ORIGINAL PAPERS

Adv Clin Exp Med 2015, **24**, 3, 419–427
DOI: 10.17219/acem/23362

ISSN 1899–5276

Rafał Muchacki[1, A–C, F], Ryszard Szkilnik[2, A, B, F],
Jolanta Malinowska-Borowska[3, D–F], Aleksandra Żelazko[3, C, E, F],
Łukasz Lewkowicz[3, D–F], Przemysław G. Nowak[3, A, C–D, F]

Impairment in Pain Perception in Adult Rats Lesioned as Neonates with 5.7-Dihydroxytryptamine

[1] Beskid Oncology Center, John Paul II City Hospital in Bielsko-Biała, Poland
[2] Department of Basic Medical Sciences, Public Health Faculty, Medical University of Silesia, Bytom, Poland
[3] Toxicology and Drug Addiction Division, Department of Toxicology and Occupational Health Protection, Public Health Faculty, Medical University of Silesia, Katowice, Poland

A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. Whereas some studies have demonstrated the essential role of 5-hydroxytryptamine (5-HT) in tramadol and acetaminophen analgesia, other research has presented conflicting results. To dispel doubts, some aspects of the involvement of 5-HT in the antinociceptive properties of these drugs remain to be clarified.
Objectives. The aim of this study was to determine whether the serotoninergic system dysfunction produced by neonatal 5-HT lesion in rats may affect the antinociceptive effects of tramadol and acetaminophen administered in adulthood.
Material and Methods. Three days after birth, the control rats were pretreated with desipramine HCl (20 mg/kg *i.p.*) 30 min before intraventricular saline – vehicle injection. A separate group received 5.7-DHT; 2 × 35 μg in each lateral ventricle. At the age of 8 weeks, 5-HT and 5-hydroxyidoleaceticacid (5-HIAA) concentrations were determined in the thalamus and spinal cord by an HPLC/ED method. The antinociceptive effects of tramadol (20 mg/kg *i.p.*) or acetaminophen (100 mg/kg *i.p.*) were evaluated by a battery of tests.
Results. 5.7-DHT lesioning was associated with a reduction in 5-HT and 5-HIAA content of the thalamus (> 85% and > 90%) and spinal cord (> 58% and 70%). Neonatal 5.7-DHT treatment produced a significant reduction in the antinociceptive effect of tramadol in the hot plate, tail-immersion, paw withdrawal and writhing tests. In the formalin hind paw test, the results were ambiguous. 5-HT lesion was also associated with a decrease in the analgesic effect of acetaminophen in the hot plate and writhing tests. A similar relationship wasn't found in the other assessments conducted with the use of acetaminophen.
Conclusions. The present study provides evidence that (1) an intact serotoninergic system is required for the adequate antinociceptive action of tramadol, and (2) the serotoninergic system exerts a negligible influence on acetaminophen-induced analgesia in rats. We hypothesize that similar abnormalities in nociception may occur in patients with 5-HT dysfunction (e.g. depression), so these results should be complied in analgesic dosage adjustment (**Adv Clin Exp Med 2015, 24, 3, 419–427**).

Key words: tramadol, acetaminophen, serotonin, lesion, rats.

Pain is defined as an increased sensitivity to harmful stimulations and it can occur through both peripheral and central mechanisms. Endogenous opioids generate analgesic signals in the periaqueductal gray and these signals are projected to the rostral ventromedial medulla to be thereafter relayed to the dorsal horn of the spinal cord, which is called the descending analgesia circuit. Available literature data suggests that neurons producing serotonin (5-hydroxytryptamine, 5-HT) also contribute to the descending pain modulatory system [1]. Many studies have demonstrated reciprocal interactions between μ-opioid and adrenergic, and serotoninergic mediated mechanisms [2, 3].

However, 5-HT is an important mediator in pain modulation with its distinct receptor subtypes located at different levels of the nervous system, the sites of actions as well as underlying mechanism have not been rigorously clarified. Importantly, it has been demonstrated that the serotoninergic system may participate in the antinociceptive action of many compounds (e.g. morphine, nefopam, imipramine, desipramine and others) [4–6].

Tramadol is an analgesic drug, and its mechanism of action is believed to be mediated by the μ-opioid receptor. A further action of tramadol has been identified as blocking the reuptake of 5-HT. Despite being introduced into clinical practice in the 70's, the mechanism and biological action of this drug is still a matter of concern [7]. Likewise, the non-opiate drug nefopam acts principally by inhibiting 5-HT, norepinephrine (NE) and dopamine (DA) uptake, and its analgesic activity could be modulated by the α_1- and α_2-adrenergic receptors, the dopaminergic D_2 receptors, and the serotonergic 5-HT_{1B} and 5-HT_{2C} receptor subtypes [8, 9]. It should be kept in mind that the therapeutic effects of antidepressant drugs (e.g. desipramine) have been previously attributed to the facilitation of central monoaminergic neurotransmission. As a matter of fact, they also produce analgesia by increasing the activity of noradrenergic and serotonergic (inhibiting 5-HT and NE reuptake) projections descending from the brain to the spinal cord to modulate the release of endogenous opioid peptides, but the analgesic effect occurs at lower doses and with an earlier onset than pure antidepressant effects [10]. It is also worth knowing that, although one of the primary mechanisms of acetaminophen (paracetamol), induced analgesic and antihyperalgesic effects, relies on cyclooxygenase inhibition, experimental studies have reported on the contribution of spinal 5-HT receptors to the antinociceptive effects of this drug [11]. Recent clinical data indicates altered function in serotoninergic transmission in patients suffering from depression and insomnia, and furthermore, it has been demonstrated that 5-HT affects pain sensitivity in humans [12]. Taking the above into consideration, we hypothesized that 5-HT dysfunction may result in impairment of the antinociceptive effect evoked by the examined analgesics. Therefore, the general aim of this study was to determine whether serotoninergic system lesion in rats may affect the antinociceptive effect of tramadol and acetaminophen, two commonly prescribed and administered drugs.

