

# Research into analgesic effect of ondansetron in persistent pain model in rats with central noradrenergic system lesion

Wojciech Roczniak<sup>1</sup>, Joanna M. Oświęcimska<sup>2</sup>, Barbara Brodziak-Dopierała<sup>3</sup>, Elżbieta Cipora<sup>1</sup>, Przemysław G. Nowak<sup>4</sup>, Magdalena Babuśka-Roczniak<sup>1</sup>

<sup>1</sup> The Jan Grodek Higher Vocational State School in Sanok, Medical Institute, Poland

<sup>2</sup> Department of Paediatrics, School of Medicine with the Division of Dentistry in Zabrze, Medical University of Silesia, Katowice, Poland

<sup>3</sup> Medical University of Silesia School of Pharmacy with the Division of Laboratory Medicine Department of Toxicology, Sosnowiec, Poland

<sup>4</sup> Toxicology and Drug Addiction Division, Department of Toxicology and Health Protection, Public Health Faculty, Medical University of Silesia, Katowice, Poland

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## Abstract

**Introduction.** Many known substances affecting the serotonergic system induce definite physiological effects, including those which are therapeutic. For instance, the enhanced serotonergic transmission due to decreased functions of autoreceptors and increased inhibitory functions of postsynaptic 5-HT<sub>1A</sub> is associated with antidepressant effect. The central serotonergic system takes part in the regulation of many bodily functions, such as sleep, wakefulness, blood pressure, pain perception or sexual behaviours. Moreover, it is involved in the pathogenesis of depression, anxiety, addictions, migraine and other headaches. In pain therapy, not only typical analgesics are used, but also substances without obvious analgesic effect, thus allowing potential pharmacological modulation of analgesic activity in the treatment of pain.

**Objective.** The aim of the study was to determine whether a chemical lesion to the central noradrenergic system at an early stage of individual development alters reactivity of 5-HT<sub>3</sub> receptors in adult rats.

**Materials and method.** The study used newborn and adult Wistar rats aged 8–10 weeks. Behavioural tests (writhing test, formalin assay) were used to assess the analgesic action of ondansetron as a 5-HT<sub>3</sub> receptor antagonist.

**Results.** The analgesic effect of ondansetron (1.0 mg/kg b.w., i.p.) in the writhing test was weak and short. Pain intensity score after ondansetron injection (1.0 mg/kg b.w., i.p) was 2–3 points and did not differ significantly between the study groups.

**Conclusions.** Damage to the central noradrenergic system at an early stage of individual development has no effect on the antinociceptive effects of the serotonin (5-HT<sub>3</sub>) receptor antagonist, ondansetron, in the persistent pain model.

## Key words

central serotonergic system, lesion, central noradrenergic system, ondansetron, analgesic effect

## INTRODUCTION

The central serotonergic system takes part in the regulation of many life functions, such as sleep, wakefulness, blood pressure, pain perception or sexual behaviours. Moreover, it is involved in the pathogenesis of depression, anxiety, addictions, migraine and other headaches. Serotonin (5-hydroxytryptamine, 5-HT) is thought to be the major mood-regulating factor. Even trace amounts of this neurotransmitter affect mood, appetite, sleep and pain tolerance. A drop in its level may cause 'addictive' binge eating, sleeplessness, depression, aggression, low pain tolerance, and impair thermoregulatory mechanisms [1, 2]. Neuronal bodies of the central serotonergic system are located mainly in the medial part of the brain stem, in the raphe nuclei (medial dorsal and great nucleus). Their axons form projections ascending to the structures of the striatum, cerebral cortex, cerebellar cortex, hippocampus, amygdala,

thalamus, hypothalamus and projections descending to the spinal cord [3, 4].

Many known substances affecting the serotonergic system induce certain physiological as well as therapeutic effects. For instance, enhancement of serotonergic transmission through decreased functions of autoreceptors and intensification of inhibitory functions of postsynaptic 5-HT<sub>1A</sub> are associated with antidepressant effect.

