

## INVOLVEMENT OF THE SEROTONIN SYSTEM IN SSRI-INDUCED ANTINOCICEPTION

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### ABSTRACT

Serotonin specific reuptake inhibitors (SSRI) may induce antinociception; however, the mechanism of this effect is not clear. SSRIs increase 5-HT levels in neuronal synapses and facilitate serotonergic activity. In this study, therefore, the activity of para-chlorophenylalanine (pCPA), which reduces 5-HT release, and 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, were examined on the antinociceptive activity of six SSRIs, in the abdominal constriction test. The compounds studied included fluoxetine, fluvoxamine, sertraline, paroxetine, citalopram and zimelidine. The effects of pCPA and 5-HTP were also evaluated on morphine analgesia as a standard compound. All antidepressants tested demonstrated dose-inhibition of acetic acid-induced abdominal constrictions. The antinociceptive activities of morphine, fluoxetine, fluvoxamine and sertraline, but not paroxetine, citalopram and zimelidine were significantly reduced by pCPA. Subsequently, 5-HTP restored the reduced antinociception of morphine, fluoxetine and fluvoxamine caused by pCPA. Furthermore, 5-HTP increased morphine, fluoxetine, fluvoxamine and sertraline-induced antinociception. Opioid receptor antagonists have been shown to reduce the antinociception induced by morphine, fluoxetine, fluvoxamine and sertraline but not by paroxetine, citalopram and zimelidine. It can be concluded, therefore, that the serotonin system is only involved in antinociception produced by antidepressants, and their antinociception is opioid antagonist reversible.

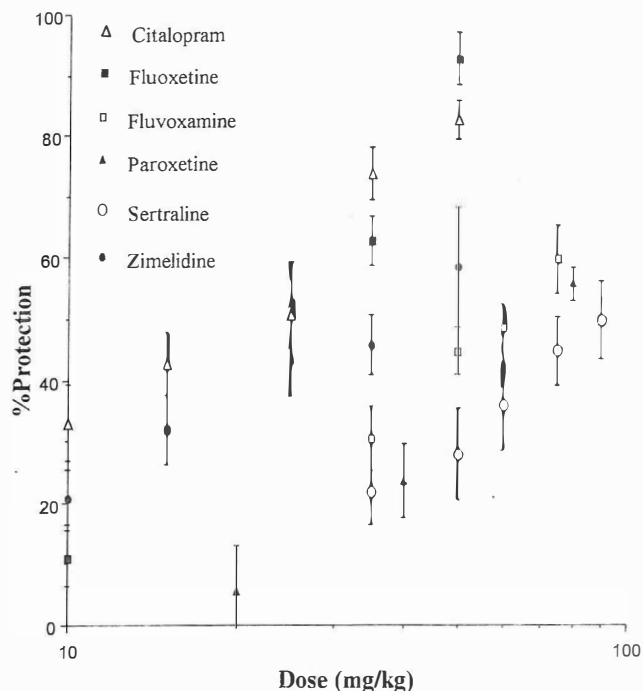
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### INTRODUCTION

It has been reported that peripherally administered 5-HT evokes hyperalgesic activity,<sup>1</sup> whereas intrathecal (i.t.) injection of 5-HT has been shown to have analgesic properties.<sup>2</sup> Serotonin specific reuptake inhibitors (SSRI) may induce antinociception;<sup>3</sup> however, the mechanism of this effect is not clear. SSRIs increase 5-HT levels in neuronal synapses and therefore facilitate serotonergic

activity.<sup>4</sup> It has been suggested, therefore, that SSRIs might induce antinociception through inhibition of 5-HT reuptake.<sup>5</sup> It should be noted that this hypothesis has not been examined in detail.

