PRACA ORYGINALNA

Inflammatory and visceral pain perception in rats lesioned with DSP-4 as neonates

Odczucie bólu zapalnego i trzewnego u szczurów z noworodkową lezją ośrodkowego układu noradrenergicznego wywołaną podaniem DSP-4

Pain examination in DSP-4 lesioned rats

Badanie bólu u szczurów z lezją DSP-4

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A B S T R A C T

BACKGROUND:
The aim of this study was to examine the impact of the central noradrenergic lesion on the inflammatory and visceral pain perception after morphine, paracetamol and nefopam administration.

MATERIAL AND METHODS:
Intact male rats were contrasted with rats in which noradrenergic system was destroyed with DSP-4; 50 mg/kg sc on the 1st and 3rd days of postnatal life. After 10 weeks, painful reactions were assessed by means of formalin and writhing test. Furthermore accumulation of L-dihydroxyphenlalanine (L-DOPA) and 5-hydroxytryptamine (5-HTP) in some parts of the brain were examined using HPLC/ED method.

RESULTS:
30 min after morphine (5.0 mg/kg sc) challenge rats were injected into the right hind paw plantar surface with 50 µl 5% formalin solution. Both groups showed the typical biphasic nocifensive response curve lasting 60 min but DSP-4 lesioned rats scored more points in the first and second phase as well as the interphase period than control group. After paracetamol (100 mg/kg ip) administration also typical biphasic nocifensive response curve were observed however no differences between control and DSP-4 treated rats were noticed. Similar results were obtained after nefopam (20 mg/kg ip) challenge. Injections of morphine evoked similar antinociception in visceral pain model in both tested groups (control and DSP-4). Paracetamol elicited lower analgesia in control than in DSP-4 rats while

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nefopam was without effect. In biochemical assay, applied analgesics modified serotonin and dopamine synthesis rate but the effect (increase or decrease) depended on examined structure (the prefrontal cortex, thalamus with hypothalamus and brain stem).

CONCLUSIONS:
Obtained results demonstrated that DSP-4 treatment in rats modified the antinociceptive effects of the analgetics with distinct pharmacological prosperities and mechanism of action. It is likely that similar nociception abnormalities may occur in patients with noradrenergic system dysfunction, so it points to the requirement of analgetics dosage adjustment.

KEY WORDS:
Noradrenergic system, lesion, nociception, morphine, paracetamol, nefopam, rats

STRESZCZENIE

WSTĘP:
Celem pracy było zbadanie wpływu lezji ośrodkowego układu noradrenergicznego na analgetyczne działanie morfiny, paracetamolu i nefopamu u szczurów.

MATERIAŁ I METODY:
Noworodki szczurze 1-go i 3-go dnia życia otrzymały neurotoksynę DSP-4 (50 mg/kg sc) natomiast kontrola 0.9% roztwór NaCl (1.0 ml/kg sc). Po osiągnięciu wieku 10-ciu tygodni wykonano test formalinowy oraz wicia, oznaczono ponadto szybkość syntezy serotoniny i dopaminy w korze przedczołowej, wzgórzu z podwzgórzem oraz pniu mózgu metodą HPLC/ED.

WYNIKI:
W teście formalinowym stwierdzono, że obie grupy badawcze wykazywały typową dwufazową reakcję bólową trwającą 60 minut, nie mniej u zwierząt z lezją reakcja ta była znamiennie silniej wyrażona niż u kontroli. Takiej zależności nie stwierdzono po podaniu paracetamolu (100 mg/kg ip) lub nefopamu (20 mg/kg ip). W teście wicia wykazano, że efekty przeciwbólowego działania morfiny nie różnią się pomiędzy badanymi grupami zwierząt. Paracetamol działał natomiast słabiej przeciwbólowo u kontroli niż w grupie DSP-4. Nefopam nie wykazywał efektu przeciwbólowego w teście wicia. W badaniach biochemicznych stwierdzono, że stosowane leki przeciwbólowe modyfikują szybkość syntezy serotoniny i dopaminy ocenianą zawartością 5-hydroksytryptaminy (5-HTP) i L-dwuhydroksyfenyloalaniny (L-DOPA). Uzyskane efekty, tj. wzrost lub zmniejszenie syntezy uzależnione były od badanej struktury mózgu (kora przedczołowa, wzgórze z podwzgórzem, pień mózgu).