In turn, increased function of the autoreceptors or postsynaptic receptors in the limbic system is connected with an anxiolytic effect. Agonists of 5-HT<sub>2</sub> receptors decrease dopamine secretion and the action of dopaminergic neurons in the limbic system, whereas their antagonists have antipsychotic, anxiolytic and antidepressant effects.

The antagonists of 5-HT<sub>3</sub> receptors are most frequently used in chemotherapy-induced nausea and vomiting. Their mechanism consists in the blockage of 5-HT<sub>3</sub> receptors, both peripherally in the endings of the vagus nerve, and centrally in the *area postrema*, and in the solitary nucleus [5, 6].

The 5-HT<sub>3</sub> receptors located centrally (within the spinothalamic tract, brain stem nuclei, thalamus and posterior horns of the spinal cord) and peripherally (within

Address for correspondence: Wojciech Roczniak, The Jan Grodek Higher Vocational State School in Sanok, Medical Institute, Mickiewiczza 21, 38-500 Sanok, Poland  
E-mail: wojciech\_roczniak@interia.pl

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nerve endings of afferent nociceptive fibres) are involved in the process of generation and perception of pain. The peripheral 5-HT<sub>3</sub> receptors have been shown to take part in pain generation induced by an inflammatory process. However, they have no effect on pain caused by mechanical or thermal stimuli. Tissue-released 5-HT, by affecting the peripheral 5-HT<sub>3</sub> receptors, sensitizes the neurons responsible for the perception of pain caused by bradykinin (pronociceptive effect) [7, 8]. In turn, the centrally released 5-HT exerts an antinociceptive effect, e.g. by stimulating 5-HT<sub>3</sub> receptors. That effect can be blocked by the administration of the central 5-HT<sub>3</sub> receptor antagonist. In the spinal cord, the 5-HT<sub>3</sub> receptors can also modulate the function of GABA-ergic interneurons involved in pain perception [9].

## OBJECTIVE

The aim of the study was to determine whether a chemical lesion to the central noradrenergic system in an early period of individual development changes the reactivity of the 5-HT<sub>3</sub> receptors in adult rats. Behavioural tests (writhing test and formalin assay) and the 5-HT<sub>3</sub> receptor antagonist, ondansetron, were used to investigate the function of these receptors.

## MATERIALS AND METHOD

The study used male Wistar newborn and adult rats aged 8–10 weeks. The animals were kept at a constant temperature of about 22°C and 12h artificial light day/night cycle: 12 h/12 h (light from 07:00 – 19:00). Throughout the experiment, the animals had free access to water and standard diet.

The experiment was approved by the local Bioethics Committee of the Silesian Medical University (Consent No. 66 of 11 December 2007).

### Performance of the CNS lesion [10, 11]

The male newborn rats were divided into 2 groups:

**Group I (Control).** The animals received zimelidine hydrochloride at a dose of 10 mg/kg b.w. s.c. in the volume of 1.0 ml/kg b.w., and after 30 minutes, 1.0 ml/kg b.w., s.c. of 0.9% NaCl solution on day 1 and day 3 of life.

**Group II (DSP-4).** The animals were given zimelidine hydrochloride at a dose of 10 mg/kg b.w. s.c. in the volume of 1.0 ml/kg b.w., and after 30 min, DSP-4 at a dose of 50 mg/kg b.w. s.c. on day 1 and day 3 of life.

Behavioural tests aimed to assess the reactivity of the central 5-HT<sub>3</sub> receptors were performed in adult 8–10 week animals, using a selective antagonist of THE 5-HT<sub>3</sub> receptor (ondansetron). The receptor ligand doses were determined based on own experience and literature data. The behavioural tests were conducted from 08:00 – 15:00; the respective groups consisted of 8–10 animals.

**Assessment of the analgesic effect of ondansetron in models of persistent pain: writhing test – visceral pain model induced by a chemical stimulus [12].** According to the method, 24h before the experiment, rats were deprived of access to food. On the day of the test, the animals were placed individually in glass cages of 400 × 300 × 200 mm, and an ethacrynic acid solution, prepared in the ratio of 3/47

weight parts of ethanol/ /water, was injected i.p. at a dose of 3.0 mg/1.0 mL/ /100g. The solution was prepared *ex tempore*. 10 min after the acid administration, observation was started and the number of writhing episodes was counted. In the literature, these episodes [8, 9] have been defined as assuming a characteristic flat body posture with a simultaneous lateral rotation of the spine and stretching of the hind paws, the so-called writhing syndrome. The episodes were counted for 60min at 10-min intervals (10–20, 20–30, 30–40, 40–50, 50–60 min), starting from intraperitoneal injection of the irritant (ethacrynic acid). Next, for each time interval the mean value was calculated for each study group (control, DSP-4).

