

A COMPARATIVE STUDY OF THE ANALGESIC ACTIVITY OF H₂ RECEPTOR ANTAGONISTS

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Summary

Histaminergic mechanisms are involved in the initiation, perception and also in the modulation of pain sensation. Cimetidine is found to be effective in relieving neuralgic and neuropathic pain. This study was undertaken to evaluate the analgesic activity of Cimetidine, Ranitidine and Famotidine. Analgesic activity was studied using thermal methods, mechanical methods and chemical methods. Only Cimetidine showed significant analgesic activity in all the parameters studied and it compared well with that of Aspirin, where as Ranitidine showed analgesic activity comparable to that of Cimetidine only in rat tail flick test. Famotidine showed a weak analgesic activity in rat tail flick test but did not show any significant analgesic activity in other three studies.

Key Words: H₂ blockers, Cimetidine, Ranitidine, Famotidine, Analgesics.

Introduction

The development and introduction of H₂-receptor antagonists has been considered as one of the most outstanding inventions of the present century and represents a major breakthrough in the medical management of peptic ulcer and other related conditions collectively known as acid peptic diseases. They are the mainstay in the treatment of peptic ulcer, reflux oesophagitis, Zollinger Ellison syndrome, Systematic mastocytosis, Stress ulcers, etc.

Since H₂ receptors are not just confined to parietal cells of the stomach but are widely distributed in various body systems and tissues like central nervous system, vascular and non vascular smooth muscle, immunocompetent 'T' lymphocytes, heart, etc and possibly mediate some regulatory or modulatory functions which remain unexplored to a large extent, it may not be surprising to find the H₂ blockers exerting a wide range of pharmacological effects other than suppressing acid output. Many such actions may form the basis for some of the therapeutic application of them in conditions other than acid peptic diseases. Thus they are finding their use in various other disease states and clinical conditions unrelated to hyperacidity.

Cimetidine and other H₂ – blockers have been reported to be useful as adjuvants to H₁-blockers in pruritus and urticaria [1], inflammatory skin disorders like eczema, Psoriasis [2] and to correct the blood pressure in hypotension and shock along with H₁ blockers[3]. Cimetidine has also been reported to be useful as an immunomodulator in hypogammaglobulinaemia and AIDS in paracetamol poisoning to minimize hepatotoxicity , multiple viral warts in children[4] and portal hypertension (Daneshmend and Roberts, 1981)[5]. Thus the therapeutic horizon of H₂ blockers is widening. Of late there has been some interest in the efficacy of cimetidine in certain pain syndromes.

Histamine in pain perception:

Histaminergic mechanisms are known to be involved in the initiation, perception and also in the modulation of pain sensation. Histamine stimulates the cutaneous branch of the sensory nerve fibre and sends pain impulses to the central nervous system[6]. In the peripheral neurons the receptors for histamine are generally of H₁ type [7].

Substance P released from the peripheral nerve endings acts as a stimulus for histamine release by interacting with mast cell receptors to induce degranulation. The liberated histamine evokes a variety of responses including antidromic, vasodilatation, neurogenic plasma extravasation, reactive hyperemia and sensitizes the sensory nerve ending producing pain. Thus histamine released in the nerve endings evokes itch in the epidermis and pain in the dermis[8].

Histaminergic mechanisms are also known to be involved in the modulation of pain sensation in the CNS [9]. Hough (1988), [10] has described that central histaminergic mechanisms involving both H₁ and H₂ receptors may be involved in the perception of pain.

Cimetidine provided the dramatic relief of pain and erythema[11] and was also found to be effective for rapid pain relief and prevention of post herpetic neuralgia in herpes zoster infection [12].

Cimetidine was effective in relieving neuralgic and neuropathic pain [13]. However there are conflicting reports about the antinociceptive effects of H₂ blockers in the conventional experimental animal models for pain. The effects of H₂ blockers on stress induced and morphine induced analgesia have been variable and inconsistent. Foot shock induced analgesia is attenuated by H₂ blockers [14]. Morphine induced antinociception is attenuated by H₂ blockers [15]. On the other hand the antinociceptive effects of opioids is potentiated by H₁ and H₂ blockers [16]; stress induced analgesia attenuated but opioid induced analgesia potentiated by cimetidine and other H₂ blockers [17], Dimaprit , a potent H₂ receptor agonist caused a significant elevation of nociceptive threshold, where as Dimaprit induced writhing was inhibited by cimetidine[18]. Epidural injection of phenol containing ranitidine induced analgesia[19]. In view of the above facts and also because of the paucity of information about the comparative efficacy of various H₂ blockers – Cimetidine, Ranitidine and Famotidine in various conventional experimental models of pain, this study was taken up.

