PRACA ORYGINALNA

Interaction between central noradrenergic system and serotoninergic 5-HT_3 receptor mediated analgesia in rats

Interakcja pomiędzy ośrodkowym układem noradrenergicznym a działaniem przeciwbólowym pośredniczonym przez receptor serotoninergiczny 5-HT_3 u szczurów

Wojciech Roczniak, Przemysław Nowak

ABSTRACT

BACKGROUND

The aim of the present study was to examine the impact of the central noradrenergic system on the serotoninergic 5-HT_3 receptor mediated analgesia in rats.

MATERIAL AND METHODS

The noradrenergic system was lesioned in male rats shortly after birth by subcutaneous (sc) injections of the neurotoxin DSP-4 [(N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (50 mg/kg x 2) given on postnatal days 1 and 3. Rats continued to be housed until they were 10 weeks old, for further experimentation. The anti-nociceptive effects of the central serotoninergic 5-HT_3 receptor agonist (1-phenylbiguanide; 7.5 mg/kg), antagonist (ondansetron; 1.0 mg/kg) and both drugs administration (intraperitoneal; ip) were examined in models of exteroceptive sensation using thermal (tail immersion and hot plate tests) and mechanical stimuli (paw pressure test). Furthermore accumulation of 5-hydroxytryptamine (5-HTP) in some parts of the brain were determined using high pressure chromatography with electrochemical detection method (HPLC/ED).

RESULTS AND CONCLUSION

In the tail immersion test we did not observe differences between control and DSP-4 treated rats as far as the anti-nociceptive effect evoked by the central serotoniergic 5-HT_3 receptor agonist (1-phenylbiguanide; 7.5 mg/kg ip) is concerned. Conversely in the hot plate test 1-phenylbiguanide (7.5 mg/kg ip) produced significantly diminished analgesic reaction in DSP-4 lesioned rats in comparison to control (in all tested intervals (20, 40, 60 and 80 min; $p < 0.05$); this effect was abolished by 5-HT_3 receptor antagonist (ondansetron; 1.0 mg/kg ip) pretreatment. Similar effects were

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observed in paw pressure test; in this case significant changes were noticed in 20 and 40 min of testing ($p < 0.05$). In biochemical assay we found that 1-phenylbiguanide significantly increased 5-HTP level in the prefrontal cortex of control rats being without effect in DSP-4 group in this regard. Ondansetron did not affect 5-HTP content when given alone but injected before 1-phenylbiguanide abolished its effect in control group. In the thalamus with hypothalamus (control) as well as in the brain stem (control and DSP-4) 1-phenylbiguanide only non-significantly elevated 5-HTP level. Ondansetron alone did not affect examined parameters but in the brain stem administered before 1-phenylbiguanide statistically lowered 5-HTP (in both tested groups) in comparison to respective controls (1-phenylbiguanide). The results of the present study indicate that the noradrenergic system participates in the analgesic properties of 5-HT_3 acting drugs integrated in the higher brain structures (e.g. thalamus, cortex) being without effect on spinal analgesia. Additionally, obtained data pointed out on the possibility of nociception disturbances (mediated by 5-HT_3 receptor) in patients with noradrenergic system dysfunction (e.g., depression and/or anxiety disorders).

KEY WORDS
serotoninergic receptor 5-HT_3, central noradrenergic system, DSP-4, analgesia, rats

ABSTRACT

WSTĘP
Badanie miało na celu określenie roli ośrodkowego układu noradrenergicznego w antynocyceptywnych efektach pośredniczonych przez receptor serotoninergiczny 5-HT_3 u szczurów.

MATERIAŁ I METODY
Noworodki szczurze szczepu Wistar 1. i 3. dnia życia otrzymały podskórnie (sc) iniekcję neurotoksyny DSP-4 [N-(2-chloroetylo)-N-etylo-2-bromo-benzylaminy] w dawce 50 mg/kg x 2 celem trwałego zniszczenia ośrodkowego układu noradrenergicznego. Zwierzęta kontrolne otrzymały 0,9% roztwór NaCl (1,0 ml/kg sc). Po osiągnięciu wieku 10 tygodni wykonano testy behawioralne oceniające czucie interoceptywne z użyciem bodźca termicznego (test imersji ogona, test gorącej płytki) oraz bodźca mechanicznego (test wycofania łapy) po dootrzewnowym (ip) podaniu agonisty ośrodkowego receptora serotoninergicznego 5-HT_3 (1-fenylbiguanidu; 7,5 mg/kg), antagonisty receptora 5-HT_3 (ondansetronu; 1,0 mg/kg) oraz łącznym podaniu obu związków. Ponadto, posługując się metodą chromatografii cieczowej wysokociśnieniowej z detekcją elektrochemiczną (HPLC/ED), oznaczono zawartość 5-hydroksytryptofanu (5-HTP) w wybranych częściach mózgu badanych zwierząt.