The writhing test was performed in the same way after administration of ondansetron (1.0mg/kg b.w., i.p.) at doses as above in the control group, and in the rats with central noradrenergic lesions. Ondansetron was injected 30 min prior to the administration of ethacrynic acid.

Based on the results, the percentage of inhibitions of writhing episodes was calculated according to the formula:

$$\% \text{ of inhibitions} = 100 - \frac{100 \times B}{A}$$

where A is the mean number of writhing episodes without the administration of the analgesic, calculated for the relevant time interval, whereas B – the mean number of writhing episodes in the respective time interval after administration of analgesic (separately for each rat)

**Formalin test – the ‘inflammatory’ pain model induced by a chemical stimulus [13].** The rats were placed singly in glass cages (400 x 300 x 200 mm) and both groups received ondansetron (1.0 mg/kg b.w., i.p.). Next, after 30 min, the animals received 50 µl of 5% formalin solution in the right paw pad. After another 5 min, the intensity of pain reaction was assessed for 70 min at 5 min intervals. Assessment was according to the following scale:

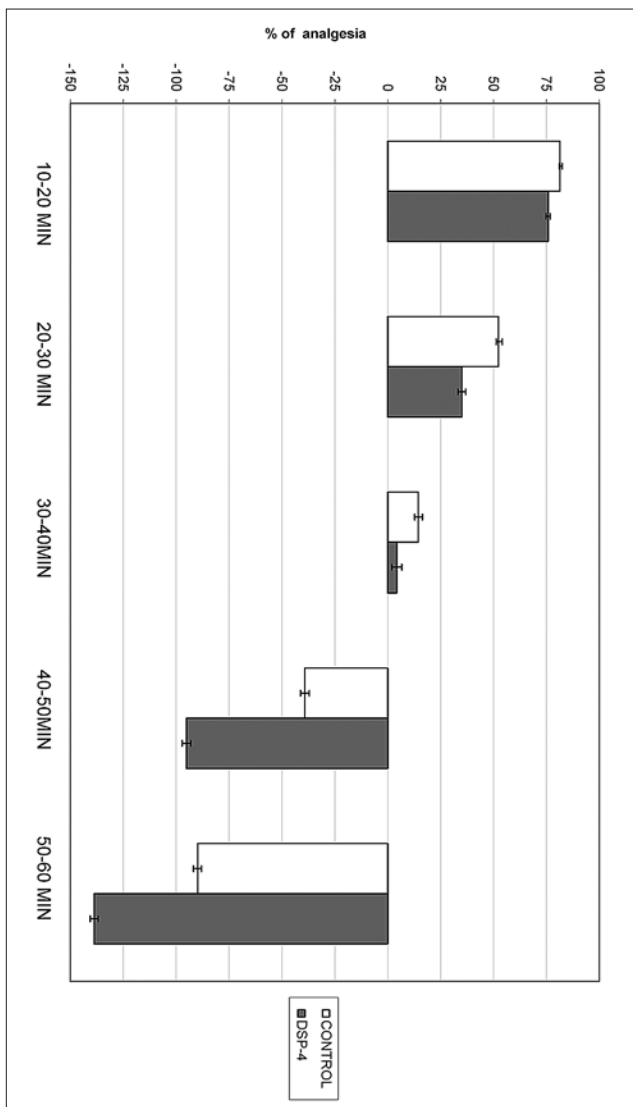
- 0 – lack of reaction;
- 1 – the animal’s paw remains on the ground, but body weight shifts to the remaining three paws;
- 2 – the paw is elevated above the ground, but body weight rests on the other three paws;
- 3 – the animal licks the raised paw, and the body weight shifts to the remaining three paws.

