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Influence of Central Noradrenergic System Lesion on the Serotoninergic 5-HT3 Receptor Mediated Analgesia in Rats

Wpływ lezji ośrodkowego układu noradrenergicznego na działanie przeciwbólowe zależne od receptorów serotoninergicznych 5-HT$_3$ u szczurów

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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. Monoaminergic pathways, impinging an adrenergic and 5-HT3 serotonin receptors, modulate nociceptive transmission, but their mechanisms and interactions has not been clarified yet.
Objectives. The study was designed to investigate the influence of the neonatal noradrenergic system lesion on the antinociceptive effect of 5-HT3 receptor ligands assessed in adult animals.
Material and Methods. Intact male rats were contrasted with rats in which noradrenergic nerve terminals were largely destroyed shortly after birth with neurotoxin DSP-4 [(N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine; 50 mg/kg × 2 subcutaneously (*sc*)], on the 1st and 3rd days of postnatal life. Control animals were injected with saline (1.0 mL/kg sc). When the rats attained 10 weeks of age, painful reactions were assessed by means of writhing and formalin tests after intraperitoneal (*ip)* administration of 1-phenylbiguanid (FBG; 7.5 mg/kg) or ondansetrone (1.0 mg/kg) with FBG (7.5 mg/kg). Morphine was used as a model analgesic drug.
Results. Injections of morphine (7.5 mg/kg *sc*) evoked similar antinociception in the visceral pain model (writhing test) in both tested groups (control and DSP-4). In control rats, a 5-HT$_3$ receptor agonist FBG (7.5 mg/kg) elicited analgesia similar to that of morphine but the effect was significantly lower in DSP-4 treated animals. A 5-HT$_3$ receptor antagonist ondansetrone (1.0 mg/kg) injected before FBG did not modify the effect in the control but suppressed it in the DSP-4 group. In the formalin test, morphine produced higher analgesia in control rats in comparison with the DSP-4 group (pain intensity score of 1 point vs. 2-3 points, respectively). Ondansetrone injected before FBG alleviated the observed effect.
Conclusions. Based on the obtained results, we concluded that the neonatal DSP-4 treatment alters the antinociceptive effects of morphine and serotoninergic 5-HT$_3$ receptor ligands. The above may explain altered (diminished) reactions of analgesics applied to patients with noradrenergic system dysfunction (e.g. depression and/or anxiety disorders) (**Adv Clin Exp Med 2013, 22, 5, 629–638**).

Key words: 5-HT3 receptor, DSP-4, analgesia, noradrenergic system, rats.

Streszczenie

Wprowadzenie. Ośrodkowe układy monoaminergiczne działając poprzez receptory adrenergiczne i 5-HT$_3$, modulują percepcję bólu, mechanizmy i interakcje między nimi nie zostały jednak ostatecznie zdefiniowane.
Cel pracy. Celem pracy była oceny wpływu lezji ośrodkowego układu noradrenergicznego wykonanej u noworodków szczurzych na działanie przeciwbólowe pośredniczone przez receptory serotoninergiczne 5-HT$_3$ oceniane u dorosłych zwierząt.
Materiał i metody. Szczurze noworodki szczepu Wistar 1. i 3. dnia życia otrzymały iniekcję neurotoksyny DSP-4

[N-(2-chloroetylo)-N-etylo-2-bromo-benzylaminy] w dawce 50 mg/kg × 2 podskórnie (*sc*) w celu trwałego zniszczenia ośrodkowego układu noradrenergicznego. Zwierzęta z grupy kontrolnej otrzymały 0,9% roztwór NaCl (1,0 ml/kg *sc*). Po osiągnięciu 10 tygodni wykonano testy oceniające przeciwbólowe działanie ligandów receptora 5-HT_3 w modelach uporczywego bólu (test wicia, test formalinowy), po dootrzewnowym (*ip*) podaniu agonisty ośrodkowego receptora serotoninergicznego 5-HT_3 (1-fenylbiguanidu FBG; 7,5 mg/kg) oraz łącznym podaniu antagonisty receptora 5-HT_3 (ondansetronu; 1,0 mg/kg) i FBG (7,5 mg/kg). Jako „modelową" substancję przeciwbólową zastosowano morfinę.

Wyniki. Morfina (7,5 mg/kg *sc*) w teście wicia wywierała silne i długotrwałe działanie analgetyczne, zarówno u szczurów kontrolnych, jak i u zwierząt z lezją DSP-4. FBG w dawce (7,5 mg/kg mc *ip*) u szczurów kontrolnych działał przeciwbólowo podobnie jak morfina. U zwierząt z lezją DSP-4 efekt ten był istotnie statystycznie słabszy. Ondansetron zastosowany 30 min przed podaniem FBG nie wpływał na działanie przeciwbólowe tego związku w grupie kontrolnej, bardzo natomiast hamował jego działanie analgetyczne u szczurów po lezji DSP-4. W teście formalinowym morfina działała silnie analgetycznie u zwierząt kontrolnych. Średnia ocena punktowa natężenia bólu nie przekraczała 1 punktu. U szczurów z lezją DSP-4 działanie przeciwbólowe morfiny było istotnie słabsze. Po podaniu FBG w analogicznej dawce do morfiny natężenie bólu w grupie kontrolnej oceniono na 2–3 punkty. Podobnie jak w przypadku morfiny, działanie analgetyczne FBG było istotnie słabsze u zwierząt z lezją DSP-4 i efekt ten był antagonizowany przez ondansetron.

