

## SEROTONERGIC MECHANISM IN IMIPRAMINE INDUCED ANTINOCICEPTION IN RAT TAIL FLICK TEST

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**Abstract :** Substantial evidence has accumulated that spinally projecting serotonergic neurons modulate nociception. However, the exact receptor subtypes that mediate the antinociceptive response of serotonin within the spinal cord continue to be a subject of debate. Therefore, we explored the effect of serotonergic system on imipramine induced antinociception by using 5-Hydroxytryptamine-3 (5HT<sub>3</sub>) receptor antagonist ondansetron and 5-Hydroxytryptamine-2 (5HT<sub>2</sub>) receptor antagonist mianserine, and depletion of brain 5-Hydroxytryptamine (5HT) with p-chlorophenyl alanine (PCPA). Male wistar strain rats were pretreated with either ondansetron (0.5 mg/kg, i.p.) or mianserine (1 mg/kg, i.p.). After 15 minutes, rats received injection of imipramine (10 mg/kg). Nociception was assessed by tail-flick method. Imipramine (2 mg, 5 mg, 10 mg, and 20 mg/kg) produce antinociceptive response in the dose dependent manner. Prior treatment with 5HT<sub>3</sub> antagonist, Ondansetron and 5HT<sub>2</sub> antagonist, mianserine reduce the antinociceptive response of imipramine. In PCPA treated rats imipramine (10 mg/kg) failed to produce antinociception. These results indicate that the 5HT plays an important role in imipramine induced antinociception.

**Key words :** imipramine                      serotonin                      antinociception

### INTRODUCTION

The involvement of 5-Hydroxytryptamine (5HT) in descending control of pain has long been recognised (1). Pharmacological evidence shows that electrical stimulation of nucleus raphe magnus (NRM) increase the release of metabolites of 5HT in the medullary dorsal horn cells in the rat spinal cord (2). Electrical or chemical stimulation of the NRM and surrounding nucleus reticularis paragigantocellularis

produces antinociception which is antagonised by intrathecally administered 5HT receptor blockers (3) and is accompanied by the efflux of 5HT from the spinal cord (4).

5HT is generally thought to be a inhibitory neurotransmitter in the dorsal horn cell. It is involved not only in the processing of central pain messages and modulating pain transmission signals in the dorsal horn but is also released at this site

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(5). It can act either by direct excitation of sensory neurones by increasing sodium permeability via 5HT<sub>3</sub> receptor activation or via G-protein coupled 5HT<sub>1</sub> and 5HT<sub>2</sub> receptors (6). Tricyclic antidepressants have been reported to have an antinociceptive effect (7, 8) and some of them have been successfully used to alleviate chronic pain (9, 10). Imipramine, a tricyclic antidepressant, also produces antinociception which is due to inhibition of 5HT re-uptake (11). Therefore, we explored the effect of serotonergic system in imipramine induced antinociception by using 5HT<sub>3</sub> receptor antagonist ondansetron and 5HT<sub>2</sub> receptor antagonist mianserine and depletion of brain 5HT with PCPA.

## METHODS

Male albino rats of wistar strain weighing 200–250 gms were divided into 5 groups. Group 1 received normal saline (0.1 ml, i.p.) and group 2 received imipramine (2 mg, 5 mg, 10 mg and 20 mg/kg, i.p.). In group 3 and 4 animals received ondansetron (0.5 mg/kg, i.p.) and mianserine (1 mg/kg, i.p.) 15 minutes before imipramine (10 mg/kg) administration. Group 5 received PCPA 50 mg/kg (i.p.) daily for 3 days followed by imipramine 10 mg/kg. The time course effect of imipramine alone and in the presence of ondansetron and mianserine and in PCPA pre-treated rats on the latency of tail flick response was studied.

In the tail flick test (thermal nociception) the rat was placed on tail flick apparatus (Techno), radiant heat was applied to a portion of the tail (about 5 cm from the tip) and the latency of tail flick response (TFL) was noted for each rat at 0

(pre-drug), 15, 30, 45, 60, 75, 90, 105 and 120 minutes intervals. The cut-off point was 20 seconds and 6 rats were used for each dose response. Percent antinociceptive response at each test time after administration of drugs was calculated by following formula:

$$\% \text{ Antinociceptive response} = \left[ \frac{T-C}{20-C} \right] \times 100$$

T-post-treatment reaction time, C-pre-treatment reaction time (control),

20-cutoff time (in this study)

None of the drugs altered the motor activity of animals as judged by rota rod test before and after drug administration.

Statistical analysis of mean values of % antinociceptive response were assessed by non-parametric one-way analysis of variance Kruskal wallis test. Mean ( $\pm$ SE) reaction time was calculated by unpaired 't' test.  $P < 0.05$  considered statistically significant. The project was approved by the institutional ethics committee.

## RESULTS

Intraperitoneal administration of Imipramine (2 mg, 5 mg, 10 mg and 20 mg/kg) caused a dose related increase in the latency of tail flick response in rats (Table I). At the peak effect (90 min) the mean ( $\pm$ SE) reaction time (TFL) of imipramine 2 mg/kg was  $5.6 \pm 1.2$  ( $P < 0.05$ ), 5 mg/kg was  $6.4 \pm 1.4$  ( $P < 0.001$ ), 10 mg/kg was  $7.2 \pm 0.05$  ( $P < 0.001$ ) and 20 mg/kg was  $7.5 \pm 0.03$  ( $P < 0.001$ ) (Table I).