In the current study therefore the activity of 5-hydroxytryptophan (5-HTP), a precursor of 5-HT, and para-chlorophenylalanine (pCPA), which specifically reduces 5-HT release,<sup>6,7</sup> were examined on the antinociceptive activity of six SSRIs in the abdominal



**Fig. 1.** Dose response antinociceptive activity of SSRI antidepressants in the abdominal constriction assay. All SSRIs were administered subcutaneously 30 min before the test. Values are mean±S.E.M. (n=8-10).

constriction test in order to probe their action on 5-HT generating systems. The compounds studied included fluoxetine (FLX), fluvoxamine (FLV), sertraline (Sert), paroxetine (Par), citalopram (Cit) and zimelidine (Zim). The effect of pCPA and 5-HTP were also evaluated on morphine analgesia as a standard compound.

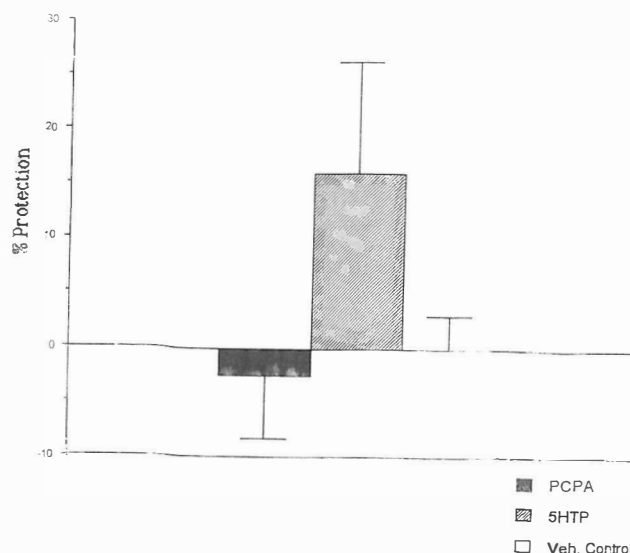
**MATERIALS AND METHODS**

Male ICI-WSP derived mice (22-25g) were employed throughout. Animals were allowed food and water *ad libitum* and were maintained at a temperature of 22.0±1.0°C on a 12/12h light/dark cycle, the experiments being performed between 9.00 and 17.00 h.

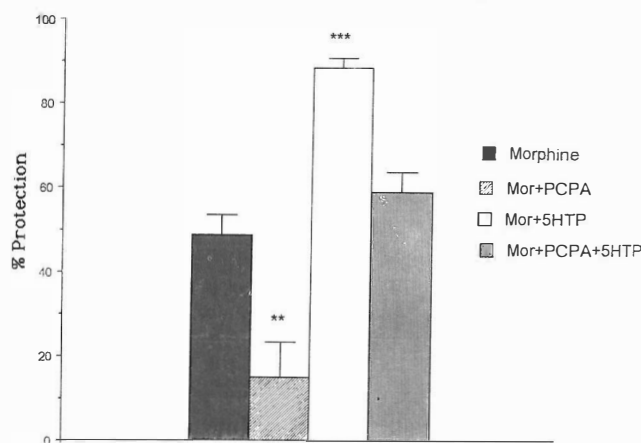
Antinociceptive activity in mice was assessed using the abdominal constriction assay.<sup>8</sup> Briefly, abdominal constrictions were elicited by intraperitoneal (ip) injection of 1% acetic acid and their incidence recorded over a 20 min period. Any decrease in abdominal constrictions compared to vehicle controls was calculated as a percentage of control values and designated % protection (as a criterion for antinociceptive activity of the compound).

$$\% \text{Protection} = 100 - \frac{\text{Writhes in drug group}}{\text{Writhes in control group}} \times 100$$

Acetic acid (BDH, UK) was administered i.p. 5 min

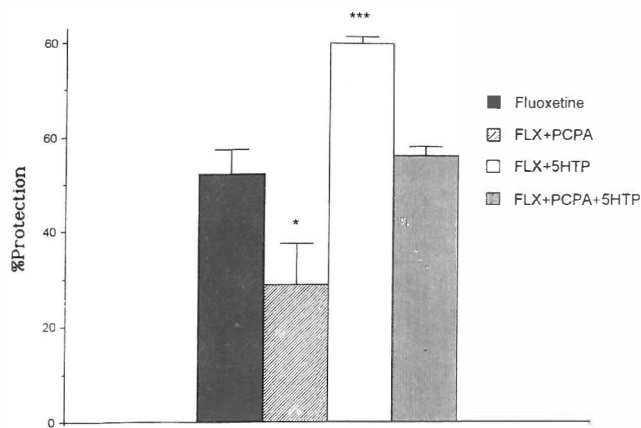


**Fig. 2.** Antinociceptive activity of pCPA (300 mg/kg) and 5-HTP (100 mg/kg) in the acetic acid abdominal constriction assay. pCPA was administered i.p. 72 h and 5-HTP 30 min before the test. Values are mean±S.E.M. (n>8). p>0.05 compared with the vehicle control group.