## RESULTS

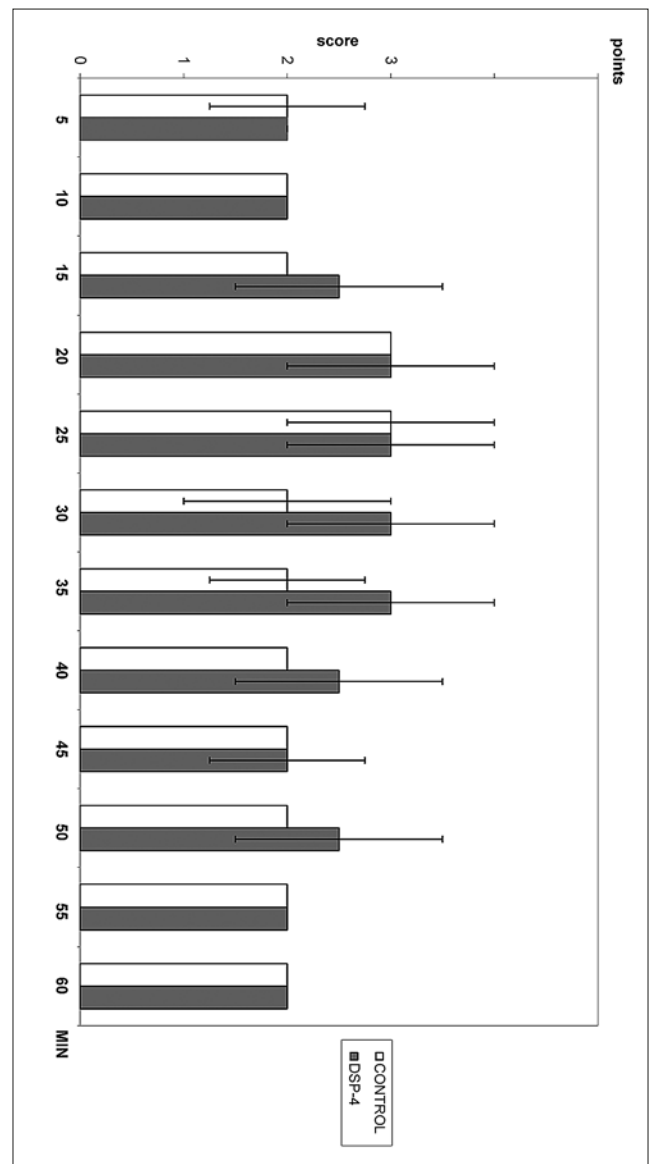
The analgesic effect of ondansetron (1.0 mg/kg b.w., i.p.) in the writhing tests was weak and of short duration. After drug administration, no significant differences in the degree of analgesia were noted between the study groups (Fig. 1).

**Formalin test.** Pain intensity score after ondansetron injection (1.0 mg/kg b.w., i.p) was 2–3 points and did not differ significantly between the study groups (Fig. 2).





**Figure 1.** Effect of DSP-4 lesion on analgesia after ondansetron (1.0 mg/kg b.w., i.p.) in the writhing test in rats ( $\bar{x} \pm \text{SEM}$ ; n=10)



**Figure 2.** Effect of DSP-4 lesion on analgesia after ondansetron (1.0 mg/kg b.w., i.p.) in the formalin test in rats ( $\bar{x} \pm \text{SEM}$ ; n=10)

## DISCUSSION

Behavioural studies conducted in the last decade have shown that the administration of 5-HT<sub>3</sub> receptor ligands to animals do not cause significant changes in their behaviour. However, these compounds modify the behavioural effects of other substances tested in the animal models of anxiety, psychoses and drug addictions [14, 15, 16, 17, 18, 19]. Nevertheless, despite many years of research, the role of 5-HT<sub>3</sub> receptors in the perception of pain has not been clearly established, and in the majority of available studies the 5-HT<sub>3</sub> ligands were applied locally (intrathecally).