The Aim and Objective of this study is to screen for analgesic activity of H₂ receptor antagonists in Albino rats and mice.

Methods

In the present work, H₂ antagonists cimetidine, ranitidine and famotidine were studied for their possible analgesic activity in the conventional experimental animal models of pain . All the three drugs were obtained in pure powder form by the kind courtesy of M/S SK&F (Cimetidine), Cadila (Ranitidine) and BPRL (Famotidine). The chemical structures and other details of these compounds have been described in the review of literature.

Acetylsalicylic acid or Aspirin (ASA) was also used as a standard analgesic drug for comparison. All these drugs were administered by mouth as a suspension in 2% gum acacia through polythene catheter. The oral route was preferred as these drugs are usually given by mouth. The animals used in the present work were albino rats and mice. These animals were bred locally in the animal house of the department. The animals were fed with the standard Lipton India animal feeds supplied in the form of pellets. They were also fed with green leafy vegetables and carrot.

The methods employed in the present work for studying the analgesic activity were:-

1. Thermal methods
 - a. Radiant heat
 - b. Contact heat
2. Mechanical method

3. Chemical method

1. Thermal Methods:

a) Radiant heat – The method used here is that of D'Amour and Smith (1941)[20], involving the use of Analgesiometer (INCO) as a source of radiant heat. Albino rats of either sex weighing between 100-150gm were used. The animals subjected to discoordination tests to exclude false positive response. Only those animals showing the response within 4-5 secs were selected and the animals showing variation in reaction time of more than one second were discarded. The selected animals were divided into 5 groups of 10 each. The tails were shaved for 3 cm from the base to ensure proper exposure of the skin to heat. The first group served as untreated control and received 2% gum acacia suspension orally (1ml/100gm body weight). The second, third and fourth groups received cimetidine (100mg/kg), ranitidine (40mg/kg) and famotidine (5mg/kg) respectively and the fifth group received acetylsalicylic acid (125mg/kg body wt). All these drugs were administered by mouth as a suspension in 2% gum acacia and the volume of the drug suspension was maintained as 1ml/100gm body wt.

The tail flick response was tested in the animals of all the groups one hour after the administration of the drug. Each animal was placed in the cylindrical restrainer present at the top of the instrument with the tail stretched and protruding over the electrically heated nichrome wire, which acts as a source of radiant heat from below, without actually coming in contact with the skin. The time required for flicking of the tail, starting from the time of switching on the instrument (onset of stimulus) was taken as the reaction time. The cut-off time was taken as 15 sec to avoid damage to the skin due to prolonged exposure to heat and the animals not showing any response even at the end of 15 sec were assumed to have complete analgesia. The observation were tabulated and the significance of difference was calculated by the student 't' test. The percent prolongation of reaction time was also calculated for graphical presentation.

b) Contact Heat Method – The method followed is that of Wolff and Macdonald (1944)[21] modified by Eddy and Leimbach (1953)[22], involving the use of mouse hot plate. Albino mice of either sex weighing 20-30 gms were used. The animals were subjected to discoordinated test to exclude the false positive response. Those animals not responding within 5 sec, either to lick the paws or to jump in an attempt to escape were discarded. The selected animals were divided into 5 groups of 10 each. The first group served as untreated control and received only 2% gum acacia suspension by mouth (0.5ml/20gm body weight). The second, third, and fourth group were given Cimetidine (100mg/kg), ranitidine (40mg/kg) and famotidine (5mg/kg) respectively, where as the 5th group received actyl salicylic acid (125mg/kg body weight). All the drugs were administered by mouth as a suspension in 2% gum acacia and the volume of suspension was maintained as 0.5ml/20gm body weight.

The temperature of the hotplate was maintained at 55.5 +_ 0.5 C. To test the response, each animal was placed on the hot plate and the time required for licking the paws or trying to jump

out in an attempt to escape, starting from the time of placing the animal on the hotplate was taken as the reaction time. The response was tested in the animals of all the groups one hour after the administration of drugs. The cut-off time was taken as 10 seconds to avoid any damage to the paws and animals not showing any response within 10 seconds were considered to have developed complete analgesia. The observations were tabulated and the significance of difference was calculated by the student 't' test. The percent prolongation of reaction time was also calculated for graphical presentation.