Material and Methods

Animals and Treatment

Newborn male Wistar rats (University Animal Department, Katowice, Poland) were divided into 2 groups. To produce lesion of the serotoninergic system, rats from the first group were administered 5.7-dihydroxytryptamine HCl (5.7-DHT; Sigma Chemicals, St Louis, MO, USA), 70 μg dissolved in 10 μL of 0.1% ascorbic acid/0.9% NaCl; 5 μL *per side*. For noradrenergic neuron protection, the rats were pretreated with 20 mg/kg desipramine HCl (Sigma Chemicals, St Louis, MO, USA) (*i.p.*) 30 min before 5.7-DHT injection. Control animals were also pretreated with desipramine HCl and administered with the vehicle (10 μL of 0.1% ascorbic acid/0.9% NaCl; 5 μL per side). The dose and the days of treatment were selected on the basis of the reports by Brus et al. [13], Nowak et al. [14] and Jośko et al. [15]. The litters remained with dams until the 21st day after birth and then were placed in individual cages. All rats had free access to food pellets and tap water and were housed in a temperature (22 ± 1°C) and light (from 6:00 AM to 6:00 PM) controlled room.

This study was reviewed, approved and controlled by the Local Bioethics Committee for Animals at the Medical University of Silesia (permission no. 78/2007 issued on 11.12.2007) and performed in accordance with the principles and guidelines described in the NIH booklet "Care and Use of Laboratory Animals". Behavioral and biochemical studies were conducted when rats reached the age of 8 weeks.

Hot Plate Test

Antinociception was assessed according to O'Callaghan and Holtzman [16], using a hot-plate instrument (COTM, Bialystok, Poland) with the plate temperature maintained at 56 ± 0.1°C.

Each rat was placed individually with all 4 paws on the plate. Then the response latency to either a hind-paw lick or a jump was recorded. In the absence of a response, the animals were quickly removed from the 56°C hot plate at 20 s (cut-off time) to avoid tissue damage. The determined latency time for each animal was converted into the percentage of analgesia according to the formula: % analgesia = [(Tx–T0)/(Tmax–T0) × 100], where Tx was the individual latency time determined at appropriate intervals after administration of the examined analgesics, T0 was the individual latency time determined before analgesic injection, and Tmax was 20 s. The analgesic effect was measured before drug administration (after saline 1.0 mL/kg *i.p.*)

and at 30, 60, 90 and 120 min after tramadol (20 mg/kg ip) (Pliva, Kraków, Poland) or acetaminophen (100 mg/kg *i.p.*) (Bristol-Meyers Squibb, UK) injection.

Tail Immersion Test

Each rat was placed in a cone restrainer, and the end of the tail was immersed 5 cm in a 58.5°C water bath. The pain threshold was measured as the time required to elicit a flick of the tail. The cut-off time was 10 s. Reaction latency (s) was used as a parameter reflecting the intensity of the pain experienced. Tail-flick results were expressed as % of analgesia = (post-drug latency − pre-drug latency) × 100/(cut-off time − pre-drug latency) [17]. The analgesic effect was measured before drug administration (after saline 1.0 mL/kg ip) and at 30, 60, 90 and 120 min after tramadol (20 mg/kg *i.p.*) and acetaminophen (100 mg/kg *i.p.*) injection.

Paw Pressure Test

A paw pressure tester (analgesimeter, probe tip diameter 1 mm; weight 25 g; Ugo Basile Milan, Italy) was used for this study. Each rat was gently wrapped in a towel and its left hind paw was placed under the weight of the apparatus, and the test was started. A brisk foot withdrawal of the hind limb after constantly increasing pressure terminated the measurement, and the pressure (in grams) was recorded. The rats were habituated to the full procedure on two consecutive days and the experiments were conducted on the third day. The mechanical threshold was always assessed three times before drug administration to obtain a mean value. A 750 g cutoff value was used to prevent tissue damage [17]. The following formula was used to count the percentage of analgesia: % analgesia = = [(100 × B)/A – 100], where A was pressure (g) as a mean value from 3 assessments before drug administration, and B was pressure (g) assessed at 30, 60, 90, 120 min after drug treatment. The analgesic effect was measured before drug administration (after saline 1.0 mL/kg *i.p.*) and at 30, 60, 90 and 120 min after tramadol (20 mg/kg *i.p.*) and acetaminophen (100 mg/kg *i.p.*).