WNIOSKI:
Wyniki niniejszych badań wskazują, że uszkodzenie ośrodkowego układu noradrenergicznego wywołane podaniem DSP-4 we wczesnym okresie rozwoju osobniczego u szczurów w różny sposób modyfikuje przeciwbólowe działanie analgetyków o odmiennym mechanizmie działania. Wydaje się, że podobne efekty mogą wystąpić u chorych z dysfunkcją układu noradrenergicznego, co może wskazywać na konieczność modyfikacji dawek stosowanych leków przeciwbólowych.

SŁOWA KLUCZOWE:
Układ noradrenergiczny, lezja, ból, morfina, paracetamol, nefopam, szczury

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 determined that neonatal DSP-4 treatment modifies the convulsions evoked by bicuculine and pentylenetetrazole in adult rats [1]. We also established that administration of vigabatrine (GABA transaminase inhibitor) 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 [2]. In another study we found that neonatally DSP-4-lesioned rats are 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 [3]. By means of this model (permanent noradrenergic dysfunction) we showed that the sensitivity to anxiolytic-like effect of benzodiazepine (diazepam) was altered [4]. 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) [5] as well as serotoninergic systems ($5\text{-}HT_{1A}$ autoreceptors desensitisation) [6, 7]. All the above indicate to a powerful effect of chemical noradrenergic terminals destruction on the other monoaminergic and GABA-ergic pathways in the rat brain. On the other hand one must cognize that norepinephrine has a prominent role in the regulation of attention, arousal, cognitive processes, anxiety, and nociception - all of which are potential targets for anaesthetic and analgesic actions. It is well-known that drugs such as etomidate, ketamine, pentobarbital, diazepam, halothane, etc., strongly influence noradrenergic system activity (e.g., NE release, NE turnover and NE content throughout brain) [8-10]. Conversely, there are only scarce literature data which suggest that the monoaminergic systems play a prominent role in pain modulation and opioid analgesia in mammals. Many of the studies performed on this subject demonstrated reciprocal interactions between μ-opioid and $_2$-adrenergic and $5\text{-}HT_1$ and $5\text{-}HT_2$ serotoninergic mediated mechanisms [11, 12]. To the best of our knowledge there are no literature data on the effect of DSP-4 treatment (in neonates) and antinociceptive effect of such analgetics as paracetamol (COX inhibitor) or nefopam (serotonin uptake inhibitor). In order to clarify the effects of DSP-4 on antinociceptive action of these analgetics, we employed two models of persistent pain (writhing test – visceral pain assessment and formalin test – inflammatory pain estimation), furthermore we looked into serotonin and dopamine brain synthesis rate to find contingent link between these catecholamines and analgesic action of studied drugs.

MATERIALS AND METHODS

ANIMALS AND TREATMENT

Wistar rats (University Animal Department, Katowice, Poland) were housed under controlled environmental conditions, in a well-ventilated room, at 22 ± 2^0C and under a 12 h light:12 h dark cycle (lights on from 7:00 a.m. to 7:00 p.m.). Animals received food and water *ad libitum*. Offspring rats were weaned at 21days, and segregated by sex. Experiments were carried out in the morning in only male rats, handled in accordance with the principles and guidelines described in the *NIH Guide for the Care and Use of Laboratory Animals*. All procedures were reviewed and approved by the Local Bioethical Committee for Animal Care. The central noradrenergic system of newborn rats was lesioned with DSP-4 (Sigma, St. Louis, MO, USA). Rats were injected on the 1st and 3rd day of postnatal life with either DSP-4 (50 mg/kg *sc*) or 0.9% NaCl (1.0 ml/kg *sc*). DSP-4 was dissolved in distilled water immediately before injection. The dose and the days of injection were chosen on the basis of the work of Brus et al. [13], and were consistently proven to reduce endogenous noradrenalin content in the prefrontal cortex and hippocampus by 95–99%. Rats continued to be housed as above until 8–10 weeks, for further experimentation.

FORMALIN TEST

Inflammator pain and analgesia were determined using the formalin test in the rat. Rats

were placed in a clear plastic chamber (30 × 30 × 30 cm^3) for 30 min to allow them to accommodate to their surroundings with a mirror placed at a 45° angle beneath the floor to allow an unobstructed view of the paws. Then they were removed for drug administration: morphine (5.0 mg/kg ip), paracetamol (100 mg/kg ip) and nefopam (20 mg/kg ip). 30 min later rats were injected into the right hind paw plantar surface subcutaneously using a 30-gauge syringe 50µl of 5% formalin solution. Animals were then returned to the chambers, and 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 [14].