Glaum et al. [20] showed that the intrathecal (i.t.) administration of the selective agonist of 5-HT<sub>3</sub> receptors, 2-methyl-5-HT, in rats had a similar analgesic effect to 5-HT in the tail immersion test, and slightly weaker than 5-HT in the hot-plate test. Previous application of selective antagonists of the 5-HT<sub>3</sub> receptor abolished the antinociceptive effect of both 5-HT and 2-methyl-5-HT. Similar results were reported by Sasaki et al. [21] in the formalin test. The analgesic effect of 5-HT<sub>3</sub> receptor stimulation in the

dorsal horn of the spinal cord has also been confirmed in electrophysiological studies. Peng et al. [22, 23] found that the response of the posterior horn neurons of the spinal cord to pain was inhibited by stimulation of the periaqueductal grey. However, the intrathecal injection of 5-HT<sub>3</sub> receptor antagonists to animals blocked this effect, which indicated the antinociceptive activation of this subtype of serotonin receptor at the spinal cord level. On the other hand, according to Xiao et al. [24], the 5-HT<sub>3</sub> serotonergic receptor is not involved in the antinociceptive effect of 5-HT. These researchers found no effect of the 5-HT<sub>3</sub> receptor antagonist injected into the spinal cord on the antinociceptive action of 5-HT. Paradoxically, other studies [25, 26, 27] revealed that the 5-HT<sub>3</sub> receptor agonists enhanced pain response.

Zeitze et al. [25] examined pain response in knock-out mice deprived of a gene encoding the 5-HT<sub>3R-A</sub> subunit in a few models of acute and chronic pain. They found that in the mutated animals the response to acute pain was similar to that in healthy animals. However, in the model of chronic pain, lack of functionally active 5-HT<sub>3</sub> receptor attenuated



the perception of pain. In turn, Giordano et al. [26] used a few antagonists of 5-HT<sub>3</sub> receptors (ICS-205-930, MDL-7222 and GR-38032F) subcutaneously, and found that these substances had no analgesic effect in the models of pain induced by thermal or mechanical stimuli, but relieved chronic pain induced by thermal stimulus. Similar findings were reported by Sufka et al. [27] who observed that ondansetron applied locally prevented the perception of pain caused by the injection of 5-HT to the animal paw.

In another study, paroxetine, a selective serotonin reuptake inhibitor (SSRI), exerted an analgesic effect similar to that of morphine in the writhing test in mice. This effect was abolished by previous administration of ondansetron (5-HT<sub>3</sub> receptor antagonists), which may indicate the involvement of spinal 5-HT<sub>3</sub> receptors in the modulation of pain perception [28].

Taking into account the above results, it seems justified that apart from typical analgesics, pain therapy should also employ substances which do not have an obvious analgesic effect, such as glyocorticosteroids [29], N-methyl-D-asparaginian (NMDA) receptor antagonists [30], endocannabinoids [31], anticonvulsants [32] and antidepressants [33], allowing pharmacological modulation of effects of analgesic drugs in the treatment of pain.

Despite the low specificity of the writhing test used in the presented study, its considerable sensitivity allows determination of the analgesic effect of both opioids and drugs with a weaker analgesic effect. Therefore, the writhing test can be used in the screening of various compounds for their potential antinociceptive action. It is believed that the behaviour of animals after intraperitoneal injection of etacrynic acid is mainly of a reflex nature, although it cannot be excluded that the visceral peritoneum also receives somatic innervation [34].

The injection of formalin into the rat paw causes a two-phase behavioural response. The first phase lasts approximately 3 min and is caused by direct nociceptor stimulation, whereas the other phase, appearing after 20–30 min of exposure to the irritant, is an inflammatory response [34]. Some authors postulate that the second phase of the formalin test also involves central mechanisms, triggered by neuronal stimulation in the first phase, although this view is not widely accepted [34, 35].

The analgesic effect of ondansetron found in the current study in the writhing test and formalin assay was weak and of short duration. The administration of this compound did not cause any significant differences in analgesia intensity between the groups of rats studied.

## CONCLUSIONS

Damage to the central noradrenergic system in the early period of individual development does not alter the antinociceptive effects of the serotonergic 5-HT<sub>3</sub> receptor antagonist, ondansetron, in the persistent pain model.

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