WYNIKI I WNIOSKI
Nie stwierdzono różnicy w antynocyceptywnym działaniu agonisty receptora serotoninergicznego 5-HT_3 1-fenylbiguanidu (7,5 mg/kg ip) w teście imersji ogona pomiędzy grupą kontrolną i DSP-4. Natomiast w teście gorącej płytki 1-fenylbiguanid (7,5 mg/kg ip) wykazywał znamiennie słabsze działanie analgetyczne u zwierząt z lezją układu noradrenergicznego wywołaną podaniem DSP-4 we wszystkich badanych przedziałach czasowych, tj. 20. 40. 60. i 80. minucie obserwacji ($p < 0,05$); efekt ten był blokowany przez antagonistę receptora 5-HT_3 ondansetron (1,0 mg/kg ip). Podobne wyniki uzyskano w teście wycofania łapy, w tym przypadku znamienność statystyczna wystąpiła w 20. oraz 40. minucie obserwacji ($p < 0,05$). W badaniach biochemicznych stwierdzono, że 1-fenylbiguanid znamiennie zwiększa zawartość 5-HTP w korze mózgowej u zwierząt kontrolnych, pozostając bez wpływu u zwierząt z lezją DSP-4. Efekt ten był blokowany podaniem antagonisty ondansetronu. Podobną tendencję zaobserwowano we wzgórzu z podwzgórzem (uzyskane różnice nie były jednak statystycznie znamienne), natomiast w pniu mózgu stosowane ligandy receptora 5-HT_3 nie wpływały na badany parametr.

Na podstawie przeprowadzonych badań wyciągnięto wnioski, iż zniszczenie ośrodkowego układu noradrenergicznego osłabia antynocyceptywne efekty pośredniczone przez receptor serotoninergiczny 5-HT_3 integrowane na poziomie wyższych struktur ośrodkowego układu nerwowego (kora mózgowa, wzgórze), natomiast pozostaje bez wpływu na procesy integrowane w rdzeniu kręgowym (brak zmian w teście imersji ogona). Wyniki badań wskazują pośrednio na możliwość wystąpienia zaburzeń transmisji bodźców bólowych pośredniczonych przez receptor 5-HT_3 u chorych z dysfunkcją ośrodkowego układu noradrenergicznego (np. u chorych z zaburzeniami lękowymi lub depresją).

SŁOWA KLUCZOWE
receptor serotoninergiczny 5-HT_3, ośrodkowy układ noradrenergiczny, DSP-4, analgezja, szczury

INTRODUCTION

5-hydroxytryptamine (serotonin; 5-HT) has been implicated in a wide variety of behavioral disorders (depression, anxiety, schizophrenia, mania, obesity, drug addiction, etc.) as well as physiological processes including circadian rhythms, thermoregulation and pain perception. The diversity of actions is attributed to the existence of numerous different receptor subtypes: seven distinct classes (5-HT_1 to 5-HT_7) have been identified so far [1, 2]. Within these receptor classes, the serotoninergic 5-HT_3 receptor is unique in its interaction with a ligand-gated ion channel that conducts monovalent cations (Na^+ and K^+) and generates excitation through noradrenaline (NA), dopamine (DA) and 5-HT release [3]. It has been established that the distribution of the 5-HT_3 receptor is widespread, including higher brain areas such as cortex and hippocampus as well as nuclei of the lower brainstem (e.g., trigeminal nucleus and dorsal vagal complex) [4]. In the spinal cord, it is localized most densely within the substantia gelatinosa [5], a superficial dorsal horn area associated principally with the processing of nociceptive sensory afferent inputs. More specifically, Huang et al. [6] demonstrated that 5-HT_3 receptor co-localizes with GABA and enkephalinergic phenotypes in the spinal horn, suggesting that 5-HT may activate these neurons via 5-HT_3 receptor and therefore affect the release of GABA and enkephaline. Despite many years of research the role of serotoninergic 5-HT_3 receptors in nociception is still controversial. Zeitz et al. [7] found, that activation of both peripheral and central 5-HT_3 receptors is pronociceptive, others [8, 9] showed a hyperalgesic effect for serotoninergic 5-HT_3 receptor agonists, but some authors reported that they had an analgesic activity [10]. Conversely, Xiao et al. [11] found that serotoninergic 5-HT_3 receptor is not involved in mediating the 5-HT-evoked anti-nociception. It is likely that complex anatomical and functional organization of the brain serotoninergic system as well as diversity of animal models and research methods applied may account for some of the differences observed between particular studies. Furthermore it has been shown that the sensation of pain is not only modified by 5-HT containing neurons but also through other neurotransmitters that form the descending antinociceptive pathways (NA and endogenous opioids such as β-endorphin and dynorphin). The pontine noradrenergic cell groups (A5, A6 [locus coeruleus, LC], and A7) are believed to constitute a key antinociceptive component of this system, as some of these neurons project to the dorsal horn of the spinal cord [12] and release NA to suppress transmission of the pain message. The activation of the descending systems markedly modifies not only the release of glutamate from the primary afferent or interneurons, but also the release of GABA and glycine. In addition, the output of nociceptive information to the higher pain center from projection neurons is also significantly altered. As a consequence, the severity of pain perception is profoundly reduced. It was also demonstrated that the alternation in NA exocytosis in the thalamus, brain stem and other nuclei alters the output of nociceptive information to the higher brain center from projection neurons [13, 14, 15]. Previously it was demonstrated that the selective noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) to rats produces marked NA terminals destruction with following neurotransmission perturbation observed in adulthoods [16]. Also other neurotransmit-