Footpad Inflammation Test

In the formalin test, the rats were placed individually in clear plastic containers (30 × 30 × 30 cm^3) for 30 min to allow them to accommodate to their new surroundings. A mirror was placed at a 45° angle beneath the chamber to provide an unobstructed view of the rat's paws. The animals were then removed and injected with tramadol (20 mg/kg *i.p.*) or acetaminophen (100 mg/kg *i.p.*) and placed back into the containers. 30 min later, the rats were injected in the right hind paw plantar surface subcutaneously (sc) using a 30-gauge syringe 50 μL of 5% formalin solution. The animals were then returned to the chambers, and the nociceptive behavior was observed immediately after formalin injection. Nociceptive behavior was quantified using the scale 0–3 points. Formalin-induced pain is biphasic. The initial acute phase (0–10 min) is followed by a relatively short quiescent period, which is then followed by a prolonged tonic response (15–60 min). A reduction of formalin-induced behavior observed after administration of a given drug is interpreted as an analgesic response [17, 18].

Writhing Test

Control and 5.7-DHT lesioned rats (deprived of food 24 h before testing) were placed individually in clear Plexiglas containers (40 × 30 × 20 cm) and allowed to accommodate for 30 min. Then rats were injected with saline (1.0 mL/100g *ip*) and 30 min later, administered with ethacrynic acid solution 3.0 mg/1 mL/100 g (in the left lower quadrant of the abdomen). Ethacrynic solution was prepared ex tempore by mixing 3/47 ethanol/water. The rats were returned to their chambers and 10 min later contractions of abdominal musculature (writhes) were counted (contractions of the abdomen, twisting and turning of the trunk, arching of the back and extension of the hind limbs) for the following 60 min with divisions of 10 min intervals (10–20, 20–30, 30–40, etc.). The rats were used once and killed immediately after testing [17]. According to the same paradigm, a separate groups of rats (control and 5.7-DHT) were tested after tramadol (20 mg/kg ip) and acetaminophen (100 mg/kg *i.p.*), respectively. The antinociceptive effect was expressed as the percentage decrease in the number of writhes and was calculated according to the formula: % inhibition of writhing = [100 – (100 × B)/A], where A was the mean number of writhes in the saline-treated control and 5.7-DHT rats for an appropriate observation period, and B was the mean number of writhes in the drug-treated rats counted at the appropriate observation intervals.

Assay of Biogenic Amines and Their Metabolites

The rats were injected with saline (1.0 mL/kg *i.p.*), tramadol (20 mg/kg *i.p.*) or acetaminophen (100 mg/kg *i.p.*) and 60 min later decapitated. The thalamus and spinal cord were rapidly dissected and placed on dry ice, and weighed and stored at –70°C for pending

assay. The levels of 5-HT and its metabolite (5-HIAA) were assessed using a high pressure liquid chromatography with the electrochemical detection method (HPLC/ED). Samples were homogenized for 15–20 s in ice-cold trichloroacetic acid (0.1 M) containing 0.05 mM ascorbic acid. After centrifugation (5,000 g, 5 min), supernatants were filtered through 0.2 μm cellulose membranes (Titan MSF Microspin filters, Scientific Resources Inc., Eatontown, UK) and injected into a system that consisted of a thermally controlled ASI-100 autosampler (Thermo Scientific, UK). For separation and detection of biogenic amines, a precolumn Hypersil BDS C18, 10 mm × 4 mm and 3 μm (ThermoQuest, GB), column Hypersil BDS C18, 250 mm × 4 mm, 6 mm, 3 μm (ThermoQuest, GB) and an electrochemical detector analytic cell 5010 Coulochem (ESA, Inc., USA) were used. The mobile phase was composed of 1.7 mM 1-octanesulfonic acid, 25 μM EDTA, 100 μL trietylamine/1000 mL, and 10% acetonitrile in 75 mM phosphate buffer at pH 3. The flow rate was 0.6 mL/min. The applied potential was E1 = –175 mV and E2 = + 250 mV. The chromatograms were automatically integrated by universal chromatographic interface UCI-100. The data was quantified using the area under the peaks and external standards, using Chromeleon software (Dionex, Germany). The obtained results of the catecholamine assay were presented in ng per gram of wet tissue (ng/g) [19, 20].

Data Analysis

Differences between groups were assessed by a *t*-Student test. A p value < 0.01 and < 0.05 was taken as the level of significance.

Results

Hot-Plate Test

Tramadol (20 mg/kg *i.p.*) elicited a lower antinociceptive effect in 5.7-DHT rats in comparison to control animals, and the differences were significant at 60, 90 and 120 min of observation ($p < 0.05$; $p < 0.01$ and $p < 0.01$, respectively) (Fig. 1).

Also, the acetaminophen produced a lower antinociceptive effect in the 5.7-DHT group in comparison to control rats, and the effect was significant at all observation time points, i.e.: 30, 60, 90 and 120 min ($p < 0.01$; $p < 0.05$; $p < 0.01$ and $p < 0.05$, respectively) (Fig. 2).