Wnioski. Uszkodzenie ośrodkowego układu noradrenergicznego zmienia przeciwbólowe działanie morfiny oraz agonisty receptorów 5-HT_3. Powyższe może pośrednio tłumaczyć zmienioną (zmniejszoną) reakcję na leki przeciwbólowe u pacjentów z zaburzeniami ośrodkowego układu noradrenergicznego (np. u chorych na depresje lub mających zaburzenia lękowe) **(Adv Clin Exp Med. 2013, 22, 5, 629–638).**

Słowa kluczowe: receptor serotoninergiczny 5-HT_3, DSP-4, analgezja, układ noradrenergiczny, szczury.

Chronic pain causes very rapid adaptive changes in the CNS involving the activation of a number of systems both supporting and inhibiting pain sensation. Considering the cause of pain, nociceptive, neurogenic and psychogenic pains can be distinguished [1]. Nociceptive pain is induced by the stimulation of pain receptors (nociceptors) in the peripheral nervous system that gives rise to the signals conducted by two types of nerve fibers: fast myelinated *A* δ axons and free unmyelinated *C* fibers. Type *C* fibers are very thin and susceptible to damage, do not have myelin sheaths and conduct pain stimuli very slowly.

Their activation triggers a gradually increasing dull or burning pain. The fibers are very large and they branch, innervating large areas of the body, making it impossible for the patient to localize the pain [2]. They conduct electrical impulses induced by mechanical, thermal and chemical stimuli, and there are different types of receptors expressed at terminals – predominantly the opioid ones [3]. These receptors are inactive until being excited by a number of substances released from damaged tissues, such as bradykinin, histamine, 5-HT, ATP, potassium ions, leukotrienes and prostaglandins [4], which cause the release of opioids affecting the opioid receptors. The most important post-synaptic receptors mediating the perception of pain are: NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxy-5-methyl-4--isoxazolepropionic acid) and NK-1 for substance *P*. The modulating effect on the release of glutamate and substance *P* is exerted by presynaptic receptors: opioid δ and μ, adrenergic α2, $GABA_B$ and serotonin 5-HT_2 and 5-HT_3 receptors. The opioid receptors are located in both the presynaptic membrane and in a smaller amount in the post-synaptic membrane. These neurotransmitter systems may therefore provide targets for pharmacological modification of pain signal conduction [5].

The central noradrenergic system mediates the inhibition of pain, particularly chronic pain [6]. There is evidence showing its involvement in the mechanism of the phenomena accompanying chronic pain, such as anxiety and depression. It has been shown that functional hypothyroidism of the noradrenergic neurons giving projections to the cerebral cortex and the limbic system plays a major role in the pathogenesis of endogenous depression. Noradrenaline (NA) is a neurotransmitter released from axonal terminals of neurons located in locus coeruleus, which reach almost all areas of the brain. It is involved in the control of neuronal activity and is responsible for the formation of different behavioral states. It also participates in the regulation of hormonal activities, learning and appetite processes [7–11]. Similarly, the central serotonergic system is involved in the regulation of many bodily functions such as sleep, wakefulness, blood pressure, pain perception and sexual behaviors. It is also assumed to be involved in the pathogenesis of depression, anxiety and addiction, as well as migraine and other headaches.

One of the receptors of this system is the 5-HT_3 receptor built, like other ionotropic receptors, out of four transmembrane segments (M1-M4) forming the pentameric structure. Biochemical and structural properties of the 5-HT_3 receptor show high intertissue and interspecies variation caused by differences in its structure and properties in various animal species. The density of the 5-HT_3 receptors in the CNS is limited. Their

expression has been found in the lower part of the brain stem (i.e. dorsal nucleus of the vagus nerve, solitary tract nucleus) and the local area postrema. In the limbic system these receptors are located in the hippocampus, amygdala and habenular triangle. Furthermore, they have also been detected in the cerebral cortex, rhinencephalon, olfactory bulb and in the posterior corners of the spinal cord. Stimulation of the 5-HT3 receptor is associated with activated functions of many neurons as a result of enhanced release of dopamine, NA, GA-BA, and cholecystokinin (CCK) [12, 13]. It has also been demonstrated to be involved in the cognitive processes [14, 15].

Behavioral studies have shown that the administration of 5-HT_3 receptor ligands to animals does not result in significant changes in their behavior. However, the use of these compounds modifies behavioral effects of other substances tested in animal models of anxiety, psychosis and drug addictions [16–21].

Materials and Methods

Animals

The study used newborn and adult male Wistar rats aged 8-10 weeks. The animals were kept in a room at a constant temperature of around 22°C and 12 h/12 h artificial light-dark cycle (light from 7:00 a.m. to 7:00 p.m.). Throughout the experiments the animals were given free access to water and received standard diet. Newborn rats were divided into two groups:

Group I: Control. Animals received zimelidine dihydrochloride at a dose of 10 mg/kg b.w. (*sc*) in a volume of 1.0 mL/kg b.w., and after 30 min 1.0 mL/kg b.w. (sc) of 0.9% NaCl solution on the 1st and 3rd day of life.