ter systems in the brain e.g., serotoninergic and GABA-ergic are functionally affected by DSP-4 treatment. As a matter of fact, Dąbrowska et al. [17, 18] found that chemical lesioning of noradrenergic neurons (with DSP-4) results in desensitization of serotoninergic 5-HT_{1A} autoreceptors. Bortel et al. [19, 20] demonstrated that administration of GABA transaminase inhibitor (vigabatrine) resulted in a enhanced GABA release in the prefrontal cortex in DSP-4 rats in comparison to control. On the other hand, transmitters such as NA, 5-HT and GABA which originate in periaqueductal gray, raphe nuclei, and LC are most clearly implicated in inhibitory descending pathways and all are the key brain stem sites for control of nociception transmission in the spinal cord. To the best of our knowledge there are no literature data on the effect of DSP-4 treatment (in neonates) and serotoninergic 5-HT_3 receptor ligands mediated analgesia. In attempt to clarify this issue we employed models of exteroceptive sensation using thermal (tail immersion and hot plate tests) and mechanical stimuli (paw pressure test), furthermore we examined the effect of DSP-4 administration on 5-HTP level in some parts of the brain [21].

MATERIAL AND METHODS

Animals and treatment

Male Wistar rats were obtained from University Animals Department (Katowice, Poland) and were housed in a well-ventilated room, at 22 ± 2^{0}C under a 12h light:12h dark cycle (lights on 7:00 a.m. to 7:00 p.m.), and with free access to food and water. Rats were weaned at 21 days. All procedures, reviewed and approved by the Institutional Animal Care Committee, are in accord with principles and guideline described in the NIH booklet *Care and Use of Laboratory Animals*. The central noradrenergic system of newborn rats was destroyed with DSP-4 (Sigma, St. Louis, MO, USA). On the 1 st and 3 rd day of postnatal life rats were administered with either DSP-4 (50 mg/kg sc) or 0.9% NaCl (1,0 ml/kg sc). DSP-4 was dissolved in 0.9% NaCl immediately before injection. Zimelidine (10 mg/kg sc) was applied to newborn rats (30 min before DSP-4) for prevention 5-HT neurons destruction. Rats continued to be housed as above until 10 weeks, for further experimentation. All procedures were approved by the Local Bioethical Committee for Animal Care (certificate nr 66/2007). Experiments were carried out in the morning and the animals were used only once.

Tail immersion test [22]

Anti-nociception was evaluated by measuring response latencies in the warm water tail-immersion (tail-flick) assay. Each rat was placed in a cone restrainer, and the end of the tail was immersed 5 cm in a 56°C water bath. Response latencies were measured as the amount of time the animal took to respond to the thermal stimuli. The response was defined as the removal of the tail from the warm water. 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. The determined latency time for each animal was converted to the percentage of analgesia according to the formula:

$$\% \text{ analgesia} = \frac{T_x - T_o}{T_{max} - T_o} \times 100$$

T_x – the individual latency time determined at appropriate intervals after examined analgesics administration, T_o individual latency time determined before analgesics injection, T_{max} – 10 s

The analgesic effect was measured before drug administration (after saline 1.0 ml/kg ip) and at 20, 40, 60 and 80 min after 1-phenylbiguanide (7.5 mg/kg ip), ondanserton (1.0 mg/kg ip) or both drugs injection.

Hot plate [23]

Anti-nociception was assessed using a hot plate instrument (COTM, Bialystok, Poland) with the plate temperature maintained at 56 ± 0.1°C. The rat was placed with all four paws on the plate, and the latency time to licking or shaking a hind limb was measured. The cut-off time was set at 20 s to avoid tissue damage. The determined latency time for each animal was converted to the percentage of analgesia according to the formula:

$$\% \text{ analgesia} = \frac{T_x - T_o}{T_{max} - T_o} \times 100$$

T_x – the individual latency time determined at appropriate intervals after examined analgesics

administration, T_o individual latency time determined before analgesics injection, T_{max} 20 s
The analgesic effect was measured before drug administration (after saline 1.0 ml/kg ip) and at 20, 40, 60 and 80 min after 1-phenylbiguanide (7.5 mg/kg ip), ondanserton (1.0 mg/kg ip) or both drugs injection.

Paw pressure test [24]

Nociceptive thresholds in rats were determined by a modification of the Randall-Selitto method, the paw-pressure vocalization test, in which a constantly increasing pressure is applied to the hind paw until the rat squeaks. The Ugo Basil analgesimeter (probe tip diameter 1 mm; weight 25 g) was used. A 750-g cut-off value was used for preventing tissue damage. In brief, a constantly increasing pressure was applied to the right hind paw of the rat at the metacarpal level between the third and the fourth finger to determine the minimum stimulus necessary to evoke an obvious nociceptive response (a sharp paw withdrawal). Rats were habituated to the full procedure on two consecutive days and experiments were conducted on the third day. To ensure nociceptive threshold stability, basal nociceptive threshold was measured three times (with an interval of 30 min) on the 2 days before the planned experimental day. On the experimental day, basal nociceptive threshold was also determined three times before drug injections. The following formula was used to count the percentage of analgesia:

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

A – mean pressure (g) from 3 assessments before drug administration
B – pressure (g) assessed at 20, 40, 60, 80 min after drug treatment.
The experiments were performed in a quiet room by the same investigator blinded as to the treatment used.