Tail Immersion Test

In the tail immersion test we demonstrated that tramadol administered in a dose of 20 mg/kg *i.p.* evoked intense and long-lasting analgesia in the control group (50–90% on average). As has been noted, the effect in the 5.7-DHT group was far less pronounced and significant differences were observed in all tested intervals (30 min $p < 0.05$; 60 min $p < 0.01$; 90 min $p < 0.01$ and 120 min $p < 0.05$) (Fig. 3).

The analgesic action of acetaminophen (100 mg/kg *i.p.*) in both tested groups was much less evident (in comparison to the tramadol study) and no significance between the tested groups was found (Fig. 4).

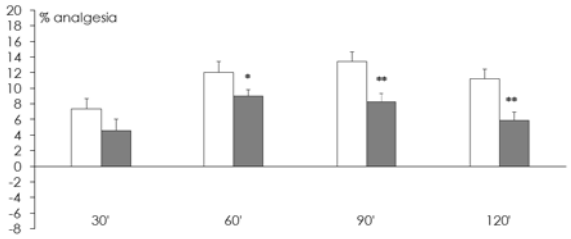

Fig. 1. Effect of 5.7-DHT treatment on analgesia assessed in hot plate test after tramadol administration (20 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

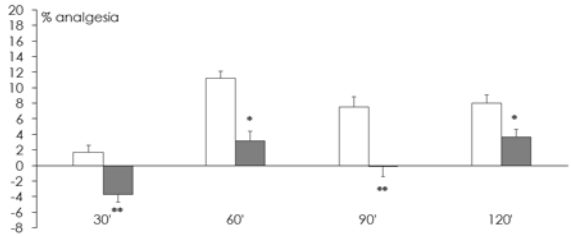

Fig. 2. Effect of 5.7-DHT treatment on analgesia assessed in hot plate test after acetaminophen administration (100 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

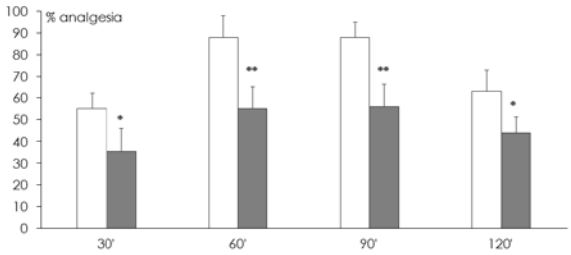

Fig. 3. Effect of 5.7-DHT treatment on analgesia assessed in tail-immersion test after tramadol administration (20 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

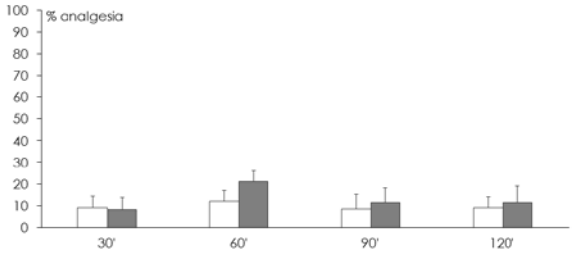

Fig. 4. Effect of 5.7-DHT treatment on analgesia assessed in tail-immersion test after acetaminophen administration (100 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

Paw Pressure Test

In the paw pressure test we showed that tramadol (20 mg/kg *i.p.*) elicited more intense analgesia in control rats in comparison to the 5.7-DHT group. The obtained difference was significant at 30, 60 and 90 min of the observation ($p < 0.05$) (Fig. 5).

The acetaminophen (100 mg/kg ip) challenge evoked similar antinociceptive action in both tested groups (Fig. 6).

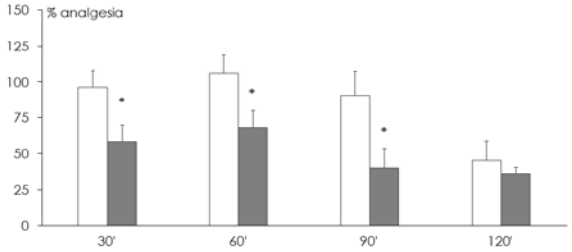

Fig. 5. Effect of 5.7-DHT treatment on analgesia assessed in paw pressure test after tramadol administration (20 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

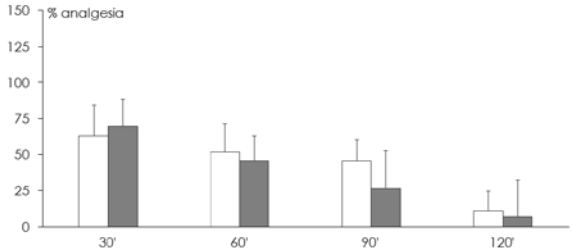

Fig. 6. Effect of 5.7-DHT treatment on analgesia assessed in paw pressure test after acetaminophen administration (100 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

Footpad Inflammation Test

To assess the effect of serotoninergic lesion on the analgesic action of the examined drugs, we compared the behavioral responses to sc injection of 50 μL (5%) of formalin into one hind paw of the control and 5.7-DHT rats. Tramadol (20 mg/kg ip) was administered 30 min before formalin administration. Both groups showed the typical biphasic nocifensive response curve lasting the 60 min of testing but the control rats scored more points (spending more time licking/biting the injected hind paw) in the second phase of the formalin test than the 5.7-DHT group ($p < 0.05$ at 25 and 30 min) (Fig. 7).