Group II: DSP-4. Animals received zimelidine dihydrochloride at a dose of 10 mg/kg b.w. (*sc*) in a volume of 1.0 mL/kg b.w., and after 30 min DSP-4 in a dose of 50 mg/kg b.w. (*sc*) on the 1st and 3rd day of life.

DSP-4 was dissolved in distilled water immediately before injection, and preceded 30 min beforehand by treatment with the selective serotonin reuptake inhibitor zimelidine (Sigma, St. Louis, MO, USA) – in order to prevent serotoninergic effects of DSP-4. All behavioral testing was conducted between 8:00 a.m. and 3:00 p.m. Each treatment group consisted of 8–10 animals. Behavioral studies (writhing test and formalin test) were performed on adult 8 to 10-week-old animals, using the following tooling materials: selective agonist of the 5-HT3 receptor (1-fenylbiguanid; Sigma, St. Louis, MO, USA) and selective antagonist of the 5-HT3 receptor (ondansetron; Sigma, St. Louis, MO, USA). Morphine was a "model" analgesic substance used. The ligand doses of the receptor tested were based on the authors' own experience and literature data. The local Ethical Committee for Animals at the Medical University of Silesia approved all experiments (permission no 66/07 issued on 11.12.2007.

Writhing Test

According to this methodology, 24 h before the experiment the 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 *ip* at a dose of 3.0 mg/1.0 mL/ /0.1 kg. The solution was prepared *ex tempore*. Observation which started 10 min after ethacrynic acid administration involved counting the writhing episodes. In the source literature [22], the episodes have been defined as assuming a characteristic flat posture with a simultaneous lateral rotation of the spine and drawing hind paws, i.e. the so called writhing syndrome. The episodes described above were counted for 60 min at 10-minute intervals (10–20, 20–30, 30–40, 40–50, 50–60 min) starting from the intraperitoneal injection of the irritant (ethacrynic acid). Then, for each time interval the mean value was calculated for each treatment group (control, DSP-4). The writhing test was performed in the manner described above after the administration of morphine (7.5 mg/kg sc), FBG (7.5 mg/kg *ip*) as well as ondansetron (1.0 mg/kg *ip*) in combination with FBG (7.5 mg/kg *ip*) in the control and in the group of animals with noradrenergic system lesions. The substances tested were injected 30 min prior to the administration of ethacrynic acid. Based on the results obtained, the percentage of writhing episode inhibitions was calculated according to the formula:

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

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

Formalin Test

The current study was conducted by placing rats separately in 400 × 300 × 200 mm glass

cages and involved the administration of morphine (7.5 mg/kg sc), FBG (7.5 mg/kg *ip*) and ondansetron (1.0 mg/kg *ip*) in combination with FBG (7.5 mg/kg *ip*) to both groups of rats. After 30 min, the rats were injected with 50 mL of 5% formalin solution in the right paw pad. After a further 5 min for 70 min at 5-minute intervals pain intensity reaction was assessed. For this purpose a 3-point scale was used as follows: 0 – no response; 1 – the animal's paw remains on the ground, but body weight shifts to the other three legs; 2 – the paw is raised above the ground, and body weight rests on the other three legs, 3 – the animal licks its raised paw and the weight shifts to the other three legs.

Statistical Analysis

Statistical analysis was performed using Statistica 6.0 software (StatSoft Inc., Tulsa, Oklahoma, USA). Normal data distribution was assessed using Kolmogorov-Smirnov test and the homogeneity of variance using Levene's test. Comparisons between the examined groups were performed using the Student's-*t* test for independent samples and *U* Mann-Whitney test, if data distribution was not normal or they were assessed in point scale. All results were considered as statistically significant at $p < 0.05$. On figures all data are presented as mean ± SEM.

Results

Writhing Test

In the writhing test, morphine (7.5 mg/kg *sc*) exerted a strong and long-lasting analgesic effect, both in control rats and those with a DSP-4 lesion (Fig. 1). FBG at an equivalent dose (7.5 mg/kg *ip*) in control rats had an analgesic effect similar to that of morphine. In animals with DSP-4 lesion this effect was statistically significantly weaker, and 30 min following FBG administration, an opposite (pronociceptive) effect was observed (Fig. 2).

Ondansetron applied 30 min before FBG administration did not affect FBG analgesic effect in the control group, whereas it strongly inhibited the analgesic effect in rats with DSP-4 lesions (Fig. 3).

Formalin Test

Morphine (7.5 mg/kg *sc*) in the formalin test had a strong analgesic effect in the control animals. The average score of writhing intensity did not exceed 1 point. In rats with DSP-4 lesion, the analgesic effect of morphine was significantly weaker (Fig. 4).