5-HTP assay (an indirect method to assess 5-HT synthesis rate) [25, 26, 27]

For 5-hydroxytryptophan (5-HTP) assay control and DSP-4 rats were injected with saline (0.9% NaCl) 1.0 ml/kg ip and after 60 min with aromatic amino acids inhibitor – hydroxybenzylhydrazine (NSD-1015) 100 mg/kg ip. The separate groups of control and DSP-4 rats were administered with 1-phenylbiguanide (7.5 mg/kg ip) (30 min before NSD-1015), onadansetron (1.0 mg/kg ip) (60 min before NSD-1015) or concomitantly ondansetron (60 min before NSD-1015) with 1-phenylbiguanide (30 min before NSD-1015). 30 minutes after NSD-1015 injection animals were sacrificed by decapitation, and their brains immediately excised, and placed on ice. The prefrontal cortex, thalamus with hypothalamus and brain stem were separated, and placed on dry ice. Then, tissues were weighed and stored at -70°C, pending assay. In the examined parts of brain the content of 5-HTP – precursor of 5-HT were estimated by means of a high performance liquid chromatography with electrochemical detection (HPLC/EC). Samples were homogenized for 15-20 sec 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 GB) and injected onto the HPLC/ED column. The mobile phase was composed of: 75 mM NaH_2PO_4, 1.7 mM 1-octanesulphonic acid, 5 µM EDTA (Avocado, Research Chemicals Ltd), 100 µl triethylamine (Sigma), 9.5 % acetonitrile (Lab-Scan), pH 3 adjusted with phosphoric acid (Fluka). The flow rate was maintained at 0.7 ml/min, at a temperature of 22°C, and the oxidation potential was fixed at +700 mV, 10 nA/V sensitivity. The instrumentation included an electrochemical detector with flow cell, piston pump, manometric module (Gilson, France), thermostat for column (Dionex, Germany), precolumn Hypersil BDS C18, 10x4 mm, 3 µm and column Hypersil BDS C18, 250x4.6 mm, 3 µm (ThermoQuest GB). Peaks were automatically integrated by universal chromatographic interface UCI-100. Data were expressed as ng/g wet tissue.

Data Analysis

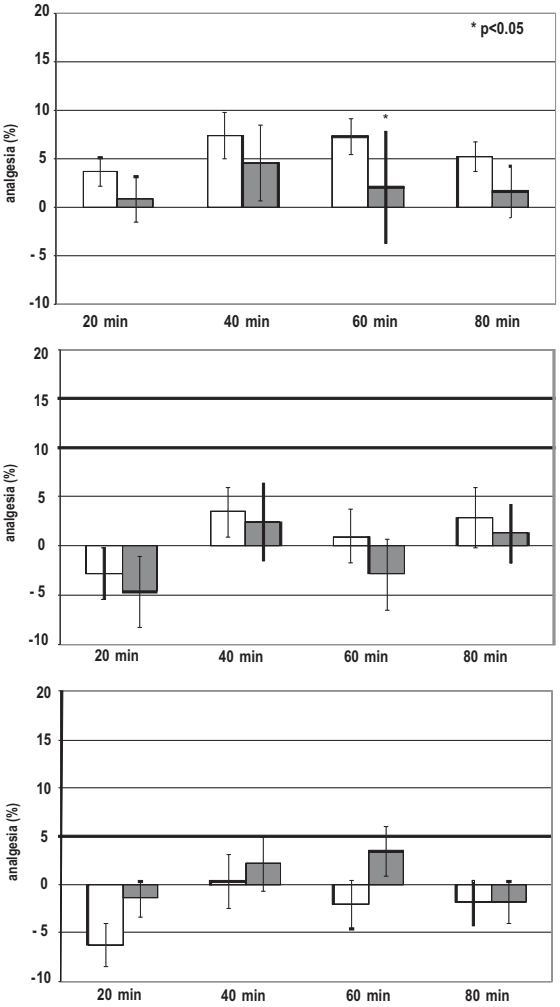
Group differences were assessed by an analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls. A P value <0.05 was taken as the level of significant difference.

RESULTS

Tail immersion test

It was demonstrated that 5-HT_3 receptor agonist 1-phenylbiguanide (7,5 mg/kg ip) injected 30 min before testing produced similar anti-

nociceptive response in control and DSP-4 treated rats. The only significantly diminished reaction (in DSP-4 group) was observed in 60 min. Ondanserton (1,0 mg/kg ip) – a 5-HT_3 receptor antagonist did not show antinociceptive reaction in both tested groups. Also concomitant 1-phenylbiguanid and ondanserton apply was without effect in this regard.