After acetaminophen administration (100 mg/kg ip) a typical biphasic nocifensive response curve was also observed, however no differences between the control and 5.7-DHT treated rats were noticed (Fig. 8).

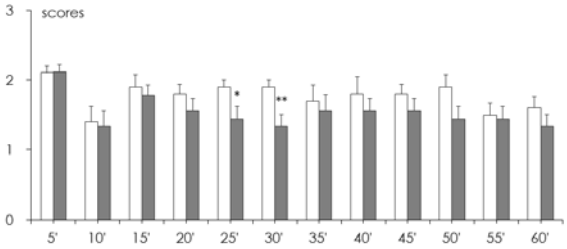

Fig. 7. Effect of 5.7-DHT treatment on analgesia assessed in footpad inflammation test after tramadol administration (20 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

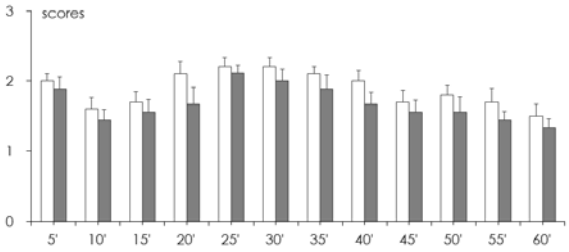

Fig. 8. Effect of 5.7-DHT treatment on analgesia assessed in footpad inflammation test after acetaminophen administration (100 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

Writhing Test

Injections of tramadol (20 mg/kg *i.p.*) elicited lower analgesia in the 5.7-DHT group in comparison to control rats. The effect was significant at 20–30, 30–40 and 50–60 intervals of observation ($p < 0.05$) (Fig. 9).

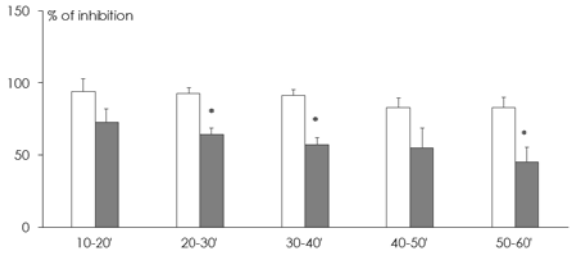

Fig. 9. Effect of 5.7-DHT treatment on analgesia assessed in writhing test after tramadol administration (20 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

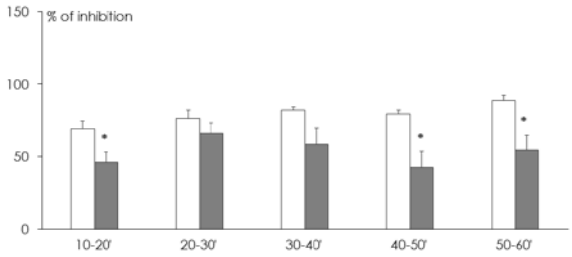

Fig. 10. Effect of 5.7-DHT treatment on analgesia assessed in writhing test after acetaminophen administration (100 mg/kg *i.p.*) in rats (n = 10)

control
5.7-DHT

* $p < 0.05$; ** $p < 0.01$ control vs. 5.7-DHT.

Acetaminophen administered at a dose of 100 mg/kg *i.p.* also reduced writhes in both experimental groups, but to a much greater extent in the control group. The effect was significant at 10–20, 40–50 and 50–60 intervals of testing ($p < 0.05$) (Fig. 10).

Biogenic Amines and Their Metabolites Content

In the HPLC/ED assay we demonstrated that 5.7-DHT icv injection to neonates (3 days after birth) resulted in a marked decrease in 5-HT and 5-HIAA levels in the thalamus and spinal cord in comparison to vehicle treated rats (differences significant at $p < 0.01$). Tramadol (20 mg/kg *i.p.*) administered 60 min before decapitation did not affect 5-HT and 5-HIAA (in both tested structures) in the control and 5.7-DHT rats in comparison to their respective controls. Conversely, acetaminophen (100 mg/kg) decreased 5-HIAA in comparison to the control group (5.7-DHT – saline) but there was no effect on 5-HT in this regard (Table 1).

Discussion

The major finding of the present study is that neonatal 5.7-DHT treatment – resulting in permanent destruction of serotoninergic inputs to the spinal cord and thalamic areas – diminishes tramadol- and to some extent acetaminophen-induced antinociception in adult rats.

As has been noted, administration of 5.7-DHT neurotoxin caused a prominent reduction in 5-HT and its metabolite (5-HIAA) content of the thalamus and spinal cord, two crucial structures involved in pain perception. In the thalamus, 5-HT and 5-HIAA were reduced to 15% and 10% in the 5.7-DHT treated group in comparison to control animals, whereas in the spinal cord to 42% (5-HT) and 30% (5-HIAA), respectively. In agreement with previous studies [5], we concluded that our treatment caused a substantial reduction in 5-HT and 5-HIAA (Table 1). These findings confirmed that 5.7-DHT has a demolishing effect on the central serotonergic system in rats (both in the brain and spinal cord) [4, 14].

