Fleetwood-Waker SM, Mitchel R, Hope PJ, Molony V, Iggo A: An alpha-2 receptor mediates the selective inhibition of noradrenaline of nociceptive responses of identified dorsal horn neurons. Brain Res 247 (Suppl.): 37-84, 1985.
11. Handley SL, Spencer PSJ: Analgesic activity after intracerebral injection in the mouse. Br J Pharmac 35: 361-362, 1969.
12. Hayes AG, Skingle M, Tyers MB: Antagonism of alpha-adrenoceptor agonist-induced antinociception in the rat. Neuropharmacol 25: 397-402, 1986.
13. Howe JR, Wang JY, Yaksh TL: Selective antagonism of the antinociceptive effect of intrathecally applied alpha-adrenergic agonists by intrathecal prazocin and intrathecal yohimbine. J Pharmacol Exp Ther 224: 552-558, 1983.
14. Kawabata A, Kasamatsu K, Umeda N, Tagaki H: The noradrenaline precursor L-threo-3, 4-dihydroxyphenylserine exhibits antinociceptive antivity via central α-adrenoceptors in the mouse. Br J Pharmacol 111: 503-508, 1994.
15. Korf J, Bunney BS, Aghajanian GK: Noradrenergic neurons: morphine inhibition of spontaneous activity. Eur J Pharmac 30: 165-169, 1974.
16. Kuraishi Y, Hirota N, Sato Y, Kaneko S, Satoh M, Tagaki H: Noradrenergic inhibition of the release of substance P from the primary afferents in the rabbit spinal dorsal horn. Brain Res 359: 177-182, 1985.
17. Langer SZ: Presynaptic regulation of the release of catecholamines. Pharmacol Rev 32: 337-362, 1981.
18. Little HJ, Rees JMH: Naloxone antagonism of sympathomimetic analgesia: In: Van Ree JM, Terenius L, (eds.), Characteristics and Function of Opioids. Amsterdam: Elsevier, pp. 433-434, 1987.
19. Miller J, Williams GV: Effects of iontophoresis of noradrenaline and stimulation of the periaqueductal gray on single-unit acitivity in the rat superficial dorsal horn. J Comp Neurol 287: 119-133, 1989.
20. O'Keefe J: Spinal cord mechanisms subserving pain perception. Masters Thesis, McGill University, 1964.
21. Paalzow GHM, Paalzow LK: Separate noradrenergic receptors could mediate clonidine-induced analgesia. J Pharmacol Exp Ther 223: 795-800, 1982.
22. Paxinos G, Watson C: The Rat Brain in Stereotaxic Coordinates. New York: Academic Press, 1985.
23. Popick FR: Application of a new intraventricular injection technique in rat brain norepinephrine studies. Life Sci 18: 197-204, 1976.
24. Reddy SV, Maderdrut JL, Yaksh TL: Spinal cord pharmacology of adrenergic agonist-mediated antinociception. J Pharmacol Exp Ther 213: 525,533, 1980.
25. Sagen J, Proudfit HK: Effect of intrathecally administered noradrenergic antagonists on nociception in the rat. Brain Res 310: 295-301, 1984.
26. Semnanian S, Shafizadeh M, Hajisayah S, Zarindast M: The assesment of GABAergic and alpha-adrenergic receptors' effect on tonic pain. 7th World Congress on Pain. Abst. No. S-451, 1993.
27. Tasker RAR, Melzack R: Different alpha-receptor subtypes are involved in clonidine-produced analgesia in different pain tests. Life Sci 44: 9-17, 1989.
28. Tjolsen A, Lund A, Hole K: The role of descending noradrenergic systems in regulation of nociception; the effects of intrathecally administered α-adrenoceptor antagonists and clonidine. Pain 43: 113-120, 1990.
29. Wheeler-Aceto H, Porreca F, Cowan A: The rat paw formalin test: comparison of noxious agents. Pain 40(2): 229-238, 1990.
30. Wilcox GL, Clarsson KH, Jochim A, Jurna I: Mutual potentiation of antinociceptive effects of morphine and clonidine on motor and sensory responses in rat spinal cord. Brain Res 405: 84-93, 1987.