WRITHING TEST

Control and DSP-4 rats (deprived of food 24 h before testing) were placed individually in clear plexiglas boxes (40 x 30 x 20 cm) and allowed to acclimatize for 30 min. Then injected with saline (1.0 ml/100g *ip*) and 30 min later, administered with etacrinic acid solution 3.0mg/1ml/100g (in the left lower quadrant of the abdomen). Etacrinic solution was prepared ex tempore 3/47ethanol/water. Rat were returned to the chamber 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 division on 10 min intervals (10-20, 20-30, 30-40, etc.). Rat were used once and then killed immediately [15]. According to the same paradigm, separate groups of rats (control and DSP-4) were tested after analgesics treatment, morphine (5.0 mg/kg *ip*), acetaminophen (100 mg/kg *ip*) and nefopam (20 mg/kg *ip*) respectively. The degree of antinociception was expressed as the percentage decrease in the number of writhes and was calculated according to the formula:

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

A – the mean number of writhes in saline-treated control and DSP-4 rats for appropriate observation period

B – the mean number of writhes in drug-treated rats counted for appropriate observation interval

L-DOPA AND 5-HTP ASSAY (AN INDIRECT METHOD TO ASSESS DOPAMINE AND SEROTONIN SYNTHESIS RATE)

For L-dihydroxyphenylalanine (L-DOPA) and 5-hydroxytryptophan (5-HTP) estimation DSP-4 and control were injected with saline (0.9% NaCl) 1.0 ml/kg *ip* and after 30 min with aromatic amino acids inhibitor – hydroxybenzylhydrazine (NSD-1015) 100 mg/kg *ip* [16]. The separate groups of control and DSP-4 rats were administered with morphine (5.0 mg/kg *ip*), paracetamol (100 mg/kg *ip*) or nefopam (20 mg/kg *ip*) and after 30 min with NSD-1015 100 mg/kg *ip*. 30 minutes after the second 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 L-DOPA, precursor of dopamine, and 5-HTP – precursor of serotonin were estimated by means of a Gilson high performance liquid chromatography with electrochemical detection (HPLC/EC). In brief, 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. Peaks were automatically integrated by universal chromatographic interface UCI-100. The instrumentation included an electrochemical detector model 141 with flow cell, piston pump model 302 with head 5SC, manometric

module model 802 (Gilson, France), thermostat for STH 595 column (Dionex, Germany), precolumn Hypersil BDS C18, 10x4 mm, 3 µm and chromatographic column Hypersil BDS C18, 250x4.6 mm, 3 µm (ThermoQuest GB).

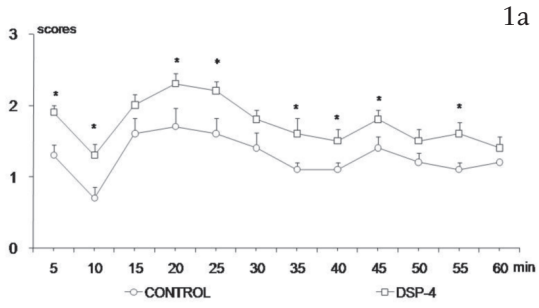
DATA ANALYSIS

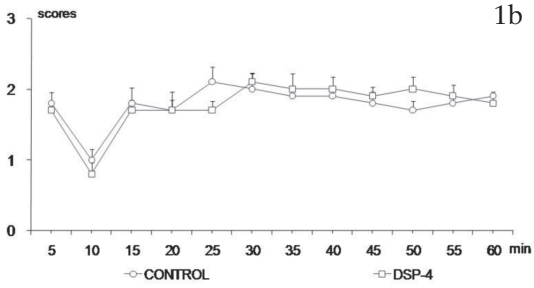
Group differences were assessed by an analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls. A P value <0.05 was taken as the level of significant difference.