2. Mechanical Method:

Tail Clip method: The method of Bianchi and Franceschini (1954)[23] was followed in this procedure. Albino mice of either sex weighing 20-30gm were used. The animals were subjected to discoordination test to exclude false positive response. The animals not responding within 5 secs to remove the clip were discarded. The selected animals were divided into 5 groups of 10 each. The first group served as untreated control and received only 2% gum acacia suspension by mouth (0.5ml/20gm body wt). The second, third, fourth and fifth groups received Cimetidine (100mg/kg), ranitidine (40mg/kg), famotidine (5mg/kg) and acetyl salicylic acid (125mg/kg body wt) respectively by oral route as a suspension in 2% gum acacia. The volume of the suspension for each animal was maintained at 0.5ml/20gm body wt. A bulldog clamp with rubber sleeves was applied to the base of the tail as a mechanical stimulus and the time taken to show the response of trying or attempting to remove the clip by the animal from the moment it was applied was taken as the reaction time. The same bulldog clamp was used for all the animals throughout to ensure uniform pressure.

The response was tested in each animal of all the groups one hour after administration of the drugs/control. Cut-off time was taken as 10 sec to avoid crush injury to the tail due to excessive pressure and animals not showing any response within 10 secs were assumed to have developed complete analgesia. The observations were tabulated and the significance of the difference was calculated by the student 't' test. The percent prolongation of reaction time was also calculated for graphical presentation.

3. Chemical Method:

Acetic acid writhing test:

In this test the method of Vander-Wende and Margolia (1956)[24], modified by Siegmund et al (1957)[25] and Witkin et al (1961)[26] was followed and acetic acid (0.6%) was used as a chemical irritant to induce writhing. Albino mice of either sex weighing 20-30 gms were used. The animals were divided into 5 groups of 10 each. The first group served as control and received only 2% gum acacia suspension by mouth, 0.5ml/20gm body wt. The second, third, fourth and fifth groups received Cimetidine (100mg/kg), ranitidine (40mg/kg), famotidine (5mg/kg) and acetylsalicylic acid (125mg/kg body wt) respectively as a suspension in 2% gum acacia by mouth. The volume of the suspension for each animal was maintained at 0.5ml/20gm

body wt. One hour after the administration of the drugs/control, 0.6% aqueous solution of acetic acid was injected intraperitoneally in the dose of 1ml/100gm body weight. Writhing movements characterized by intermittent contraction of abdominal muscles with extension of the hind limbs and twisting of the trunks were produced within 3 to 10 minutes after injection of acetic acid. The writhing movements in each animal were counted from 5th to 25th minutes by placing each animal separately in a bell jar and total number of writhing movements over a period of 20 mnts were recorded. The observations were tabulated and significance of the difference was calculated by student 't' test. The percentage reduction in the number of writhing movements was also calculated for graphical presentation.

Results:

TABLE-1

Thermal Method – (Radiant Heat) – Rat tail flick method

No. of animals used in each group (n)= 10

	Groups (n=10)	Reaction time in sec (Mean +/- S.E)	'P' value	Percentage prolongation of reaction time (%)
1.	Control	3.3 ±0.367	-	-
2.	Cimetidine (100mg/kg)	8.7 ±0.560	< 0.001	62.068
3.	Ranitidine (40mg/kg)	7.4 ± 0.686	< 0.001	55.40
4.	Famotidine (5mg/kg)	4.8 ±0.351	< 0.01	31.25
5.	Aspirin (125mg/kg)	9.4 ± 0.340	< 0.001	64.89

In this parameter all the three H₂- blockers have shown analgesic activity and increased the pain threshold as indicated by the prolongation of the reaction time. Among the three compounds cimetidine showed the most significant analgesic activity (P<0.001) and produced 62% prolongation of the reaction time, which was almost comparable to acetylsalicylic acid (ASA). Ranitidine and famotidine produced 55.4% and 31.25% prolongation of the reaction time respectively.