**Fig. 3.** Effect of pCPA (300 mg/kg) or 5-HTP (100 mg/kg) on morphine-induced antinociception, in the acetic acid abdominal constriction assay. pCPA was administered i.p. 72 h and 5-HTP 30 min before the test (n>8). Morphine (0.5 mg/kg s.c.) was administered with vehicle as a 30 min pre-treatment before acetic acid challenge. Values are mean±S.E.M. (n>8). \*\*p<0.01 compared with the morphine+vehicle group. \*\*\*p<0.001 compared with the morphine+vehicle group.

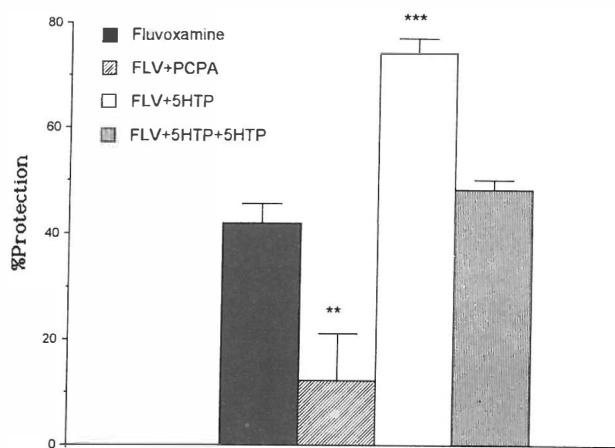
before the test. Morphine (AAH Pharmaceuticals, UK) was dissolved in normal saline, and SSRIs including fluoxetine (Lilly), fluvoxamine (Duphar), sertraline (Pfizer), paroxetine (Smith Kline Beecham), citalopram (Lundbeck) and zimelidine (Astra) were all dissolved in Tween 80 (5%



**Fig. 4.** Effect of pCPA (300 mg/kg) or 5-HTP (100 mg/kg) on fluoxetine-induced antinociception in the acetic acid abdominal constriction assay. pCPA was administered i.p. 72 h and 5-HTP 30 min before the test. Fluoxetine (27 mg/kg) was administered s.c. with vehicle as a 30 min pre-treatment before the test. Values are mean $\pm$ S.E.M. (n>8).

\* $p < 0.05$  compared with the fluoxetine+vehicle group.

\*\*\* $p < 0.001$  compared with the fluoxetine+vehicle group.



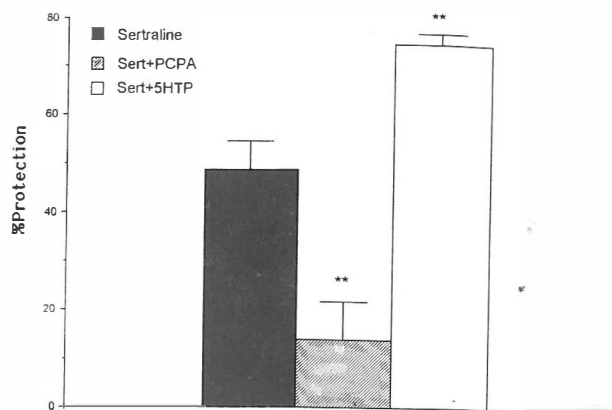
**Fig. 5.** Effect of pCPA or 5-HTP on fluvoxamine-induced antinociception. pCPA (300 mg/kg) was administered i.p. 72 h, and 5-HTP 30 min pre-treatment. Fluvoxamine (60 mg/kg) was administered s.c. with vehicle 30 min before the abdominal constriction test. Values are mean $\pm$ S.E.M. (n>8).