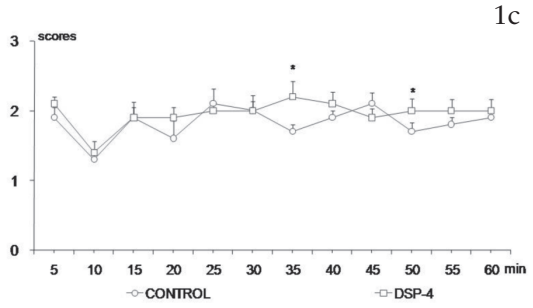
RESULTS

FORMALIN TEST

To assess the effect of noradrenergic lesion on analgesic action of morphine we compared the behavioral responses to *sc* injection of 25 µl (5%) of formalin into one hind paw of control and DSP-4 rats. Morphine (5.0 mg/kg *sc*) was administered 30 min before formalin apply. Both groups showed the typical biphasic nocifensive response curve lasting 60 min of testing but DSP-4 lesioned rats scored more points (spending more time licking/biting the injected hind paw) in the first and second phase as well as the interphase period of the formalin test than control group ($p < 0.05$ at 5, 10, 20, 25 35, 40, 45 and 55 min) (Fig. 1a). After paracetamol (100 mg/kg *ip*) administration also typical biphasic nocifensive response curve were observed however no differences between control and DSP-4 treated rats were noticed (Fig. 1b). Similar results were obtained after nefopam (20 mg/kg *ip*) administration (Fig. 1c).







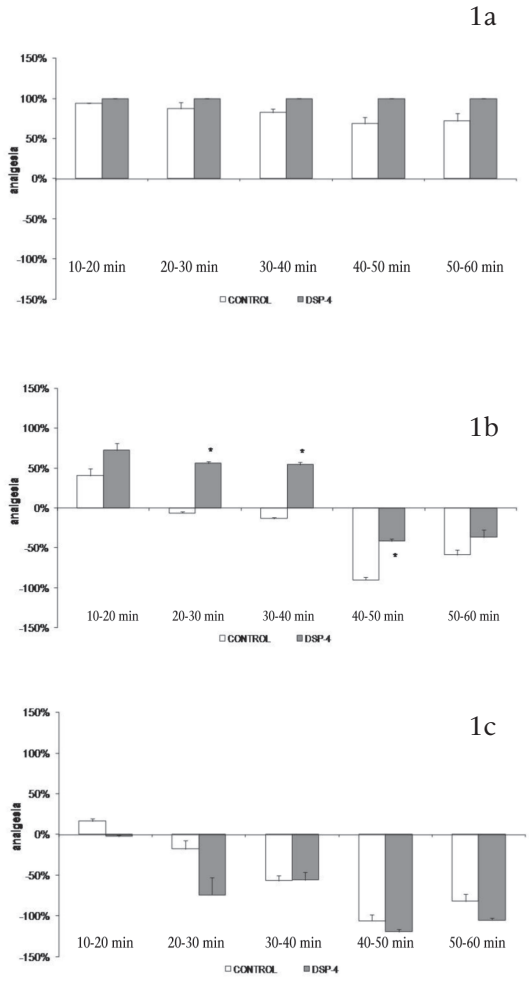
Rycina 1. Wpływ podania DSP-4 na przeciwbólowe efekty morfiny (5.0 mg/kg sc) (Ryc. 1a), paracetamolu (100 mg/kg ip) (Ryc. 1b) oraz nefopamu (20 mg/kg ip) (Ryc. 1c)w teście formalinowym u szczurów (n=10).

Figure 1. Effect of neonatal DSP-4 treatment on analgesia assessed in the formalin test after morphine (5.0 mg/kg *sc*) (Fig. 1a), paracetamol (100 mg/kg) *ip* (Fig. 1b) and nefopam (20 mg/kg *ip*) (Fig. 1c) administration in adult rats (n=10).

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

WRITHING TEST

Injections of morphine (5.0 mg/kg *sc*) evoked similar antinociception in visceral pain model in both tested groups (control and DSP-4) (Fig. 2a). Acetaminophen (100 mg/kg *ip*) elicited lower analgesia in control in comparison to DSP-4 rats, the effect was significant at 20-30, 30-40 and 40-50 intervals of observation (Fig. 2b). Nefopam administered in a dose of 20 mg/kg *ip* did not reduce writhes in both experimental groups, moreover even pronociceptive action of this drug was observed starting with 30 min of testing (Fig. 2c).