TABLE-2

Thermal Method – (Contact Heat) – Mouse – Hot plate method

No. of animals used in each group (n)= 10

	Groups (n=10)	Reaction time in sec (Mean +/- S.E)	'P' value	Percentage prolongation of reaction time (%)
1.	Control	4.9±0.315	-	-
2.	Cimetidine (100mg/kg)	6.9±0.348	< 0.001	40.81
3.	Ranitidine (40mg/kg)	5.4 ± 0.434	< 0.1	10.25
4.	Famotidine (5mg/kg)	5.3±0.396	< 0.1	8.16
5.	Aspirin (125mg/kg)	7.2 ± 0.490	< 0.001	46.93

In this parameter only cimetidine has shown some analgesic activity as suggested by the prolongation of reaction time in response to the noxious stimulus of contact heat. The percent prolongation of the reaction time compared to the control was 40.81%. Where as ASA produced 46.93%. Though the analgesic activity of cimetidine in this parameter was some what lesser compared to ASA, it was statistically highly significant ($P < 0.001$). The analgesic activity of ranitidine and famotidine was very weak as suggested by 10.2% and 8.16% prolongation of reaction time respectively and it was statistically insignificant ($P > 0.1$).

TABLE-3**Mechanical Method – Mouse – tail clip method**

No. of animals used in each group (n) = 10

	Groups (n=10)	Reaction time in sec (Mean +/- S.E)	'P' value	Percentage prolongation of reaction time (%)
1.	Control	3.6±0.267	-	-
2.	Cimetidine (100mg/kg)	8.1 ±0.434	< 0.001	55.55
3.	Ranitidine (40mg/kg)	4.4± 0.221	< 0.05	18.18
4.	Famotidine (5mg/kg)	4.3±0.300	< 0.05	16.27
5.	Aspirin (125mg/kg)	8.7 ± 0.367	< 0.001	58.62

In this parameter also only cimetidine has shown analgesic activity with 55.55% prolongation of the reaction time which was almost comparable to ASA (58.62%) and it was statistically highly significant ($P < 0.001$). Ranitidine and famotidine showed weak analgesic activity with 18.18% and 16.27% prolongation of the reaction time respectively.

TABLE-4**Chemical Method – (Mouse-Acetic acid writhing test)**

No. of animals used in each group (n)= 10

	Groups (n=10)	Reaction time in sec (Mean +/- S.E)	'P' value	Percentage prolongation of reaction time (%)
1.	Control	45.7±2.368	-	-
2.	Cimetidine (100mg/kg)	18.2±0.953	< 0.001	60.175
3.	Ranitidine (40mg/kg)	37.8 ± 1.915	< 0.01	17.28
4.	Famotidine (5mg/kg)	45.1±4.501	>0.5	1.33
5.	Aspirin (125mg/kg)	13.6± 1.222	< 0.001	70.24

In this parameter cimetidine and ranitidine have shown analgesic activity as indicated by reduction in the number of writhing movements. The analgesic activity of cimetidine was more significant ($P < 0.001$) with 60.17% reduction in the number of writhing movements compared to control where as ranitidine produced 17.28% reduction in the number of writhing movements. Famotidine did not show any significant analgesic activity ($P > 0.5$).

Discussion

Only cimetidine showed significant analgesic activity in all the parameters studied and it compared very well with that of ASA where as ranitidine showed analgesic activity comparable to that of cimetidine only in rat tail flick test. Famotidine showed a weak analgesic activity in rat tail flick test but did not show any significant analgesic activity in other three parameters. Hence the present study suggests that among the three H₂-blockers only cimetidine has good analgesic activity comparable to that of ASA.

Histaminergic mechanisms are known to be involved in the initiation, perception and also in the modulation of pain sensation [9], the peripheral mechanisms in the initiation and the central mechanisms in the perception and modulation of pain. Histamine is known to stimulate the nerve endings in the cutaneous branch of sensory nerve fibres and send pain impulses to the CNS. Thus histamine appears to be involved in the initiation of pain sensation in the peripheral neurons [8]. The receptors for histamine in the peripheral neurons are generally of H₁-type [7]. However H₂-receptors are also likely to be involved in initiating pain sensation. Dimaprit (a highly selective H₂- agonist) induced writhing in rats was inhibited by cimetidine suggesting that even H₂-receptors may be involved in evoking pain sensation[18].