\*\* $p < 0.01$  compared with the fluvoxamine+vehicle group.

\*\*\* $p < 0.001$  compared with the fluvoxamine+vehicle group.

in saline) and injected s.c. 30 min before the test. Naloxone (Sigma, UK) and Mr2266BS (Boehringer Ingelheim, UK) were dissolved in normal saline and administered s.c. 5 min before acetic acid injection. pCPA and 5-HTP were dissolved in 1% CMC and administered i.p. 72h and 30 min before the test, respectively.

To measure the antinociceptive activity of SSRIs, different doses of each compound were administered s.c. and their antinociception calculated as mentioned above. Then, their  $ED_{50}$  doses were employed to evaluate the effects of pCPA and 5-HTP on their antinociception.



**Fig. 6.** Effect of pCPA or 5-HTP on sertraline-induced antinociception. pCPA (300 mg/kg) was administered i.p. 72 h, and 5-HTP 30 min pre-treatment. Sertraline (87 mg/kg) was administered s.c. with vehicle 30 min before acetic acid abdominal constriction test. Values are mean $\pm$ S.E.M. (n>8).

\*\* $p < 0.01$  compared with the sertraline+vehicle group.

Analgesic results were analyzed employing one way analysis of variance (ANOVA) and post hoc Tukey's tests.  $ED_{50}$  values of drugs to induce antinociception and their 95% confidence intervals were determined using linear regression. In all cases  $p < 0.05$  was considered as significantly different.

## RESULTS

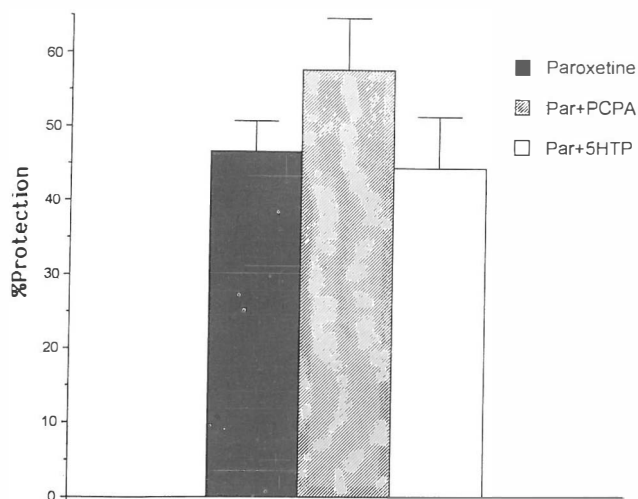
### Antinociceptive activity of SSRIs and morphine in the abdominal constriction test

Citalopram (10-50 mg/kg), fluoxetine (10-50 mg/kg), zimelidine (10-50 mg/kg), fluvoxamine (35-75 mg/kg), paroxetine (20-80 mg/kg), and sertraline (35-90 mg/kg) all produced dose-related inhibition of acetic acid-induced abdominal constriction in mice (Fig. 1). Their log dose-antinociceptive relationships possessed positive linear trends with respective correlation coefficients of 0.98, 0.93, 0.97, 0.99, 0.99, and 0.92. Their  $ED_{50}$  values (with confidence intervals) were 21.74 (14.4-27.3), 27 (19.7-36.1), 38.65 (27-75), 60.46 (54-68), 71 (64.7-80), and 87.7 (81.3-97.3) mg/kg. Morphine also induced antinociception in a same fashion as SSRIs with an  $ED_{50}$  value of 0.49 (0.35-0.66) mg/kg.