According to the literature data, tramadol has a dual mechanism of action: (1) connected with the μ-opioid receptor, and (2) bound with the blocking of the reuptake of 5-HT [7]. In our study it was shown that 5.7-DHT treatment significantly decreased the antinociceptive effect of tramadol (20 mg/kg *i.p.*) in the hot-plate, tail-immersion, paw pressure and writhing tests

Table 1. Effect of 5.7-DHT treatment on 5-HT and 5-HIAA content in the thalamus and spinal cord in adult rats (n = 7–8)

Structure	Biogenic amines (ng/g wet tissue)	Treatment					
		saline		tramadol		acetaminophen	
		control	5,7-DHT	control	5,7-DHT	control	5,7-DHT
Thalamus	5-HT	697.2 ± 40.2	100.0 ± 6.5#	694.6 ± 55.4	76.7 ± 14.5#	707.7 ± 57.5	46.6 ± 4.1#*
	5-HIAA	312.3 ± 23.1	32.4 ± 3.4#	279.2 ± 23.5	23.3 ± 3.6#	278.4 ± 25.5	16.6 ± 2.1#*
Spinal cord	5-HT	607.5 ± 55.3	254.1 ± 29.0#	703.6 ± 29.6	231.8 ± 50.4#	699.6 ± 52.4	170.5 ± 56.4#*
	5-HIAA	419.0 ± 35.1	123.0 ± 28.0#	395.4 ± 31.0	105.8 ± 22.2#	428.0 ± 56.1	55.2 ± 7.1#*

(Fig. 1–6, 9, 10). Ambiguous results were obtained in the formalin test (Fig. 7). Yanarates et al. [21] found that an intrathecal injection of 5.7-DHT (lesion of spinal 5-HT) resulted in a significant reduction of systemically administered tramadol and its metabolite (M1) antinociceptive and antihyperalgesic effects in the radiant heat tail-flick and plantar incision tests in mice. In contrast, Rojas-Corrales et al. [22] demonstrated that inhibition of the 5-HT neurotransmission attained by a 5-HT_{1A} agonist (8-OH-DPAT) challenge reduced the analgesic effect of tramadol in two nociceptive tests: a hot plate test in mice and a plantar test in rats. Dürsteler et al. [23] showed that ondansetrone (5-HT_3 receptor agonist) antagonized the analgesic effects of tramadol in acetic acid writhing tests in mice whereas Oliva et al. [24], employing the formalin test in mice, found that the analgesic effect of tramadol was prevented by the 5-HT_2 receptor the antagonist ketanserin. They concluded that the serotonergic pathway is responsible for the antinociceptive effect of tramadol in phase 2 of the formalin test, and that this effect is mediated by 5-HT_2 receptors. Others demonstrated that the lesion of the dorsal raphe nucleus (DRN) by 5.7-DHT antagonized the antidepressant-like effects of tramadol assessed in some unpredictable chronic mild stress tests in mice [25]. Taking the above into consideration and keeping in mind the results of the present study, we can conclude that serotonergic system integrity is critically involved not only in the analgesic but also in the antidepressant-like effects of tramadol.

For the past several decades numerous studies have indicated that acetaminophen was regarded as a preferential inhibitor of central cyclooxygenases to peripheral ones, but the inhibition of cyclooxygenase is not sufficient to explain its antinociceptive activity. Some reports imply that the analgesic effect of acetaminophen might come from the interaction of other multiplied neurotransmitters, like those involved in serotonergic, opioidergic, noradrenergic, cholinergic, and nitric oxide synthase systems [26, 27]. One of the hypotheses relies on its positive action on the inhibitory activity of the descending serotonergic pathways on spinal nociceptive processing, which has been supported by different groups [28, 29]. Indeed, the lesion of the bulbospinal descending serotonergic pathways abolishes the antinociceptive action of acetaminophen [30]. In our experiment it was shown that the 5.7-DHT lesion visibly affected the antinociceptive effect of acetaminophen (100 mg/kg *i.p.*) examined in the hot plate and writhing tests being at the same time without effect on other measurements (Fig. 2 and 10). This is to some extent in accordance with Pini et al. [28] who showed that depletion of brain 5-HT with p-chlorophenylalanine (p-CPA) prevented the antinociceptive effect of acetaminophen in the hot-plate test and in the first phase of the formalin response in rats. Conversely, the same group of scientists demonstrated that pre-treatment with ondansetron at doses of 2 and 4 mg/kg (5-HT_3 receptor antagonist) did not affect the antinociceptive activity of acetaminophen in the hot-plate test and in the paw pressure test in rats [31]. Bonnefont et al. [29] found that the oral administration of acetaminophen (400 mg/kg) reduced nociceptive behavior in both phases of the formalin test in rats which was totally blocked by an intrathecal injection of a selective 5-HT_{1A} receptor antagonist (WAY 100,635). In our experiments we did not observe such effect (Fig. 8). The discrepancy may be explained by the fact that a much lower dose of acetaminophen was used in the present study. Others have also demonstrated that the fixed combination of aspirin, acetaminophen and caffeine produced a significant reduction in extracellular DA and a dramatic increase in NA release from the striatal slices suggesting that the mechanism of this analgesic combination is based on the modulation of catecholaminergic neurotransmission [32]. Also, acetaminophen administration significantly increased 5-HT and NA levels in the posterior cortex, hypothalamus, striatum, hippocampus and brain stem, but not spinal cord which is more or less in line with our results (no effect of acetaminophen in the thalamus and spinal cord on 5-HT and 5-HIAA in control but a decrease in 5-HT and 5-HIAA in 5.7-DHT rats) [33]. Altogether, the data cited above and the results of the present study suggest that acetaminophen affects central monoaminergic neurotransmission, thereby it can be concluded that monoamines (including 5-HT) might to some extent participate in its analgesic action.