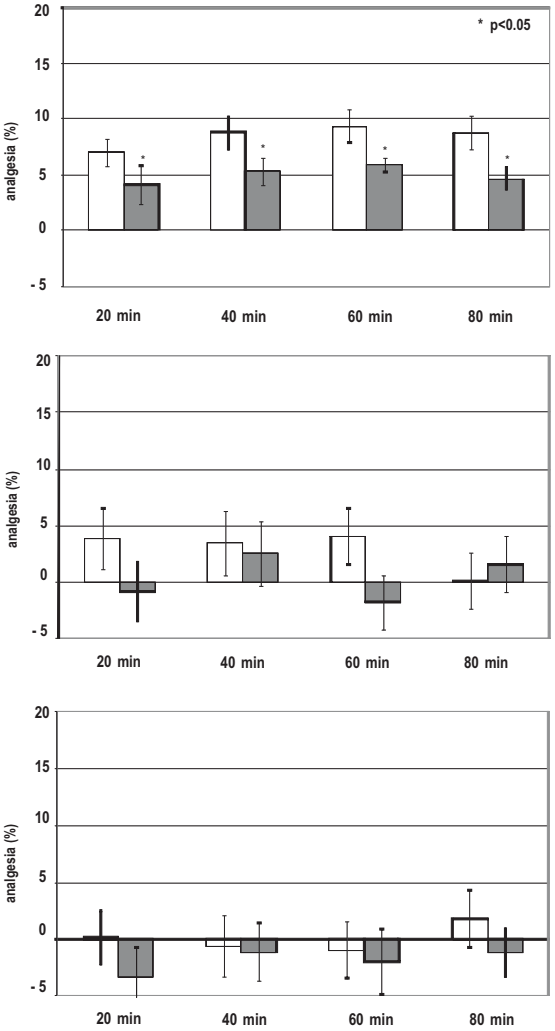
Ryc. 1. Wpływ podania DSP-4 (50 mg/kg sc) na antynocyceptywne efekty 1-fenylbiguanidu (7,5 mg/kg ip) (Ryc. 1a), ondansetronu (1,0 mg/kg ip) (Ryc. 1b) lub 1-fenylbiguanidu (7,5 mg/kg ip) z ondansetronem (1,0 mg/kg ip) (Ryc. 1c) w teście imersji ogona u szczurów (x ± SEM; n=8).

Fig. 1. Effect of DSP-4 (50 mg/kg sc) treatment on anti-nociception effects assessed in tail-immersion test after 1-phenylbiguanide (7.5 mg/kg ip) (Fig. 1a), ondansetron (1.0 mg/kg ip) (Fig. 1b) or 1-phenylbiguanide (7.5 mg/kg ip) with ondansetron (1.0 mg/kg ip) (Fig. 1c) in rats (x ± SEM; n=8).

Objaśnienia (Explanations):
□ Kontrola (Control)
▒ DSP-4
* p < 0.05 Kontrola (Control) *vs.* DSP-4

Hot plate test

1-phenylbiguanide (7.5 mg/kg ip) elicited lower anti-nociception effects in DSP-4 rats in comparison to control rats and the effect was significant at 20, 40, 60 and 80 min of the observation (Fig. 2a). Ondansetron (1.0 mg/kg ip) alone did not evoked antinociceptive reaction in both tested groups (Fig. 2b) but administered 30 min before 1-phenylbiguanide (7.5 mg/kg ip) injection totally abolished this effect in both tested groups (Fig. 2c).



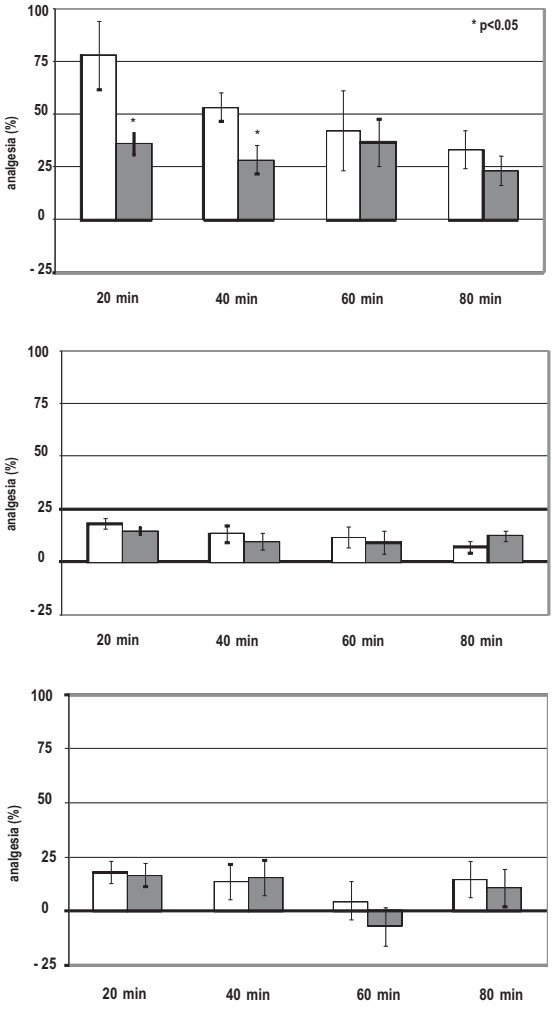
Ryc. 2. Wpływ podania DSP-4 (50 mg/kg sc) na antynocyceptywne efekty 1-fenylbiguanidu (7,5 mg/kg ip) (Ryc. 2a), ondansetronu (1,0 mg/kg ip) (Ryc. 2b) lub 1-fenylbiguanidu (7,5 mg/kg ip) z ondansetronem (1,0 mg/kg ip) (Ryc. 1c) w teście gorącej płytki u szczurów (x ± SEM; n=8).

