Effects of 5-HT$_7$ receptor ligands in rats suffering from chronic neuropathic pain

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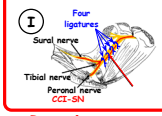
Introduction

- Neuropathic pain, characterized by long-lasting exaggerated pain behaviors such as hyperalgesia/allodynia (generally resistant to classical analgesic drugs [10]), presents a high degree of comorbidity with depression [1], notably in complex disorders such as fibromyalgia.
- The 5-HT$_7$ type of serotonin receptors (5-HT$_7$-R) is involved in pain control at peripheral, spinal and/or supraspinal levels [5], but its role in the modulation of nociceptive transmission is still unclear. In fact, conflicting data have been reported to date using selective agonists and antagonists [3, 4, 7, 9].
- 5-HT$_7$-R blockade is also known to exert antidepressant- and anxiolytic-like effects in validated animal models [6].
- Thus 5-HT$_7$-R might be potential targets for concomitant alleviation of pain and depression. In the present studies, attention was particularly devoted to the possible implication of 5-HT$_7$-R in the modulation of chronic pain in neuropathic rats.

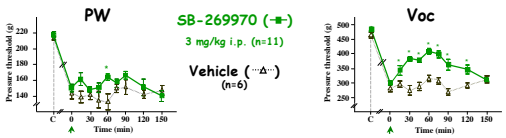
Materials and Methods



- Adult male Sprague-Dawley rats underwent unilateral chronic constriction injury (*CCI*) to the sciatic nerve [2] (Fig. I).
- In sciatic nerve-ligatured rats, mechanical nociceptive thresholds were measured using the Randall and Selitto paradigm which consists of applying increasing pressures (Ugo Basile analgesimeter) to the nerve-injured hind paw until paw withdrawal (PW) and then a squeak (Vocalization ; Voc) are obtained [8] (Fig. II).
- Threshold values were determined i) two days before surgery, and ii) 14 days later, when hyper-responsiveness to mechanical stimulation had fully developed. Drugs were injected two weeks after surgery.
- Each point is the mean ± S.E.M. of the number of independent determinations indicated in parentheses.
- * p < 0.05 as compared with thresholds measured before treatment (arrow), one way ANOVA, Dunnett's test.

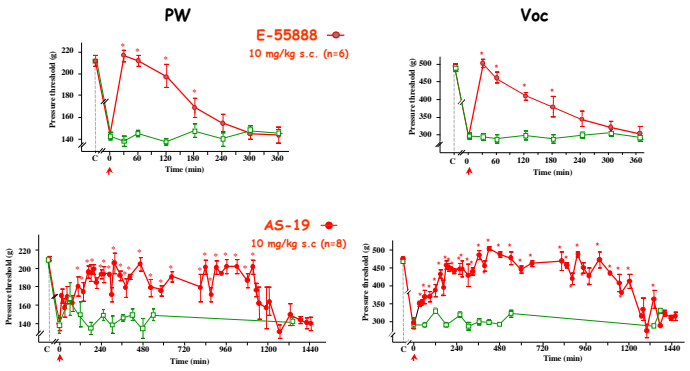
Results

1A Effects of 5-HT$_7$-R blockade



- 5-HT$_7$ receptor blockade by the antagonist SB-269970 increased pressure threshold to trigger vocalization but not paw withdrawal (Fig. 1A).
- 5-HT$_7$ receptor activation by the agonists E-55888 and AS-19 markedly increased thresholds to evoke nociceptive responses to hindpaw pressure. SB-269970 prevented the effects of both agonists (Fig. 1B).
- Blockade of GABA$_A$-R, GABA$_B$-R and opioid-R by <u>systemic administration</u> of bicuculline, phaclofen and naloxone, respectively, did not affect E-55888-induced anti-allodynia/anti-hyperalgesia (Fig. 2 A,B,C).
- In contrast, GABA$_A$ receptor blockade at spinal level (by <u>intrathecal administration</u> of bicuculline) completely prevented the effects of E-55888 (Fig. 3).

1B Effects of 5-HT$_7$-R activation

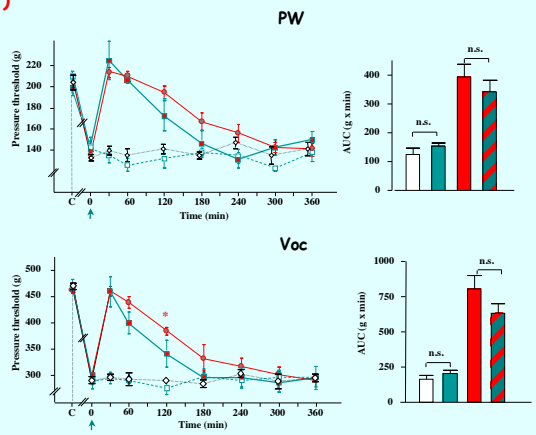


-□- 5-HT$_7$ -R blockade by SB-269970 3 mg/kg + 5-HT$_7$ -R agonist (n=6, 5, 4)

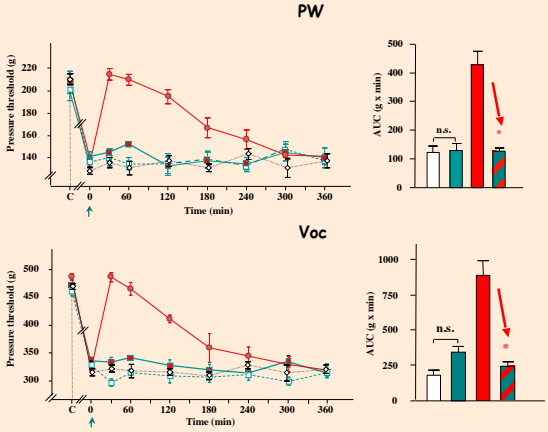
2 Which mechanisms underlie the anti-hyperalgesic effects of 5-HT$_7$-R activation ?