After administering FBG in a dose equivalent to that of morphine, pain intensity in the control

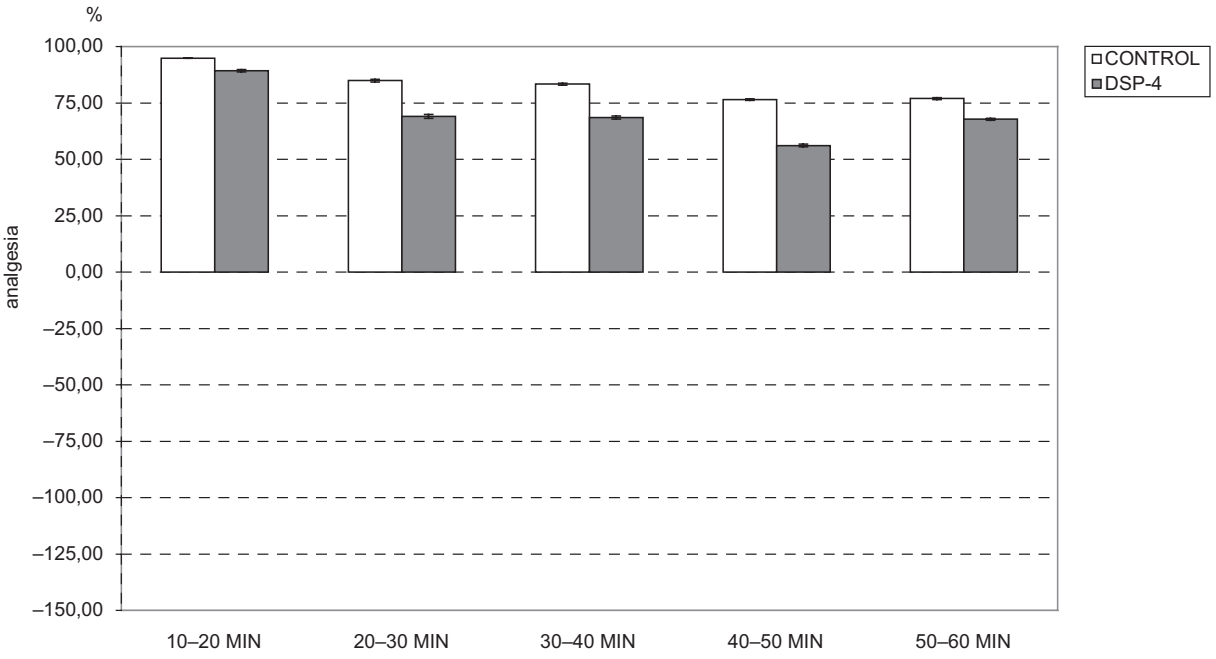

Fig. 1. Effect of DSP-4 lesion on analgesia assessed in writhing test after morphine (7.5 mg/kg *sc*) in rats ($\bar{x}$ ± SEM; n = 10)

Ryc. 1. Ocena wpływu lezji DSP-4 na działanie przeciwbólowe morfiny (7,5 mg/kg *sc*) w teście wicia u szczurów ($\bar{x}$ ± SEM; n = 10)

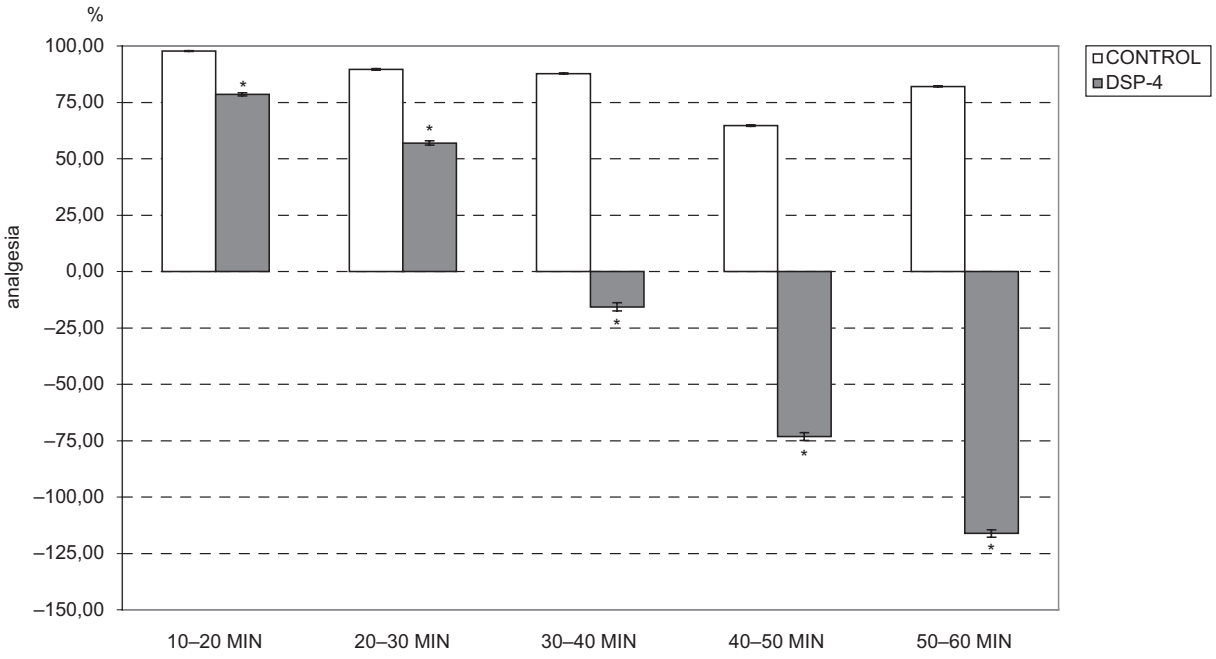

Fig. 2. Effect of DSP-4 lesion on analgesia assessed in writhing test after FBG (7.5 mg/kg *sc*) in rats ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, control *vs.* DSP-4