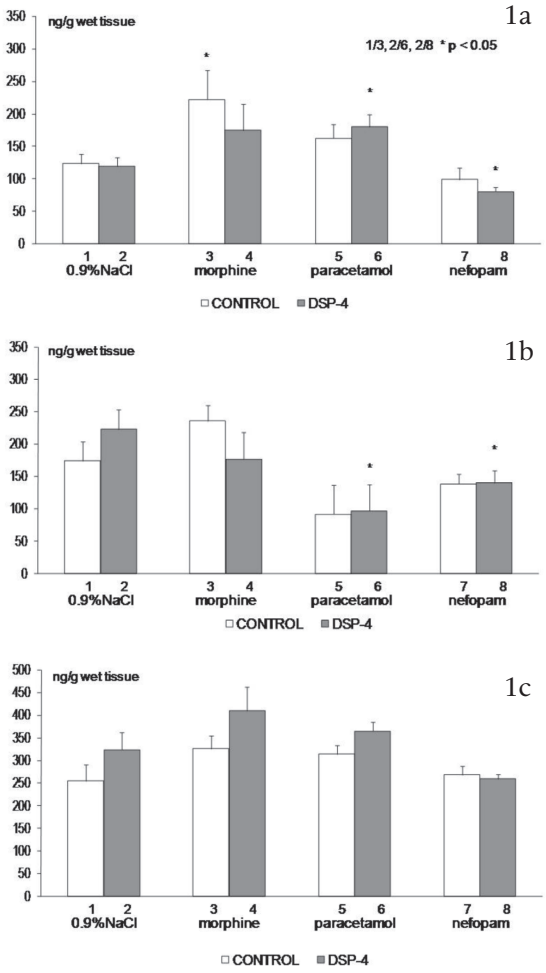
Rycina 2. Wpływ podania DSP-4 na przeciwbólowe efekty morfiny (5.0 mg/kg *sc*) (Ryc. 2a), paracetamolu (100 mg/kg *ip*) (Ryc. 2b) oraz nefopamu (20 mg/kg *ip*) (Ryc. 2c) w teście wicia u szczurów (n=10),

Figure 2. Effect of neonatal DSP-4 treatment on analgesia assessed in the writhing test after morphine (5.0 mg/kg *sc*) (Fig. 2a), paracetamol (100 mg/kg *ip*) (Fig. 2b) and nefopam (20 mg/kg *ip*) (Fig. 2c) administration in adult rats (n=10).

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

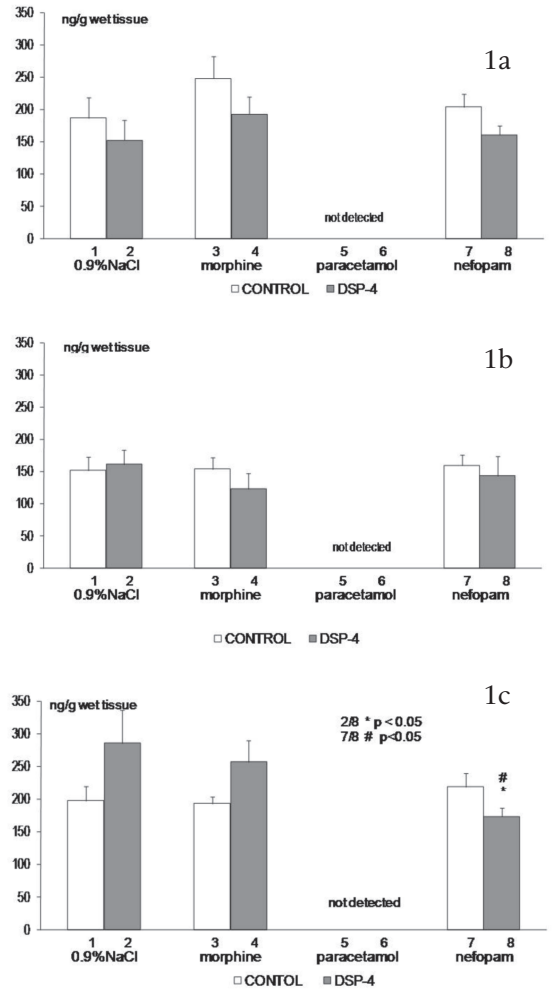
5-HTP AND L-DOPA CONTENT

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% saline). Morphine (5.0 mg/kg *sc*) significantly increased 5-HTP level only in the prefrontal cortex of control rats ($p<0.05$). Paracetamol (100 mg/kg *ip*) elevated 5-HTP content in DSP-4 group in the prefrontal cortex but diminished in the thalamus with hypothalamus, at the same time no effect was observed in all tested brain parts of control animals. Conversely, nefopam decreased 5-HTP content in the prefrontal cortex and thalamus with hypothalamus of DSP-4 rats but no effect was noted in the brain stem. Nefopam did not affect accumulation of 5-HTP in control rats (Fig. 3a – 3c). Also equally high levels of L-DOPA in all examined parts of the brain were noted between control and DSP-4 lesioned animals after saline administration. Morphine (5.0 mg/kg *sc*) did not affect



Rycina 3. Wpływ podania DSP-4 na zawartość 5-HTP w korze przedczołowej (Ryc. 3a), wzgórzu z podwzgórzem (Ryc. 3b) oraz pniu mózgu (Ryc. 3c) po podaniu morfiny (5.0 mg/kg *sc*), paracetamolu (100 mg/kg *ip*) oraz nefopamu (20 mg/kg *ip*) u szczurów (n=5 - 6).