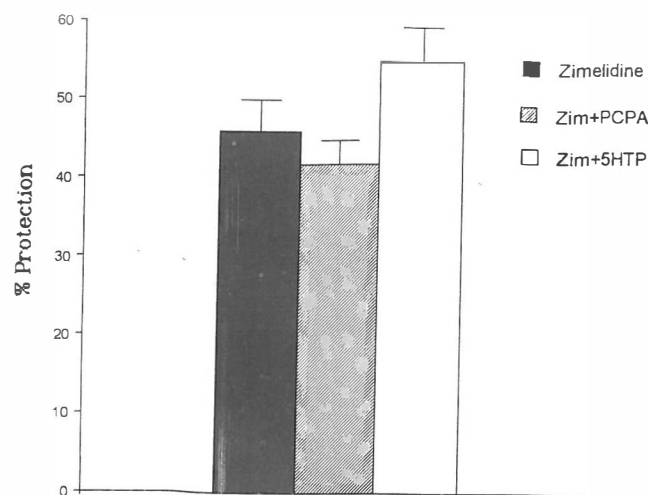
### Evaluation of antinociceptive activities of pCPA and 5-HTP in the abdominal constriction assay

As seen in Fig. 2, neither pCPA (300 mg/kg, i.p. 72 h pre-treatment), nor 5-HTP (100 mg/kg, i.p. 30 min pre-treatment) produced significant alterations in nociceptive threshold, compared to the vehicle control group. Also, the protection against constrictions displayed in animals receiving pCPA was not significantly different from that

## Serotonin and SSRI-Induced Antinociception



**Fig. 7.** Effect of pCPA (300 mg/kg) or 5-HTP (100mg/kg) on paroxetine-induced antinociception in the acetic acid abdominal constriction assay. pCPA was administered i.p. 72 h, and 5-HTP 30 min before the test (n>8). Paroxetine (70 mg/kg s.c.) was administered with vehicle as a 30 min pre-treatment before acetic acid challenge. Values are mean±S.E.M. (n>8).  $p>0.05$  compared with the paroxetine+vehicle group.



**Fig. 8.** Effect of pCPA or 5-HTP on zimelidine-induced antinociception in the acetic acid abdominal constriction assay. pCPA (300 mg/kg) was administered i.p. 72 h, and 5-HTP (100 mg/kg) 30 min before the test (n>8). Zimelidine (38 mg/kg s.c.) was administered with vehicle as a 30 min pre-treatment. Values are mean±S.E.M. (n>8).  $p>0.05$  compared with the zimelidine+vehicle group.

seen in the 5-HTP treated group ( $p>0.05$ ).

### Effect of pCPA and 5-HTP on antinociception induced by morphine (0.5 mg/kg), fluoxetine (27 mg/kg), fluvoxamine (60 mg/kg) and sertraline (87 mg/kg)

Pre-treatment with pCPA, 72 h before the abdominal constriction test, significantly attenuated the antinociception induced by s.c. administration ( $ED_{50}$  doses) of morphine from  $48.8\pm 4.7$  to  $15.1\pm 8.3$ , fluoxetine from  $52.1\pm 5.1$  to  $28.8\pm 8.6$ , fluvoxamine from  $42.0\pm 3.7$  to  $12.2\pm 8.8$  and sertraline from  $48.9\pm 5.6$  to  $13.87\pm 7.9$  (Figs. 3,4,5 and 6). Conversely, administration of 5-HTP (100 mg/kg) 30 min before acetic acid in pCPA treated animals restored morphine, fluoxetine and fluvoxamine analgesia (Figs. 3,4 and 5). Injection of 5-HTP 30 min before the test in mice also increased the analgesic activity of morphine, fluoxetine, fluvoxamine and sertraline (Figs. 3,4,5 and 6).

### Effects of pCPA and 5-HTP on antinociception induced by paroxetine, zimelidine and citalopram

pCPA (300 mg/kg i.p.) 72 h before testing had no effect on the antinociception induced by  $ED_{50}$  doses of paroxetine (60 mg/kg) and zimelidine (38 mg/kg) in the abdominal constriction assay. Similarly, 100 mg/kg i.p. 5-HTP, administered 30 min before testing, did not change the antinociceptive activity of the above-mentioned antidepressants ( $p>0.05$ ) (Figs. 7,8). In the case of animals which received 5-HTP with citalopram, there was evidence of tremorogenic activity which masked any assessment of abdominal constrictions. Therefore this experiment was

terminated, and consequently no results were available.