Ryc. 2. Ocena wpływu lezji DSP-4 na działanie przeciwbólowe FBG (7,5 mg/kg *ip*) w teście wicia u szczurów ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, badanie kontrolne *vs.* DSP-4

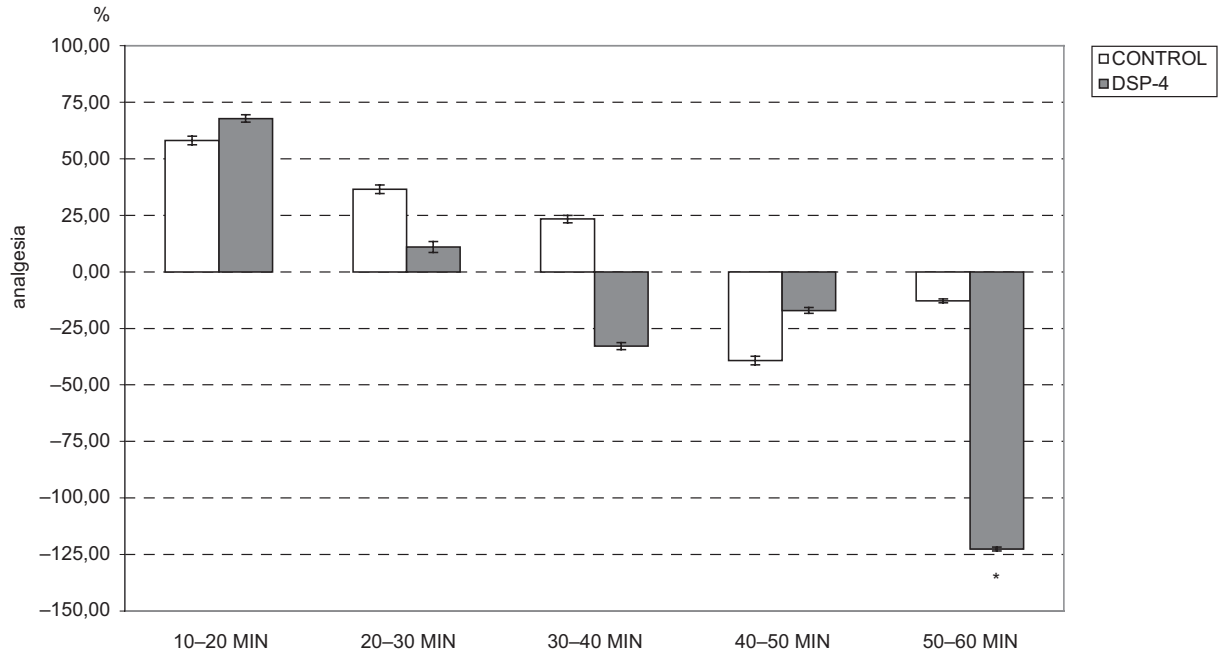

Fig. 3. Effect of DSP-4 lesion on analgesia assessed in writhing test after concomitant FBG (7.5 mg/kg *sc*) and ondansetrone (1,0 mg/kg *ip*) in rats ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, control *vs.* DSP-4

Ryc. 3. Ocena wpływu lezji DSP-4 na działanie przeciwbólowe FBG (7,5 mg/kg *ip*) i ondansetronu (1,0 mg/kg *ip*) zastosowanych łącznie w teście wicia u szczurów ($\bar{x}$ ± SEM; n = 10).* p < 0.05, badanie kontrolne *vs.* DSP-4

group was estimated at 2–3 points. Similarly to morphine, the analgesic effect of FBG was significantly lower in animals with DSP-4 lesion (Fig. 5).

The use of ondansetron (1.0 mg/kg *ip*) before FBG injection (7.5 mg/kg *ip*) in the formalin test resulted in the elimination of the previously observed stronger analgesic effect in the group of rats with DSP-4 lesions (Fig. 6).