Fig. 2. Effect of DSP-4 (50 mg/kg sc) treatment on anti-nociception effects assessed in hot plate test after 1-phenylbiguanide (7.5 mg/kg ip) (Fig. 2a), ondansetron (1.0 mg/kg ip) (Fig. 2b) or 1-phenylbiguanide (7.5 mg/kg ip) with ondansetron (1.0 mg/kg ip) (Fig. 2c) in rats (x ± SEM; n=8).

Objaśnienia jak w Ryc. 1 (Explanations as in Figure 1)

Paw pressure test

Before drugs injection, withdrawal thresholds of intact and DSP-4 rats were 98 ±9.3 g and 90 ±12.9 g respectively (means from all measurements n=24 for each examined group). Serotoninergic 5-HT_3 receptor agonist (1-phenylbiguanide in a dose of 7.5 mg/kg ip) evoked lower anti-nociception response in DSP-4 treated rats in comparison to control and the effect was significant at 20 and 40 min of testing (Fig. 3a). Serotoninergic 5-HT_3 receptor antagonist (ondansetron in a dose of 1.0 mg/kg ip) did not elicit anti-nociceptive reaction in both tested groups (Fig. 3b) but administered 30 min before 1-phenylbiguanide (7.5 mg/kg ip) apply abolished this effect in both tested groups (Fig. 3c).



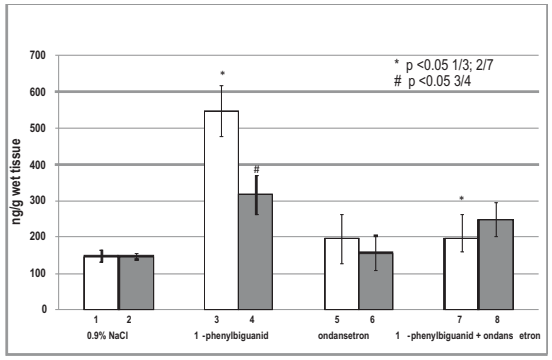
Ryc. 3. Wpływ podania DSP-4 (50 mg/kg sc) na antynocycepty-wne efekty 1-fenylbiguanidu (7,5 mg/kg ip) (Ryc. 3a), ondanset-ronu (1,0 mg/kg ip) (Ryc. 3b) lub 1-fenylbiguanidu (7,5 mg/kg ip) z ondansetronem (1,0 mg/kg ip) (Ryc. 3c) w teście wycofania łapy u szczurów (x ± SEM; n=8).

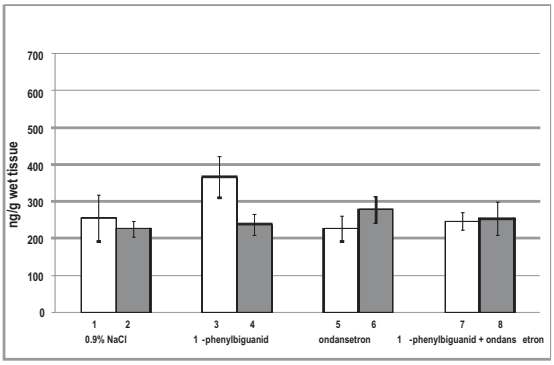
Fig. 3. Effect of DSP-4 (50 mg/kg sc) treatment on anti-nociception effects assessed in paw withdrawal test after 1-phenylbiguanid (7.5 mg/kg ip) (Fig. 3a), ondansetron (1.0 mg/kg ip) (Fig. 3b) or 1-phenylbiguanide (7.5 mg/kg ip) with ondansetron (1.0 mg/kg ip) (Fig. 3c) in rats (x ± SEM; n=8).