In the present study only cimetidine appears to have significant analgesic activity in the conventional experimental models of pain. Even for other pain syndromes cimetidine seems to be more effective than ranitidine and famotidine. Cimetidine effectively relieved the pain of trigeminal neuralgia but ranitidine and famotidine were ineffective [27].

Based on our observations and also in the light of the present evidence and available reports in the literature it is very difficult to explain the discrepancy in the analgesic activity of the three H₂-blockers. However there seems to be little correlation between their analgesic activity and their ability to block H₂ receptors. Perhaps the analgesic activity may be unrelated to the H₂-receptor blockade. This is consistent with an earlier observation reported in the literature [28]. The analgesic activity of cimetidine and other H₂-blockers may be probably due to a complex central mechanism of action, though the possibility of a peripheral component of action cannot be ruled out.[18]. Generally the H₂ – blockers because of their poor lipid solubility attain low concentration in the CNS but the CSF level of cimetidine is higher than that of other H₂-blockers [29].

Cimetidine was known to bind with imidazoline recognition sites in the guinea- pig and rat brain with a higher affinity than towards H₂-receptors [30], suggesting that distinct binding sites or

receptors existed for the imidazoline compounds. The demonstration of the existence of the imidazoline preferring receptors (IPRs), the specific binding sites for the drugs having imidazoline structure, has emerged as a fascinating concept in neuropharmacology and has aroused much scientific curiosity in recent times [31]. Many imidazoline drugs i.e. drugs having an imidazoline structure like clonidine, dexmedetomidine, tolazoline etc, are known to bind specifically with these receptors. Though the functional involvement of these receptors in the CNS is yet to be clearly established, it may be possible that the CNS effects of some imidazoline drugs may be mediated through these receptors. The centrally acting alpha-2 agonists having imidazoline structure like clonidine and dexmedetomidine are known to produce many actions like centrally induced hypotension, vagotonia, sedation, analgesia etc., which cannot be completely explained by alpha-2 receptor stimulation. These drugs produce analgesia when given by oral, transdermal, intravenous, intrathecal and epidural routes and their analgesic action poorly correlates with alpha-2 receptor stimulation. Clonidine induced antinociception was abolished by Idazoxan, a selective ligand at IPR but not by alpha-2-blockers [32]. Thus the analgesic effect of clonidine, dexmedetomidine and other imidazoline appears to be mediated through imidazoline receptors. It may be quite interesting, tempting and reasonable to speculate that Cimetidine having an imidazole structure and also known to bind avidly with IPRs may produce its antinociceptive effect through these receptors, analogous to clonidine and dexmedetomidine. This fact may also try to explain the weaker analgesic effect observed with non- imidazole H₂-blockers like ranitidine and famotidine.

However this aspect remains to be elucidated and if confirmed it may be an exciting possibility of initiating the development of very effective and potent imidazoline analgesic agents and open up a new horizon in analgesic therapy.

Thus, the present work, though of preliminary nature, suggests that H₂-blockers and Cimetidine in particular have good analgesic potential and further elaborate research work involving more number of animals and different experimental models of pain in a wide range of animal species including non-rodents and primates to elucidate the exact molecular and biochemical mechanism of action and to develop more effective compounds, seems to be worth undertaking.

References

1. Bleehen SS. Cimetidine and Chlorpheniramine in the treatment of chronic idiopathic urticaria: a multicentre randomized double-blind study. *Br.J.Dermatol*, 1987,117,81-88.
2. Nielsen HJ. Histamine and Histamine type-2 receptor antagonists in Psoriasis. Mechanisms and speculations. *Dan.Med.Bull*, 1991, 38(6), 478-80.
3. Lorenz W, Doenicke A, Schoning B, et al, H₁+H₂ receptor antagonists for premedication in anaesthesia and surgery: A critical view based on randomized clinical trials with Haemacel and various antiallergic drugs. *Agents and actions*,1980,10,114-124.
4. Orlow SJ, Paller A, Cimetidine therapy for multiple warts in children. *J.Am.Acad.Dermatol*, 1993; 28:794-6.