## DISCUSSION

The serotonin reuptake inhibitor compounds tested in this study all demonstrated dose-related inhibition of acetic acid-induced abdominal constrictions. Several clinical and laboratory studies also report inherent antinociceptive activity for antidepressants.<sup>10,11,14</sup> Citalopram was the most and sertraline the least potent SSRI tested in inducing antinociception. Their potencies in relation to morphine were as follows: citalopram 2.33%, fluoxetine 1.81%, zimelidine 1.27%, fluvoxamine 0.81%, paroxetine 0.69% and sertraline 0.56%.

The study of Shaw and colleagues<sup>15</sup> provides good evidence for involvement of stimulus intensity as a complicating factor in evaluating antinociceptive response. The use of a 55°C thermal stimulus, for instance, yields a model which, although retaining the ability to detect morphine, is insensitive to many analgesics. This implies that the stimuli employed in such assays exceed those encountered in clinical pain and that some antinociceptive activities would not be therefore detected, thus limiting the predictive validity of these assays. In the present study, the abdominal constriction assay was chosen as the most appropriate test for detection of the weaker antinociceptive activity induced by antidepressants.<sup>12</sup>

Modulation of nociceptive processing seems to depend on the recruitment of intrinsic systems, which mediate their effects by opiate and non-opiate systems. Emphasis has

been put on the role played by opioids and the monoamines 5-hydroxytryptamine (5-HT), and noradrenaline (NA), in mediating the effects of intrinsic antinociceptive systems.

Reuptake of monoamines is considered to be a major known component of the pharmacology of antidepressants.<sup>4</sup> Therefore, attempts to explain the direct antinociceptive effect of antidepressants in pharmacological terms center mostly on how these compounds interact with neurotransmitter systems in the brain and spinal cord.<sup>10,12</sup>

The results obtained in the present study showed that pCPA had no inherent effect on antinociceptive threshold. However, the antinociceptive activities of morphine, fluoxetine, fluvoxamine and sertraline but not paroxetine, citalopram and zimelidine were significantly reduced by pCPA. Subsequently, 5-HTP restored the reduced antinociception of morphine, fluoxetine and fluvoxamine caused by pCPA. It was noteworthy that 5-HTP increased morphine, fluoxetine, fluvoxamine and sertraline-induced antinociception, although the possibility cannot be excluded that this may have been an additive effect. On the other hand, administration of this 5-HT precursor had no effect on paroxetine, citalopram and zimelidine antinociception, which might tend to negate the idea of an additive effect for all SSRIs in the presence of 5-HTP.

Significant involvement of 5-HT in antidepressant activity and antinociception, particularly in the specific serotonin reuptake inhibitor types, has been considered.<sup>5</sup> This consideration has arisen from various pieces of evidence. Firstly, all SSRIs inhibit serotonin reuptake, thus increasing intra-synaptic 5-HT levels in the CNS,<sup>4</sup> and most compounds of this type have been shown to elicit antinociceptive activities.<sup>12</sup> Secondly, the involvement of 5-HT in regulating the transmission of nociceptive information at various levels of the peripheral and central nervous system is undisputed.<sup>1</sup> Moreover, neuronal systems containing serotonin appear not only to be involved in regulation of segmental nociceptive input at a spinal and supraspinal level, but also in mediating the antinociceptive action of morphine and that resulting from electrical stimulation of certain brain stem nuclei.<sup>9</sup>

Attenuation of fluoxetine, fluvoxamine and sertraline antinociception by pCPA and enhancement of this action by 5-HTP tends to confirm the involvement of 5-HT in SSRI antinociception. However, the current data demonstrates that paroxetine, citalopram and zimelidine antinociception are not modified by these two compounds, suggesting that involvement of 5-HT in SSRI antinociception may not be universal. It should be noted that other neurotransmitters such as acetylcholine, dopamine, and GABA are also involved in antinociception.<sup>16</sup> However, it is not clear to what extent these parameters have interfered in the results of SSRI induced antinociception.