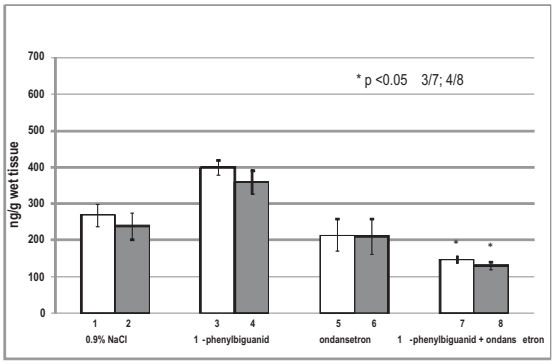
Objaśnienia jak w Ryc. 1 (Explanations as in Figure 1)

5-HTP assay

Equally high levels of 5-HTP in the prefrontal cortex, thalamus with hypothalamus and brain stem were observed between control and DSP-4 lesioned animals (after 0.9% NaCl injection) (Fig. 4a, 4b, 4c). 1-phenylbiguanide in a dose of 7.5 mg/kg ip significantly increased 5-HTP level in the prefrontal cortex of control rats ($p < 0.05$) being without effect in DSP-4 group in this regard. Ondansetron (1.0 mg/kg ip) did not affect 5-HTP content when given alone but injected before 1-phenylbiguanide abolished its effect in control group (Fig. 4a). In the thalamus with hypothalamus (control) as well as in the brain stem (control and DSP-4) 1-phenylbiguanide non-significantly elevated 5-HTP level (Fig. 4b, 4c). Ondansetron alone did not affect examined parameters but in brain stem administered before 1-phenylbiguanide statistically lowered 5-HTP (in both tested groups) in comparison to respective controls (1-phenylbiguanide) (Fig. 4c).







Ryc. 4. Wpływ DSP-4 (50 mg/kg sc) na zawartość 5-HTP w korze przedczołowej, wzgórzu z podwzgórzem i pniu mózgu po podaniu 1-fenylbiguanidu (7,5 mg/kg ip) (Ryc. 4a), ondansetronu (1,0 mg/kg ip) (Ryc. 4b) lub 1-fenylbiguanidu (7,5 mg/kg ip) z ondansetronem (1,0 mg/kg ip) (Ryc. 4c) w teście imersji ogona u szczurów (x ± SEM; n=5-6).

Fig. 4. Effect of DSP-4 (50 mg/kg sc) treatment on 5-HTP level in the prefrontal cortex, thalamus with hypothalamus and brain stem after 1-phenylbiguanide (7.5 mg/kg ip) (Fig. 4a), ondansetron (1.0 mg/kg ip) (Fig. 4b) or 1-phenylbiguanide (75 mg/kg ip) with ondansetron (1.0 mg/kg ip) (Fig. 4c) administration in rats (x ± SEM; n=5-6)

Objaśnienia jak w Ryc. 1 (Explanations as in Figure 1).

DISCUSSION

In the present study, we demonstrated that first (1) serotoninergic 5-HT_3 receptor agonist (1-phenylbiguanide) administered intraperitoneally elicits anti-nociceptive effect examined in three models of exteroceptive sensation and second (2) DSP-4 treatment to newborn rats essentially affects serotoninergic 5-HT_3 receptor mediated analgesia that is integrated in higher brain structures being without effect on spinal analgesia.

The main controversy as far as 5-HT_3 receptor mediated analgesia is concerned is that the majority of studies examined its role only after local (intrathecal) 5-HT_3 ligands administration. For example, in rodent behavioral studies, the serotoninergic 5-HT_3 receptor agonist, 2-methyl-5-HT mimicked anti-nociceptive effects of 5-HT in tail flick and hot plate tests and intrathecal application of a selective serotoninergic 5-HT_3 receptor antagonist blocked 5-HT-induced anti-nociception [28]. Also intrathecal administration of 2-methyl-5-HT has been shown to exert analgesia in the formalin test [10]. Bardin et al. [29] found that an local (intrathecal) injection of the 1-phenylbiguanide induced significant anti-nociceptive effects in the mechanical pain test. Furthermore electrophysiological studies recording the activities of dorsal horn neurons have indicated that anti-nociception is induced by the activation of serotoninergic 5-HT_3 receptors in the dorsal horn. Peng et al. [30, 31] demonstrated that responses of dorsal horn neurons to noxious stimuli are inhibited by electrical stimulation of the periaqueductal gray matter and this inhibition is attenuated by spinal application of 5-HT_3 receptors antagonists. Accordingly, these studies suggest that serotoninergic 5-HT_3 receptors located in the dorsal horn are involved in anti-nociception. To the best of our knowledge the present study is the first which demonstrated anti-nociceptive effects of peripherally 1-phenylbiguanide administration. Dukat and Wesołowska [32] showed that MD-354 (m-chlorophenylguanidine) a 5-HT3/alpha2B-adrenoceptor ligand administered subcutaneously was inactive as an analgesic in the mouse tail-flick assay. However, it should be noted that MD-354 is only partial 5-HT_3 receptor agonist and cited authors used mice not rats in experiment. The above may, at least in part, explain the discrepancy with our results.