Figure 3. Effect of neonatal DSP-4 treatment on the 5-HTP level in the prefrontal cortex (Fig. 3a), thalamus with hypothalamus (Fig. 3b) and brain stem (Fig. 3c) after morphine (5.0 mg/kg *sc*), paracetamol (100 mg/kg *ip*) and nefopam (20 mg/kg *ip*) in adult rats (n=5-6).



Rycina 4. Wpływ podania DSP-4 na zawartość L-DOPA w korze przedczołowej (Ryc. 4a), wzgórzu z podwzgórzem (Ryc. 4b) oraz pniu mózgu (Ryc. 4c) po podaniu morfiny (5.0 mg/kg *sc*), paracetamolu (100 mg/kg *ip*) oraz nefopamu (20 mg/kg *ip*) u szczurów (n=5 - 6).

Figure 4. Effect of neonatal DSP-4 treatment on the L-DOPA level in the prefrontal cortex (Fig. 4a), thalamus with hypothalamus (Fig. 4b) and brain stem (Fig. 4c) after morphine (5.0 mg/kg *sc*), paracetamol (100 mg/kg *ip*) and nefopam (20 mg/kg *ip*) in adult rats (n=5-6).

L-DOPA level in all tested structures of both experimental groups of rats. Nefopam (20 mg/kg ip) reduced L-DOPA only in the brain stem of DSP-4 treated animals in comparison to control (after nefopam administration) and DSP-4 (after saline injection) (Fig. 4a – 4c).

DISCUSSION

In the present work, we demonstrated that DSP-4 treatment influences morphine and paracetamol induced antinociception (depending on model used in a specific test) being at the same time without effect on nefopam mediated analgesia. It is also suggested that at least in part, serotoninergic system seems to be involved in perturbed analgesic effects of morphine and paracetamol observed in the DSP-4 treated rats.

BACKGROUND

It is worth knowing that morphine antinociception is often evaluated using innate reflex responses to high-intensity phasic thermal stimulation (tail flick, paw withdrawal). These methods are problematic for investigation of morphine, because high-intensity thermal stimuli elicit responses that correlate with excitation of A-δ nociceptors that are relatively resistant to attenuation by morphine. Inflammatory pain in humans and rodents produces sensations and nocifensive responses dominated by input from unmyelinated C nociceptors that are highly sensitive to attenuation by systemic morphine. For these reasons in the current study we employed two models of persistent pain: writhing test – for visceral pain assessment and formalin test for inflammatory pain estimation.

BEHAVIORAL STUDIES

In DSP-4 lesioned rats diminished morphine (5.0 mg/kg *sc*) analgesia in the formalin test was observed while in writhing assay no significant changes were noted (Fig. 1a and 2a). This is in line with Korzeniewska-Rybicka et al. [15] who also found that pretreatment of rats either with DSP-4 or *p*-chlorophenylalanine (*p*-CPA) (serotonin synthesis inhibitor) did not cause any statistical changes in rats behavior in writhing test. Conversely, the same authors found that, the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, potently and in a dose-dependent way inhibited rat behavior stimulated by pain. This receptor subtype is found in a great quantity in the dorsal horns of the spinal cord, and it is localized there mostly at the postsynaptic sites. 5-HT_{1A} receptors are also present in the other brain structures, including raphe nuclei, at both pre and postsynaptic sites. Their stimulation by selective agonists was reported either to enhance or inhibit the reactions to pain, depending on animal model of pain perception [17]. Perhaps in this specific model (visceral pain) destruction of noradrenergic system (in