TABLE I: Effect of ondansetron, mianserine and PCPA on tail flick latency (TFL, Sec, M ± SE) changes of imipramine.

Drugs (i.p.)	0'	15'	30'	45'	60'	75'	90'	105'	120'
Control (saline)	2.8±0.7	2.8±1.2	2.9±1.1	3.1±1.3	3±0.09	3.2±0.17	3.±1.0	3.1±1.01	3±0.09
Imipramine 2 mg/kg	3.7±1.09	3.8±0.09	4.3±0.05*	4.6±1.1*	5.1±1.19*	5.9±0.5*	5.6±1.2*	5±0.6*	4±1.0
5 mg/kg	3.9±0.6	4.7±1.4*	4.7±1.0*	5.3±1.7*	5.8±0.07*	6.2±1.7**	6.4±1.4**	5.7±1.3*	5.7±1.31*
10 mg/kg	3.3±1.01	4.2±1.0*	4.9±0.02*	5.8±1.01*	6.3±1.11*	6.6±0.21**	7.2±0.05	6.9±0.6**	6.7±0.7**
20 mg/kg	3.5±1.11	4.5±0.9*	5±1.2*	5.9±0.23*	6.6±0.15*	7.3±0.5**	7.5±0.03**	7.7±0.7**	7.2±0.1**
Ondansetron (0.5 mg/kg) + Imipramine (10 mg/kg)	3.2±0.16	4.1±0.15*	3.9±0.16	3.6±0.11	3.1±0.26	3.2±0.16	3.1±0.14	3±0.21	3.2±0.21
Mianserine (1 mg/kg) + Imipramine (10 mg/kg)	3.6±0.11	3.9±0.16	4.98±0.50*	5±0.5*	5.7±0.12*	4.6±1.2	4.4±0.51	4.2±0.6	4.1±0.6
PCPA (50 mg/kg/day×3ds) + Imipramine (10 mg/kg)	3.3±1.12	3.1±1.0	3.3±0.11	3.3±1.0	3.4±0.55	3.3±0.11	3.4±0.66	3.3±0.71	3.4±0.33

n = 6  
 \*P<0.05 as compared to control  
 \*\*P<0.001 as compared to control

Imipramine 10 mg/kg was chosen for subsequent experiments. In order to study the role of 5HT subtype receptors in antinociceptive effect of imipramine, ondansetron (5HT<sub>3</sub> antagonist, 0.5 mg/kg, i.p.) or mianserine (5HT<sub>2</sub> antagonist, 1 mg/kg, i.p.) were administered 15 minutes before Imipramine (10 mg/kg) and the changes in the latency of tail flick response were recorded. Participation of serotonergic mechanisms was further confirmed by studying the effect of Imipramine in PCPA pre-treated rats.

Pretreatment with ondansetron (0.05 mg/kg) and mianserine (1 mg/kg) reduced the antinociceptive effect of imipramine (10 mg/kg) which was more with ondansetron (Table I, Fig. 1). Ondansetron and mianserine on their own did not modify the latency of tail flick response. In PCPA treated rats imipramine also failed to produce antinociceptive response (Table I, Fig. 1).

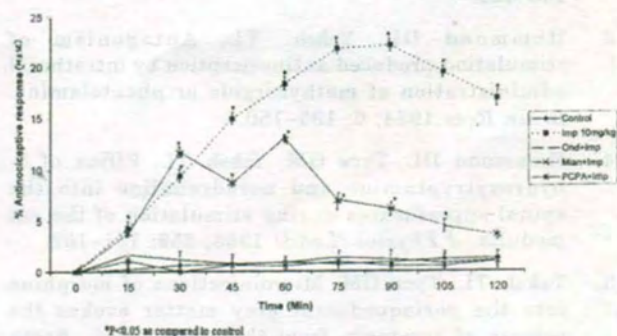


Fig. 1: Effect of Ondansetron (OND), Mianserine (MIAN) and PCPA on Imipramine (IMP) antinociception (Mean ± S.E.).

## DISCUSSION

Substantial evidence has accumulated that spinally projecting serotonergic neurones modulate nociception (9–12). However, the 5HT receptors subtypes that mediate the antinociceptive response of serotonin within the spinal cord continue to be a subject of debate. This is partly due to the fact that serotonin has multiple and often opposing effects mediated by multiple receptor subtype (6). It can either act via ligand gated 5HT<sub>3</sub> receptor activation or G Protein coupled 5HT<sub>1</sub>, 5HT<sub>2</sub> receptors (6). However, antagonism of the 5HT<sub>3</sub> receptor subtype reverses the antinociceptive effect of spinally administered serotonin (13).

Therefore, we studied the effects of the 5HT<sub>3</sub> receptor antagonist ondansetron and 5HT<sub>2</sub> antagonist mianserine and PCPA pre-treated rats on imipramine-induced antinociception.

Our study demonstrates the involvement of serotonergic mechanism in imipramine antinociception. Both 5HT<sub>3</sub> receptor antagonist ondansetron and 5HT<sub>2</sub> antagonist mianserine decrease the antinociceptive response of imipramine. Further pretreatment with PCPA blocked with antinociceptive response of imipramine. Our finding thus confirm the serotonergic involvement in imipramine induced antinociception.

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