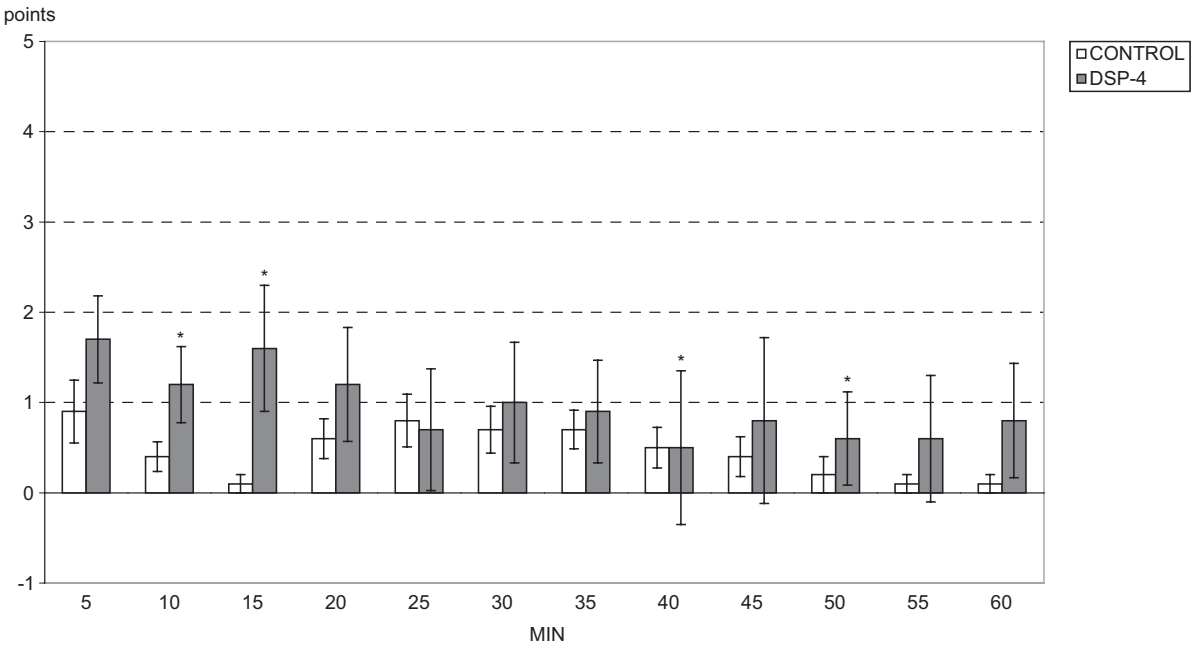

Fig. 4. Effect of DSP-4 lesion on analgesia assessed in formalin test after morphine (7.5 mg/kg *sc*) ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, control *vs.* DSP-4

Ryc. 4. Ocena wpływu lezji DSP-4 na działanie przeciwbólowe morfiny (7,5 mg/kg *sc)* w teście formalinowym u szczurów ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, badanie kontrolne *vs.* DSP-4

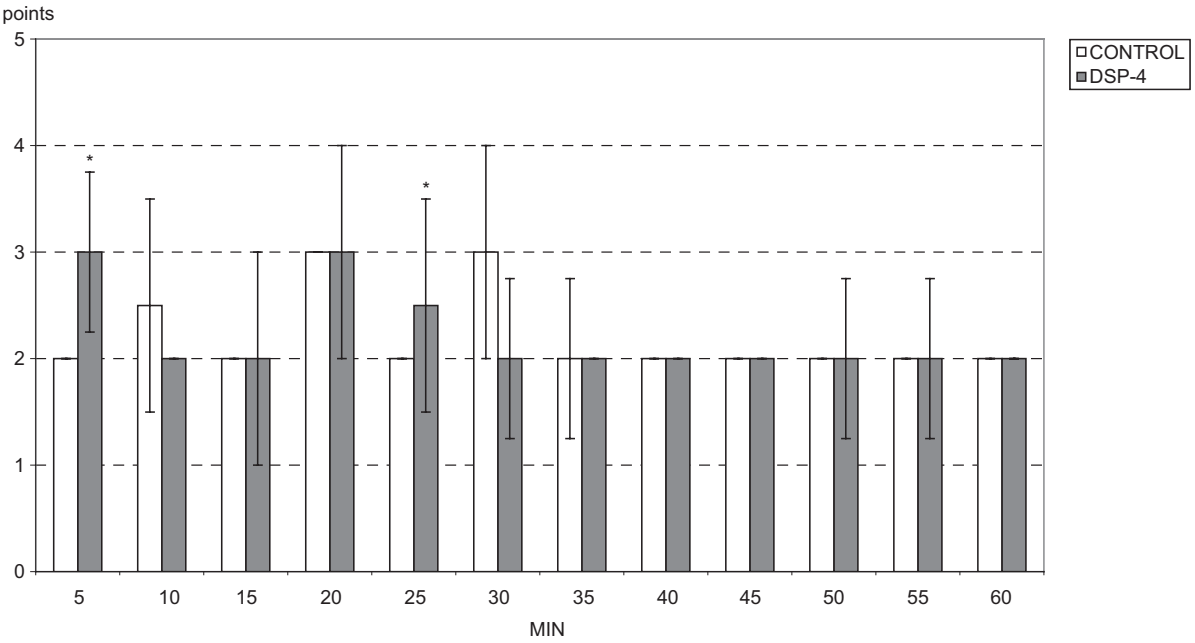

Fig. 5. Effect of DSP-4 lesion on analgesia assessed in formalin test after FBG (7.5 mg/kg *sc*) ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, control *vs.* DSP-4