Implication (?) of:

Systemic administration



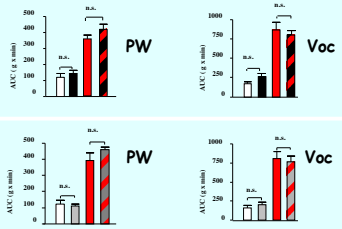
Intrathecal administration

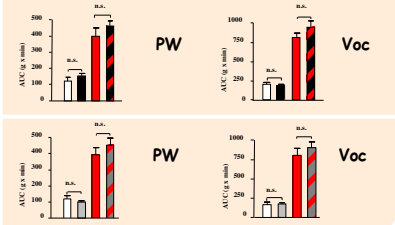


2A GABA$_A$-R (bicuculline)

2B GABA$_B$-R (phaclofen)

2C Opioid-R (naloxone)





- Vehicle i.v. or i.t + vehicle s.c. (n=6)
- Vehicle i.v. or i.t + E-55888 10 mg/kg s.c. (n=6)

GABA$_A$-R
- Bicuculline 0.5 mg/kg i.v. or 3 µg i.t. + vehicle s.c. (n=6)
- Bicuculline 0.5 mg/kg i.v. or 3 µg i.t. + E-55888 10 mg/kg s.c. (n=6)

GABA$_B$-R
- Phaclofen 2 mg/kg s.c. or 5 µg i.t. + vehicle s.c. (n=6)
- Phaclofen 2 mg/kg s.c. or 5 µg i.t. + E-55888 10 mg/kg s.c; (n=6)

Opioid-R
- Naloxone 1 mg/kg s.c. or 10 µg i.t. + vehicle s.c. (n=6)
- Naloxone 1 mg/kg s.c. or 10 µg i.t. + E-55888 10 mg/kg s.c. (n=6)

Conclusion

- In rats suffering from neuropathic pain, the anti-hyperalgesic effect of 5-HT$_7$-R blockade by SB-269970 suggests that *tonic activation* of excitatory 5-HT$_7$-R (on primary afferent fibers ?) by endogenous 5-HT contributes (but to a limited extent) to hyperalgesia.
- The marked anti-hyperalgesic effects of 5-HT$_7$-R agonists might be mediated through stimulation of other 5-HT$_7$-R, which are *not tonically activated* by endogenous 5-HT.
- The complete blockade by intrathecally-administered bicuculline, but not phaclofen and naloxone, of the anti-hyperalgesic effects of 5-HT$_7$-R stimulation by E-55888 strongly supports the idea that – *non tonically activated* – 5-HT$_7$-R are those expressed by spinal GABAergic interneurons [5]. At this level, 5-HT$_7$-R-mediated excitation probably promotes GABA release and subsequent GABA$_A$-R activation.
- These data suggest that spinal 5-HT$_7$-R are promising targets for alleviating neuropathic pain.

References

[1] Bair MJ, Robinson RL, Katon W and Kroenke K (2003) Depression and pain comorbidity. *Arch. Intern. Med.* **163**: 2433-2445.
[2] Bennett GJ and Xie YKA (1988) Peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. *Pain* **33**: 87-107.
[3] Branchat A, Romero L, Garcia M, Pujol M, Burgueno J, Torrens A, Hamon M, Baeyens JM, Buschmann H, Zamanillo D, and Vela JM (2009) 5-HT$_7$ receptor activation inhibits mechanical hypersensitivity secondary to capsaicin sensitization in mice. *Pain* **141**: 239-247
[4] Dogrul A and Seyrek M (2006) Systemic morphine produces antinociception mediated by spinal 5-HT$_7$, but not 5-HT$_5$, and 5-HT$_6$ receptors in the spinal cord. *Br J Pharmacol* **149**:498-505.
[5] Doly S, Fischer J, Brisorgueil MJ, Verge D and Conrath M (2005) Pre- and postsynaptic localization of the 5-HT$_7$ receptor in rat dorsal spinal cord : immunocytochemical evidence. *J Comp Neurol* **490**:256-269.
[6] Hedlund PB (2009) The 5-HT$_7$ receptor and disorders of the nervous system: an overview. *Psychopharmacology* **206**: 345-354.
[7] Meuser T, Pietruck C, Gabriel A, Xie GX, Lim KJ and Palmer PP (2002) 5-HT$_7$ receptors are involved in mediating 5-HT-induced activation of rat primary afferent neurons. *Life Sci* **71**: 2279- 2289.
[8] Randall LO and Selitto JJ (1957) A method for measurement of analgesic activity on inflamed tissue. *Arch Int Pharmacodyn Ther* **111**: 409-419.
[9] Rocha-Gonzalez HI, Meneses A, Carlton SM and Granados-Soto V (2005) Pronociceptive role of peripheral and spinal 5-HT$_7$ receptors in the formalin test. *Pain* **117**:182-192.
[10] Woolf CJ and Mannion RJ (1999) Neuropathic pain: aetiology, symptoms, mechanisms, and management. *Lancet* **353**:1959-1964.

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