Fluoxetine, fluvoxamine and sertraline-induced

antinociception were significantly reduced by naloxone, a general opioid antagonist and also Mr2266BS, a  $\kappa$  receptor preferring antagonist.<sup>12</sup> In contrast, paroxetine, citalopram and zimelidine antinociception were not modified by these two opioid receptor antagonists. These data demonstrate an opioid-like activity for fluoxetine, fluvoxamine and sertraline.

From the results here, therefore, it could be concluded that 5-HT activity is probably implicated only in visceral antinociception produced by those SSRIs which are naloxone sensitive. In this regard, opioid antinociception has also been established as being serotonin-dependent.<sup>13</sup> This would be in accordance with the dependency of an opioid-like SSRI involving 5-HT in its analgesic effects.

## REFERENCES

1. Taiwo YO, Levine JD: Serotonin is a directly-acting hyperalgesic agent in the rat. *Neuroscience* 48 (2): 485-490, 1992.
2. Sawynok J, Reid A: Interactions of descending serotonergic systems with other neurotransmitters in the modulation of nociception. *Behav Brain Res* 73(1-2): 63-8, 1996.
3. Rafieian-Kopaei M, Sewell RDE: Newer antidepressants: analgesia and relative monoamine reuptake inhibitory potency. *J Pharm Pharmacol (Suppl. 2)* 46: 1088, 1994.
4. Hyttel J: Pharmacological characterization of selective serotonin reuptake inhibitors (SSRIs). *Int Clinical Psychopharmacol (Suppl. 9)* 1: 19-26, 1994.
5. Ardid D, Marty H, Fialip J, Privat AM, Eschaliere A, Lavarenne J: Comparative effects of different uptake inhibitor antidepressants in two pain tests in mice. *Fund Clin Pharmacol* 6: 75-82, 1992.
6. Matsumoto K, Mizowaki M, Suchitra T, Murakami Y, Takayama H, Sakai S, Amin N, Watanabe H: Central antinociceptive effects of mitragynine in mice: contribution of descending noradrenergic and serotonergic systems. *Eur J Pharmacol* 317(1): 75-81, 1996.
7. Pini LA, Sandrini M, Vitale G: The antinociceptive action of paracetamol is associated with changes in the serotonergic system in the rat brain. *Eur J Pharmacol* 308 (1): 31-40, 1996.
8. Rafieian-Kopaei M, Sewell RDE: Opioid tolerance and  $K^+_{ATP}$  channel mediated antinociception. *Analgesia* 1(4-6): 667-670, 1995.
9. Kato M, Taguchi K, Hagiwara Y, Kubo T: Effects of electrical stimulation and morphine microinjection into periaqueductal gray on 5-hydroxyindole oxidation current in spinal cord of cats. *Gen Pharmacol* 27(7): 1195-201, 1996.
10. Philipp M, Fickinger M: Psychotropic drugs in the management of chronic pain syndromes. *Pharmacopsychiat* 26 (6): 221-34, 1993.
11. Schreiber S, Backer MM, Yanai J, Pick CG: The antinociceptive effect of fluvoxamine. *Eur Neuropsychopharmacol* 6 (4): 281-4, 1996.
12. Rafieian-Kopaei M, Sewell RDE: Is the opioid system

- involved in antinociceptive activity of antidepressants?  
*Iranian J Med Sci* 22 (1&2): 26-31, 1997.
13. Suh HW, Song DK, Wie MB, Jung JS, Hong HG, Choi SR, Kim YH: The reduction of antinociceptive effect of morphine administered intraventricularly is correlated with the decrease of serotonin release from the spinal cord in streptozotocin induced diabetic rats. *Gen Pharmacol* 27 (3): 445-50, 1996.
  14. Sindrup SH: Antidepressants in pain treatment. *Nordic J Psychiat (Suppl. 30)* 47: 67-73, 1993.
  15. Shaw JS, Rourke JD, Burns KM: Differential sensitivity of antinociceptive tests to opioid agonists and partial agonists. *Br J Pharmacol* 95: 578-584, 1988.
  16. Sewell R: Supraspinal and spinal monoamine-modified function and the expression of opioid antinociception. *J Psychopharmacol* 5: 352-359, 1991.