Ryc. 5. Ocena wpływu lezji DSP-4 na działanie przeciwbólowe FBG (7,5 mg/kg ip) w teście formalinowym u szczurów ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, badanie kontrolne *vs.* DSP-4

Discussion

Despite many years of research, the role of 5-HT_3 receptors in the perception of pain has not been clearly established. Glaum et al. [23] demonstrated that the intrathecal (it) administration of a selective 5-HT_3 receptor agonist, 2-methyl-5-HT, in rats had an analgesic effect similar to 5-HT in the tail immersion test, and a slightly weaker effect than in the hot-plate test. Pre-treatment with the selective 5-HT_3 receptor antagonists abolished the antinociceptive effects of both serotonin (5-HT) and 2-methyl-5-HT. Similar results were obtained by Sasaki et al. [24] in the formalin test. Other

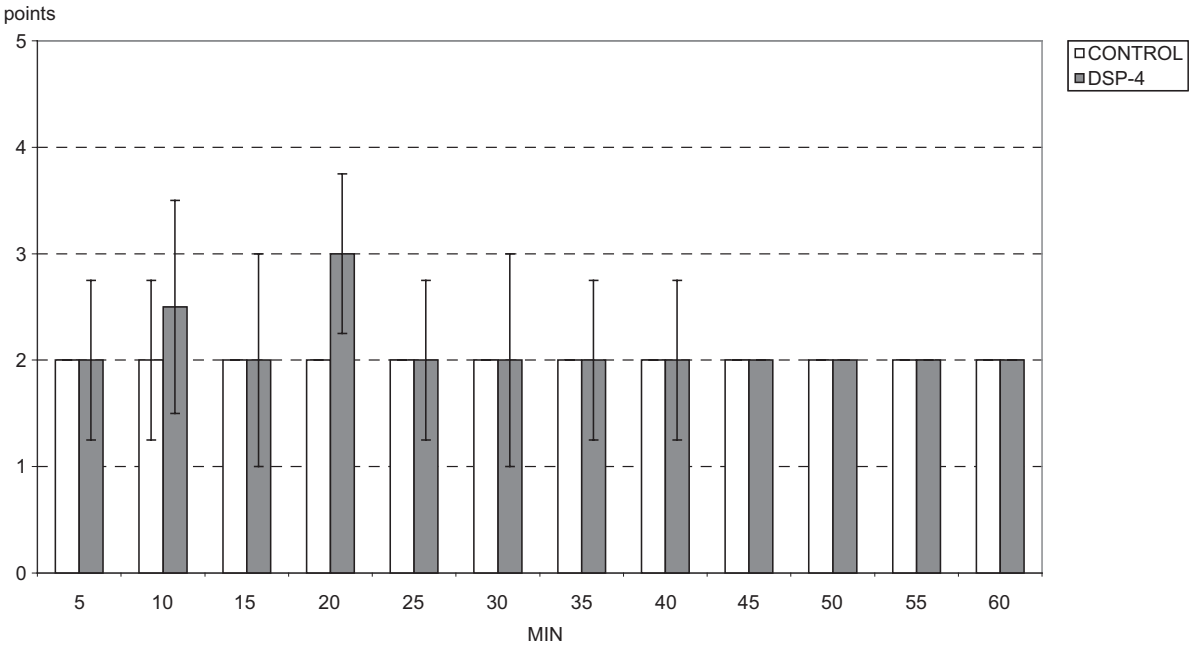

Fig. 6. Effect of DSP-4 lesion on analgesia assessed in formalin test after concomitant ondansetrone (1.0 mg/kg *ip*) and FBG (7.5 mg/kg *sc*) ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, control *vs.* DSP-4

Ryc. 6. Ocena wpływu lezji DSP-4 na działanie przeciwbólowe ondansetronu (1,0 mg/kg *ip*) i FBG (7,5 mg/kg *ip*) w teście formalinowym u szczurów ($\bar{x}$ ± SEM; n = 10). * $p < 0.05$, badanie kontrolne *vs.* DSP-4

authors showed that the intrathecal injection of FBG exerted an analgesic effect in the paw withdrawal test [25]. Similarly to the work of Glaum et al. [23], the effect was weaker than after using 5-HT. Roczniak and Nowak [26] conducted the biochemical and behavioral tests to assess the responsiveness of central 5-HT_3 receptors in rats using tooling materials, separately and jointly: selective 5-HT_3 receptor agonist (1-fenylbiguanidem) and selective 5-HT_3 antagonist (ondansetron).

The analgesic effect of 5-HT_3 receptor stimulation in the dorsal horn of the spinal cord has also been confirmed in electrophysiological studies. Peng et al. [27, 28] found that the response of neurons located in the posterior corners of the spinal cord to a painful stimulus was inhibited by the stimulation of the periaqueductal gray. However, the intrathecal injection of 5-HT_3 receptor antagonists blocked this effect, suggesting the antinociceptive activation of this serotonin receptor subtype in the spinal cord.