Summing up, the results of the current study provide evidence that (1) an intact serotoninergic system is required for proper antinociceptive action of the tramadol, and (2) the serotoninergic system exerts a negligible influence on acetaminophen analgesia in rats. It is likely that similar abnormalities in nociception may occur in patients with serotoninergic system dysfunction, so these results should be complied in analgesic dosage adjustment.

References

[1] **Argoff C:** Mechanisms of pain transmission and pharmacologic management. Curr Med Res Opin 2011, 27, 2019–2031.

[2] **Brus R, Nowak P, Szkilnik R, Mikolajun U, Kostrzewa RM:** Serotoninergics attenuate hyperlocomotor activity in rats. Potential new therapeutic strategy for hyperactivity. Neurotox Res 2004, 6, 317–325.

[3] **Lopez-Gimenez JF, Vilaro MT, Milligan G:** Morphine desensitization, internalization, and down-regulation of the mu opioid receptor is facilitated by serotonin 5-hydroxytryptamine2A receptor coactivation. Mol Pharmacol 2008, 74, 1278–1291.

[4] **Korossy E, Świerszcz M, Nowak E, Beśka M, Bałasz M, Nowak P, Szkilnik R:** Antinociceptive effects of morphine, nefopam, indomethacin and imipramine in rats with lesion of the central serotoninergic system. Ann Acad Med Siles 2009, 63, 7–24.

[5] **Świerszcz M, Nowak P, Bałasz A, Walawender I, Kasperski J, Skaba D, Nowak E, Szkilnik R:** Effect of neonatal serotonin depletion on morphine-, nefopam-, indomethacin- and imipramine-induced analgesia in tests of thermal and mechanical pain in adult rats. Adv Clin Exp Med 2010, 19, 33–41.

[6] **Roczniak W, Wróbel J, Dolczak L, Nowak P:** Influence of central noradrenergic system lesion on the serotoninergic 5-HT3 receptor mediated analgesia in rats. Adv Clin Exp Med 2013, 22, 629–638.

[7] **Reeves RR, Burke RS:** Tramadol: basic pharmacology and emerging concepts. Drugs Today (Barc) 2008, 44, 827–836.

[8] **Girard P, Coppé MC, Verniers D, Pansart Y, Gillardin JM:** Role of catecholamines and serotonin receptor subtypes in nefopam-induced antinociception. Pharmacol Res 2006, 54, 195–202.

[9] **Beśka M, Walawender I, Kasperski J, Skaba D, Nowak P, Reichman-Warmuz G, Szkilnik R:** Biological peculiarities of the analgesic drug nefopam in rats. Adv Clin Exp Med 2010, 19, 21–31.

[10] **Dharmshaktu P, Tayal V, Kalra BS:** Efficacy of antidepressants as analgesics: a review. J Clin Pharmacol 2012, 52, 6–17.

[11] **Dogrul A, Seyrek M, Akgul EO, Cayci T, Kahraman S, Bolay H:** Systemic paracetamol-induced analgesic and antihyperalgesic effects through activation of descending serotonergic pathways involving spinal 5-HT_7 receptors. Eur J Pharmacol 2012, 677, 93–101.

[12] **Ohayon MM:** Pain sensitivity, depression, and sleep deprivation: links with serotoninergic dysfunction. J Psychiatr Res 2009, 43, 1243–1245.

[13] **Brus R, Kostrzewa RM, Perry KW, Fuller RW:** Supersensitization of the oral response to SKF 38393 in neonatal 6-hydroxydopamine-lesioned rats is eliminated by neonatal 5,7-dihydroxytryptamine treatment. J Pharmacol Exp Ther 1994, 268, 231–237.

[14] **Nowak P, Nitka D, Kwieciński A, Jośko J, Drab J, Pojda-Wilczek D, Kasperski J, Kostrzewa RM, Brus R:** Neonatal co-lesion by DSP-4 and 5,7-DHT produces adulthood behavioral sensitization to dopamine D(2) receptor agonists. Pharmacol Rep 2009, 61, 311–408.

[15] **Jośko J, Drab J, Nowak P, Szkilnik R, Körossy E, Boroń D, Brus H, Kostrzewa RM, Brus R:** Thioperamide, an H_3 receptor antagonist prevents [^{3}H]glucose uptake in brain of adult rats lesioned as neonates with 5,7-dihydroxytryptamine. Neurotox Res 2011, 20, 93–96.

[16] **O'Callaghan JP, Holtzman SG:** Quantification of the analgesic activity of narcotic antagonists by a modified hotplate procedure. J Pharmacol Exp Ther 1975, 192, 497–505.