The second important findings of the present study is that DSP-4 treatment diminishes anti-nociceptive effects mediated through serotoninergic 5-HT_3 receptor agonist. Previously it has been established that immersion test assesses nociception stimuli integrated on the spinal level, in contrast to the hot plate and paw pressure tests that reflect supraspinal analgesia mechanisms [33, 34]. One can demonstrated that the tail-flick is a spinal reflex in that, at least in its shorter latency form, it persists after section or cold block of upper parts of the spinal cord [35]. From our study we learned that disruption of NA neurotransmission (by neonatal DSP-4 treatment) did not affect the serotoninergic 5-HT_3 receptor mediated perception of painful stimuli integrated on spinal level (tail immersion test; Fig. 1) but significantly altered those consolidated in the upper parts of the brain (hot plate and paw withdrawal tests; Fig. 2 and 3). One explanation of this phenomenon could be the fact that DSP-4 produced almost complete NA-denervation in the cortex, hippocampus, thalamus, etc. but at the same time significant increase in NA content in the cerebellum and brain stem occurred, probably due to noradrenergic

fiber hyperinnervation [36]. It is likely that NA-hyperinnervation of LC (in the brain stem where descending anti-nociceptive pathways originate) is a compliance mechanism that results in restoration of disrupted physiological functions of the spinal cord. It is not clear why such mechanisms did not occur in the higher brain's structures. As it was mentioned in the introduction in a long series of studies published over a period of 5 years, we showed that central noradrenergic system lesion in rats have a remarkable influence on the sensitivity status of dopaminergic, serotoninergic and GABA-ergic pathways eliciting long-term supersensitivity or desensitization of respective post- and/or presynaptic receptors depending on the model applied in a specific study. In brief, we found that neonatal DSP-4 treatment modifies the convulsions elicited by bicuculine and pentylenetetrazole administration in adult rats [37]. We also established that vigabatrine (GABA transaminase inhibitor) apply brought about an increase in GABA level in the prefrontal cortex in control and DSP-4 groups of animals; however, it contributes a 2-fold higher increase of the extracellular GABA concentration in DSP-4-lesioned rats compared to control animals [19]. In another study we found that neonatally DSP-4-lesioned rats were less vulnerable to sedative-hypnotic effects of phenobarbital and ethanol, additionally, there was no significant change in GABA concentration of the prefrontal cortex, hippocampus, cerebellum and midbrain of DSP-4-lesioned rats [20]. By means of this model (permanent noradrenergic dysfunction) we showed that the sensitivity to anxiolytic-like effect of benzodiazepine (diazepam) in rats was altered [38]. Formerly, we also found that chemical lesioning of noradrenergic neurons with DSP-4 greatly affected central dopaminergic (e.g. reactivity of dopamine D_2 and D_3 receptors) [39] as well as serotoninergic systems ($5\text{-}HT_{1A}$ autoreceptors desensitisation) [17, 18]. All the above indicate on a powerful effect of chemical noradrenergic terminals destruction on the other monoaminergic (e.g. serotoninergic) and GABA-ergic pathways in the rat brain. It is likely that DSP-4 treatment modifies reactivity of the serotoninergic $5\text{-}HT_3$ receptors involved in supraspinal pain perception. One must cognise that this is only hypothesis because we did not find any literature data concerning this issue.

As far as the 5-HT synthesis rate is concerned we demonstrated that 1-phenylbiguanide in a dose of 7.5 mg/kg ip significantly increased 5-HTP accumulation only in the prefrontal cortex in the control rats and this effect was abolished by ondansetron pretreatment. Similar but non-significant effects were observed in the thalamus with hypothalamus. In the brain stem 5-HTP accumulation after 5-HT receptor ligands administration did not differ between examined groups of rats. Johnston et al. [40] showed that some analgesic (morphine) also increased 5-HT synthesis (measured by 5-HTP accumulation) in the medial preoptic, suprachiasmatic and arcuate nuclei as well as the striatum 1 hour following its administration. Conversely, Courade et al. [41] found that paracetamol did not affect 5-HTP accumulation in rats in the posterior cortex, hypothalamus, striatum, hippocampus and brain stem in rats, 45 min after po administration (200–400 mg/kg). To the best of our knowledge there is no data on noradrenergic system destruction and serotoninergic $5\text{-}HT_3$ agonist or antagonist evoked changes in monoamine synthesis rate making the interpretation of our results difficult. On the other hand Mongeau et al. [42] demonstrated that $5\text{-}HT_3$ receptor agonist (2-methyl-5-HT) enhanced NA release in the frontal cortex and lesioning 5-HT fibers with the neurotoxin 5,7-dihydroxytryptamine did not alter the action of 2-methyl-5-HT on NA release, indicating that this effect is not attributable to an action of this $5\text{-}HT_3$ receptor agonist on 5-HT terminals. Others found that $5\text{-}HT_3$ receptor stimulation results in dopamine as well as 5-HT release [43, 44]. Conversely, it is generally accepted that drugs that increase monoamine release (e.g. amphetamine) enhanced their synthesis rate [45]. Summing up, we showed apparent 5-HT synthesis alternation in the frontal cortex and moderate in thalamus in DSP-4 group with no changes in brain stem what could indirectly confirm discussed above behavioral observation of the present study.

Concluding, obtained results revealed that the noradrenergic system participates in the analgesic activity of serotoninergic $5\text{-}HT_3$ acting drugs integrated in the higher brain structures (e.g. cortex, thalamus) being without effect on spinal analgesia. Additionally, obtained data pointed out on the possibility of nociception disturbances (mediated by serotoninergic $5\text{-}HT_3$ receptor) in patients with noradrenergic system dysfunction (e.g., depression and/or anxiety disorders).

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