The analgesic effect of the *it* administered 5-HT_3 and 2-methyl-5-HT receptor agonists has also been demonstrated in the tail immersion test in mice [29]. The authors of this study used a specific antisense oligodeoxynucleotides (AODN) blocking the synthesis of 5-HT_3 receptors in the posterior corners of the spinal cord and evaluated the effectiveness of the blockade immunohistochemically. They found the abolition of the analgesic effect of 5-HT and 2-methyl-5-HT in the animals that did not express 5-HT_3 receptor in the posterior corners of the spinal cord [30]. On the other hand, according to Xiao et al. [31], the serotoninergic 5-HT_3 receptor is not involved in the antinociceptive action of 5-HT, as no effect was found of the 5-HT_3 receptor antagonist administered by intraspinal injection on the antinociceptive effect of 5-HT. Paradoxically, other studies [32, 33] have demonstrated that the 5-HT_3 receptor agonists intensify pain response.

In the current study, the 5-HT_3 FBG serotonin receptor agonist was used systemically (ip), demonstrating its analgesic effect. Dukat and Wesołowska [34] showed that the subcutaneously injected 5-HT_3/alpha2B-adrenergic ligand, MD-354 (m-chlorophenyl guanidine) had no analgesic effect in the tail immersion test. However, it should be taken into consideration that this compound is a partial agonist of the 5-HT_3 receptor and the current study was conducted on mice, and as it was mentioned earlier, biochemical and structural properties of the 5-HT_3 receptor show high intertissue and interspecies variability.

Previous studies have demonstrated that the serotonergic and noradrenergic systems interact with each other in the modulation of pain at the spinal cord level, though little is known about central interactions of these neurotransmitters [35]. Therefore, we decided to use the previously developed model of injury (lesion) of the central noradrenergic system with DSP-4. This neurotoxin

readily crosses the blood-brain barrier and can be administered peripherally (*sc, ip*). It causes permanent inhibition of the reuptake of NA in the CNS pathways and a reduction of endogenous NA in the central and peripheral nervous system of rats. Reduction in NA content in the peripheral noradrenergic system is temporary, as some (approximately 80%) of the neurons regain the ability to re-synthesize NA 4–6 weeks after DSP-4 injection. This substance does not influence the content of catecholamines in adrenal medulla [36]. In turn, the impairment of central adrenergic neurons is permanent, and the effect of DSP-4 on the dorsal pathway neurons is stronger than on the ventral pathway neurons [37].

The molecular mechanism of the DSP-4 effect involves the inhibition of NA storage capacity in both synaptic granules of the central and peripheral noradrenergic neurons. Consequently, this leads to NA reduction in neurons, thus impairing neurotransmission [38]. DSP-4 acts in two phases: first known as the "acute phase" characterized by a rapid loss of the NA neurotransmitter and the other one, known as "neurodegenerative", associated with biochemical and morphological disorders and inhibition of DBH, the enzyme converting DA to NA [39]. DSP-4 has the ability to inhibit MAO activity. [40] NA reuptake inhibition in peripheral and central neurons occurs rapidly (within an hour after injection of the neurotoxin) and is dose-dependent. DSP-4 acts on NA content after administration of 20 mg/kg *ip*, and the maximum effect is achieved following the administration of 100 mg/kg *ip*. It should be added that if prior to DSP-4 administration animals were injected with NA reuptake inhibitor such as desipramine, the neurotoxin action was ineffective [41].

In the model of chemically-induced chronic visceral pain (writhing test), FBG showed a significantly less potent analgesic effect in animals with a lesion in the noradrenergic system caused by administration of DSP-4, and this effect was blocked by the 5-HT_3 antagonist – ondansetron. After local injection of an irritant, in this case formalin, into the paw of an animal with DSP-4 induced lesion of the noradrenergic system, the analgesic effect of morphine and FBG was found to be attenuated. These differences were eliminated by the use of ondansetron prior to FBG injection.

Therefore, this test can be used in the screening of various compounds for their potential antinociceptive effect. It is believed that the behavior of animals after intraperitoneal injection of ethacrynic acid is predominantly of a reflex nature, although it cannot be excluded that visceral peritoneum also receives somatic innervation [42]. The results indicate that the lesion of the noradrenergic system was associated with a strong pronociceptive effect after FBG administration. However, as this effect was not markedly blocked by ondansetron perhaps other mechanisms than those mediated by 5-HT_3 receptors are involved. The injection of formalin into the rat paw causes a biphasic behavioral response, the first of which takes about 3 min and results from direct stimulation of nociceptors, whereas the second, which appears after 20–30 min of exposure to the irritant, is an inflammatory reaction [42]. Some authors postulate that the 2nd phase of the formalin test also involves central mechanisms, activated by neuronal stimulation in the first phase, although this view is not widely accepted [42, 43]. The above results of previous studies indicate that a chemical lesion of the central noradrenergic system modifies serotonergic and GABAergic transduction in the rat brain. Therefore, it can be assumed that the administration of DSP-4 compound to neonatal rats, which damages the central noradrenergic system (mainly projections to the cortex and hippocampus), can permanently alter the reactivity of central 5-HT_3 receptors involved in the perception of pain.

These results may explain the clinical observations of pain severity in patients suffering from depressive and anxiety disorders [44], and the fact that ondansetron and its derivatives used as antiemetics, ameliorate the antinociceptive properties of analgesic drugs [45].

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