[17] **Korossy-Mruk E, Kuter K, Nowak P, Szkilnik R, Rykaczewska-Czerwinska M, Kostrzewa RM, Brus R:** Neonatal DSP-4 treatment modifies antinociceptive effects of the CB1 receptor agonist methanandamide in adult rats. Neurotox Res 2013, 23, 39–48.

[18] **Nowak P, Kowalińska-Kania M, Nowak D, Kostrzewa RM, Malinowska-Borowska J:** Antinociceptive effects of H_3 (R-methylhistamine) and GABA(B) (baclofen)-receptor ligands in an orofacial model of pain in rats. Neurotox Res 2013, 24, 258–264.

[19] **Nowak P, Szczerbak G, Biedka I, Drosik M, Kostrzewa RM, Brus R:** Effect of ketanserin and amphetamine on nigrostriatal neurotransmission and reactive oxygen species in Parkinsonian rats. *In vivo* microdialysis study. J Physiol Pharmacol 2006, 57, 583–597.

[20] **Szkilnik R, Brus R, Malinowska-Borowska J, Nowak D, Waliczek M, Kostrzewa RM, Nowak P:** Ontogenetic manganese exposure with perinatal 6-OHDA lesioning alters behavioral responses of rats to dopamine D1 and D2 agonist treatments. Environ Toxicol Pharmacol 2014, 37, 28–36.

[21] **Yanarates O, Dogrul A, Yildirim V, Sahin A, Sizlan A, Seyrek M, Akgül O, Kozak O, Kurt E, Aypar U:** Spinal 5-HT7 receptors play an important role in the antinociceptive and antihyperalgesic effects of tramadol and its metabolite, O-Desmethyltramadol, via activation of descending serotonergic pathways. Anesthesiology 2010, 112, 696–710.

[22] **Rojas-Corrales MO, Ortega-Alvaro A, Gibert-Rahola J, Roca-Vinardell A, Micó JA:** Pindolol, a beta-adrenoceptor blocker/5-hydroxytryptamine(1A/1B) antagonist, enhances the analgesic effect of tramadol. Pain 2000, 88, 119–124.

[23] **Dürsteler C, Mases A, Fernandez V, Pol O, Puig MM:** Interaction between tramadol and two anti-emetics on nociception and gastrointestinal transit in mice. Eur J Pain 2006, 10, 629–638.

[24] **Oliva P, Aurilio C, Massimo F, Grella A, Maione S, Grella E, Scafuro M, Rossi F, Berrino L:** The antinociceptive effect of tramadol in the formalin test is mediated by the serotonergic component. Eur J Pharmacol 2002, 445, 179–185.

[25] Yalcin I, Coubard S, Bodard S, Chalon S, Belzung C: Effects of 5,7-dihydroxytryptamine lesion of the dorsal raphe nucleus on the antidepressant-like action of tramadol in the unpredictable chronic mild stress in mice. Psychopharmacology 2008, 200, 497–507.
[26] Toussaint K, Yang XC, Zielinski MA, Reigle KL, Sacavage SD, Nagar S, Raffa RB: What do we (not) know about how paracetamol (acetaminophen) works? J Clin Pharm Ther 2010, 35, 617–638.
[27] Graham GG, Davies MJ, Day RO, Mohamudally A, Scott KF: The modern pharmacology of paracetamol: therapeutic actions, mechanism of action, metabolism, toxicity and recent pharmacological findings. Inflammopharmacology 2013, 21, 201–232.
[28] 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 1996, 308, 31–40.
[29] Bonnefont J, Alloui A, Chapuy E, Clottes E, Eschalier A: Orally administered paracetamol does not act locally in the rat formalin test: evidence for a supraspinal, serotonin-dependent antinociceptive mechanism. Anesthesiology 2003, 99, 976–981.
[30] Tjolsen A, Lund A, Hole K: Antinociceptive effect of paracetamol in rats is partly dependent on spinal serotonergic systems. Eur J Pharmacol 1991, 193, 193–201.
[31] Sandrini M, Pini LA, Vitale G: Differential involvement of central 5-HT1B and 5-HT3 receptor subtypes in the antinociceptive effect of paracetamol. Inflamm Res 2003, 52, 347–352.
[32] Fiebich BL, Candelario-Jalil E, Mantovani M, Heinzmann M, Akundi RS, Hüll M, Knörle R, Schnierle P, Finkenzeller G, Aicher B: Modulation of catecholamine release from rat striatal slices by the fixed combination of aspirin, paracetamol and caffeine. Pharmacol Res 2006, 53, 391–396.
[33] Courade JP, Caussade F, Martin K, Besse D, Delchambre C, Hanoun N, Hamon M, Eschalier A, Cloarec A: Effects of acetaminophen on monoaminergic systems in the rat central nervous system. Naunyn Schmiedebergs Arch Pharmacol 2001, 364, 534–537.

Address for correspondence:

Przemysław Nowak
Department of Toxicology and Health Protection
Public Health Faculty
Medical University of Silesia
Medyków 18
40-752 Katowice Ligota
Poland
Tel.: +48 32 088 743
E-mail: pnowak@sum.edu.pl

Conflict of interest: None declared

Received: 17.02.2014
Revised: 1.05.2014
Accepted: 24.06.2014