5. Daneshmend TK, Roberts CJ, H₂ receptor antagonists for portal hypertension. *Lancet*,1981;2(8242):360.
6. Crossland J, In Lewis Pharmacology 5th ed Churchill. Livingstone, Edinburgh. London Melbourne and Newyork,1980: 337.
7. Ganellin CR, Parson's ME, ed,Pharmacology of Histamine receptors, Wright PSG,Bristol London, Boston,1982:10-102.
8. Rang HP, Bevan S, Dray A, Chemical activation of nociceptive peripheral neurons. *British Medical Bulletin*.1991;47:534-548.
9. Dhasmana KM, Banerjee AK, Rating W, et al Analgesic effect of tramadol in the rat. *Acta Pharmacologica Sinica*, 1989;10(4):289-93.
10. Hough LB, Cellular localization and possible functions for brain histamine: recent progress, *Prog.Neurobiol*,1988,30;469-505.
11. Vander Spay S, Lewy DW, Levin W, Cimetidine in the treatment of herpes virus infections. 1980, *S.Afr.Med.J*,1980;58:112-116.
12. Bense L, Marcusson JA, Ramsten T, Effect of Cimetidine on Herpes Zoster infection.*Drug Intell Clin Pharm*,1987;21(10): 803-5.
13. Mc Quay HJ, Pharmacological treatment of neuralgic and neuropathic pain. *Cancer Surv*,1988;7:141-159.
14. Gogas KR, Hough LB, Glick SD, et al, Opposing actions of Cimetidine. *Brain. Res*, 1986;370:370-374.
15. Hough LB, Nalwalk JW, Modulation of morphine antinociception by antagonism of H₂ receptors in the periaqueductal gray. *Brain.Res*,1992;588:58-66.
16. Malec D, The influence of histamine receptor antagonists on antinociceptive action of narcotic analgesics.*Pol J Pharmacol Pharm*, 1987; 39(3): 229-35.
17. Robertson JA, Hough LB, Bodnar RJ. Potentiation of opioid and nonopioid forms of swim analgesia by Cimetidine. *Pharmacol Biochem Behav*, 1988; 31:107-112.
18. Scarpignato C, Del Soldato P, Cimetidine inhibition of Dimaprit induced writhing in the rat.*Pharmacology*,1986;32(4):237-240.
19. Mc Guinness, Contess, Epidural injection of a phenol containing Ranitidine preparation. *Anaesthesiology*, 1990;73(3):553-555.
20. D'Amour FE, Smith DL, A method of determining loss of pain sensation. *J Pharmacol Exp Ther*.1941; 72-74.
21. Wolff, Mac Donald, Evaluation of the analgesic action of pethidine hydrochloride. *J pharmacol Exp Ther*,1944;80:300-307.
22. Eddy NB, Leimbach D, Synthetic analgesics II.Diethienylbutenyl and Dithienylbutyl amines. *J.pharmacol.Exp.Ther*. 1953; 107: 385-393.
23. Bianchi C, Franceschini J, Experimental observations on Haffner's method for testing analgesic drugs. *British Journal of Pharmacology*,1954;9280-284.
24. Vander Wende C, Margolin S, Analgesic tests based upon experimentally induced acute abdominal pain.*Fed.Proc*,1956;15:94.

25. Siegmund EA, Cadmus RA, Golu, A method for evaluating both non-narcotic and narcotic analgesics. *Proc.soc.exp.biol.(N.Y)*,1957,95:729-731.
26. Witkin LB, Heubner CF, Galdi F, et al, Pharmacology of 2- amino-indane hydrochloride(Su 8629): A potent non-narcotic analgesic. *J.Pharmacol.Exp.Ther*,1961; 133: 400-408.
27. Rumore MM, Schlichting, Analgesic effects of antihistaminics. *Life Sci* 1985;36: 403-416.
28. Netti C, Bossa R, Galatulas I, et al, Antinociceptive effect of centrally administered Cimetidine and Dimaprit in the rat. *Pharmacology*,1984;28:262-267.
29. Courtney TP, Shaw RW, Cedar E, et al. Excretion of famotidine in Breast milk. *Br J Clin Pharmacol*, 1988;26:639.
30. Hill SJ , In progress in medicinal chemistry ,eds Ellis GP and West GB,Elsevier, Amsterdam. Newyork oxford. 1987;24: 29-84.
31. Parini A, Coupry I, Graham RM, et al, Characterization of an imidazoline/guanidinium receptive adrenergic receptor. *J Biol Chem*, 1989; 264(20): 11874-8.
32. Szelenyi I, Nickel B, Borbe HO, et al, Mode of antinociceptive action of flupirtine in the rat. *Br J Pharmacol*,1989; 97:835-842.