the contrary to serotoninergic system) has little influence on morphine mediate analgesia. On the other hand Martin et al. [18] found that 14 days after destruction of noradrenergic neurons in the locus coeruleus the behavioral response to injection of formalin into the hindpaw during the second phase of pain behavior were significantly reduced. There was no change during the first phase. These results provide evidence that acute and persistent nociception are differentially regulated by descending noradrenergic pathways. We also showed that the noradrenergic lesion visibly affected the antinociceptive effect of paracetamol (100 mg/kg *ip*) examined in the writhing test (augmentation) being at the same time without effect in the formalin test (Fig 1b and 2b). It is likely that this is the first report demonstrating that the permanent noradrenergic neurotransmission disruption affects the analgetic properties of paracetamol. It was found that the fixed combination of aspirin, paracetamol and caffeine produced a significant reduction in extracellular dopamine and a dramatic increase in noradrenalin release from the striatal slices suggesting that the mechanism of this analgesic combination is based on the modulation of catecholaminergic neurotransmission [19]. Also paracetamol administration significantly increased serotonin and noradrenalin levels in the posterior cortex, hypothalamus, striatum, hippocampus and brain stem, but not spinal cord [20]. Altogether, the cited above data and the results of the present study suggest that paracetamol affects central monoaminergic neurotransmission, thereby suggesting that monoamines (including noradrenalin) might participate in its analgesic action. On the other hand the magnitude of analgesia elicited by nefopam (20 mg/kg ip) administration was not so evident, both in control and DSP-4 treated rats in comparison to morphine or paracetamol. Perhaps the dose used in this study was too low to reveal the differences between control and lesioned rats. Nevertheless, because it is believed that the descending serotonergic and noradrenergic pathways are markedly involved in nefopam-induced analgesia, obtained results are in contrary to our expectations [21, 22]. Esposito et al. [23] found that reserpine (2.0 mg/kg) which depleted noradrenalin, serotonin and dopamine from vesicular storages, significantly reduced the antinociceptive effects of nefopam (40 mg/kg), which confirms that this analgetic acts through the monoaminergic systems. However the same authors excluded the role for serotonin or noradrenalin because the selective lesion of serotoninergic (with 5.7-DHT) or noradrenergic systems (with DSP-4) did not affect nefopam antinociception.

BIOCHEMICAL STUDIES

Johnston et al. [24] showed that morphine in a dose of 10 mg/kg *sc* increased serotonin synthesis (measured by 5-HTP accumulation) in the medial preoptic, suprachiasmatic and arcuate nuclei as well as the striatum 1 hour following its administration. Lower dose (5.0 mg/kg *sc*) stimulated serotonin synthesis only in the arcuate nuclei. In our studies we also showed that morphine in a dose of 5.0 mg/kg *sc* moderately increased 5-HTP accumulation in examined brain parts of the control and DSP-4 lesioned rats. Paracetamol (100 mg/kg *ip*) significantly increased 5-HTP content in DSP-4 group in prefrontal cortex but diminished in the thalamus with hypothalamus (also significantly) being at the same time without effect in control animals. Courade et all. [20] also 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 paracetamol or nefopam evoked changes in monoamine synthesis rate making the interpretation of our results difficult. As we showed nefopam decreased 5-HTP content in the prefrontal cortex and thalamus with hypothalamus of DSP-4 rats, no effect was observed in the brain stem. Nefopam did not affect accumulation of 5-HTP in control rats. Since the non-opiate analgesic nefopam inhibits monoamines uptake (serotonin, noradrenalin, dopamine) we expected more pronounced changes in 5-HTP and L-DOPA accumulation. However, nefopam acts mainly through the inhibitory descending pathways localized in the spinal cord so that perhaps biochemical assay of this structure would reveal some changes in serotonin or dopamine synthesis rate alternations.

CONCLUSIONS

Summing up, the results of the current study demonstrate that noradrenergic system dysfunction caused by neonatal DSP-4 treatment modified the antinociceptive effects of the analgetics with distinct pharmacological pros-

perities and mechanism of action. It is likely that similar abnormalities in nociception may occur in patients with noradrenergic system dysfunction, so it points to analgetics dosage adjustment.

REFERENCES:

1.Bortel A., Nitka D., Słomian L., Nowak P., Korossy E., Brus R., Kostrzewa RM. Neonatal noradrenergic lesion with DSP-4 modifies the convulsant effect of bicuculine and pentylenetetrazole in adult rats. Behavioral and biochemical studies. Annales Acad. Med. Siles. 2008; 62; 46-52.

2.Bortel A., Nowak P., Brus R. Neonatal DSP-4 treatment modifies GABA-ergic neurotransmission in the prefrontal cortex of adult rats. Neurotox. Res. 2008; 13: 247-252.

3.Bortel A., Słomian L., Nitka D., Swierszcz M., Jaksz M., Adamus-Sitkiewicz B., Nowak P., Jośko J., Kostrzewa R.M., Brus R. Neonatal N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) treatment modifies the vulnerability to phenobarbital- and ethanol-evoked sedative-hypnotic effects in adult rats. Pharmacol. Rep. 2008; 60: 331-338.

4.Bortel A., Świerszcz M., Jaksz M., Nitka D., Słomian L., Nowak P., Brus R.: Anxiety-like behaviour in neonatally DSP-4 treated rats. Behavioural and biochemical studies. Ann. Acad. Med. Siles. 2007; 61: 485-491.

5.Nowak P., Labus Ł., Kostrzewa R.M., Brus R. DSP-4 prevents dopamine receptor priming by quinpirole. Pharmacol. Biochem. Behav. 2006; 84: 3-7.

6.Dąbrowska J., Nowak P., Brus R. Desensitization of 5-HT(1A) autoreceptors induced by neonatal DSP-4 treatment. Eur. Neuropsychopharmacol. 2007; 17: 129-137.

7.Dabrowska J., Nowak P., Brus R. Reactivity of 5-HT(1A) receptor in adult rats after neonatal noradrenergic neurons' lesion - Implications for antidepressant-like action. Brain Res. 2008 [Epub ahead of print].

8.Hara K., Yanagihara N., Minami K., Hirano H., Sata T., Shigematsu A., Izumi F. Dual effects of intravenous anesthetics on the function of norepinephrine transporters. Anesthesiology 2000; 93: 1329-1335.

9.Hirota K., Kudo M., Kudo T., Kitayama M., Kushikata T., Lambert D.G., Matsuki A. Barbiturates inhibit K(+)-evoked noradrenaline and dopamine release from rat striatal slices--involvement of voltage sensitive Ca(2+) channels. Neurosci. Lett. 2000; 291: 175-178.

10. Paul V., Ekambaram P. Effects of sodium nitroprusside, a nitric oxide donor, on gamma-aminobutyric acid concentration in the brain and on picrotoxin-induced convulsions in combination with phenobarbitone in rats. Pharmacol. Biochem. Behav. 2005; 80: 363-370.

11. McNally G.P., Akil H. Role of corticotropin-releasing hormone in the amygdala and bed nucleus of the stria terminalis in the behavioral, pain modulatory, and endocrine consequences of opiate withdrawal. Neuroscience 2002; 112: 605-617.

12. Nayebi A.R., Charkhpour M. Role of 5-HT(1A) and 5-HT(2) receptors of dorsal and median raphe nucleus in tolerance to morphine analgesia in rats. Pharmacol. Biochem. Behav. 2006; 83: 203-207.

13. Brus R., Nowak P., Labus Ł., Bortel A., Dąbrowska J., Kostrzewa R.M. Neonatal lesion of noradrenergic neurons in rat brain: interaction with the dopaminergic system. Pol. J. Pharmacol. 2004; 56: 232.

14. Acton J., McKenna J.E., Melzack R. Amitriptyline produces analgesia in the formalin pain test. Exp. Neurol. 1992; 117: 94-96.

15. Korzeniewska-Rybicka I., Płaźnik A. Role of serotonergic and noradrenergic systems in a model of visceral pain. Pol. J. Pharmacol. 2001; 53: 475-480.

16. Carlsson A., Davis J.N., Kher W., Lindqvist M., Atack C.V. Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brain in vivo using an inhibitor of the aromatic amino acid decarboxylase. Naunyn-Schmiedeberg's Arch. Pharmacol. 1972; 275: 153-168.

17. Millan M.J. Serotonin and pain: a reappraisal of its role in the light of receptor multiplicity. Pain 1995; 7: 409–419.

18. Martin W.J., Gupta N.K., Loo C.M., Rohde D.S., Basbaum A.I. Differential effects of neurotoxic destruction of descending noradrenergic pathways on acute and persistent nociceptive processing. Pain 1999; 80: 57-65.

19. Fiebich B.L., Candelario-Jalil E., Mantovani M., Heinzmann M., Akundi R.S., 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.

20. Courade J.P., 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.

21. Girard P., Coppé M.C., Verniers D., Pansart Y., Gillardin J.M. Role of catecholamines and serotonin receptor subtypes in nefopam-induced antinociception. Pharmacol. Res. 2006; 54: 195-202.

22. Guindon J., Walczak J.S., Beaulieu P. Recent advances in the pharmacological management of pain. Drugs 2007; 67: 2121-2133.

23. Esposito E., Romandini S., Merlo-Pich E., Mennini T., Samanin R. Evidence of the involvement of dopamine in the analgesic effect of nefopam. Eur. J. Pharmacol. 1986; 128: 157-164.

24. Johnston C.A., Moore K.E. The effect of morphine on 5-hydroxytryptamine synthesis and metabolism in the striatum, and several discrete hypothalamic regions of the rat brain. J. Neural. Transm. 1983; 57: 65-73.