PRACA ORYGINALNA

# Impairment in pain perception in adult rats treated with N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) as neonates

## Zaburzenia percepcji bólu u dorosłych szczurów z lezją ośrodkowego układu noradrenergicznego wykonaną we wczesnym okresie życia pozapłodowego

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ABSTRACT

**BACKGROUND**

This study was designed to investigate the antinociceptive effect of morphine, paracetamol and nefopam in rats lesioned with DSP-4 as neonates.

**MATERIAL AND METHODS**

Intact male rats were contrasted with rats in which noradrenergic nerve terminals were largely destroyed shortly after birth with the neurotoxin DSP-4 [(N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine; 50 mg/kg sc x2], on the 1st and 3rd days of postnatal life. When rats attained 10 weeks of age, painful reactions were assessed by means of tail immersion test and paw pressure test. Also monoamine levels in some part of the brain were estimated using HPLC/ED method.

**RESULTS AND CONCLUSION**

In the tail immersion test we showed that there are no differences between antinociceptive effect evoked by morphine (5.0 mg/kg sc) and paracetamol (100 mg/kg ip) between control and DSP-4 rats. Nefopam (20 mg/kg ip) elicited only slight analgesia in control rats (~ 17 %), this effect was no longer observed in the DSP-4 treated group. In the paw pressure test we demonstrated that morphine and paracetamol produced lower analgesia in DSP-4 rats in comparison to control. Nefopam evoked slight analgesia in both tested groups. In biochemical study we showed that in DSP-4 treated rats there was a marked decrease in NA level in the prefrontal cortex

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(to 10.4 %, p<0.01), thalamus with hypothalamus (to 54.4 %, p<0.05) and spinal cord (to 12.3 %, p<0.01) in comparison to the control group. Conversely, in the cerebellum and brain stem of DSP-4 lesioned rats there was a significant increase in the NA content versus control (respectively to 171.2 % and 123.5 % of NA in controls, p<0.05). In the striatum we did not observe any changes in NA level between examined groups. Also the levels of 5-HT and its metabolite 5-HIAA were not altered by DSP-4 treatment in all tested structures with the exception of the spinal cord (approx. 40% decrease) and the level of DOPAC (also 40% reduction). In conclusion, the obtained results showed that neonatal DSP-4 treatment alters the antinociceptive effects of examined drugs (each of them with different mechanism of action). These data lead to the proposal that perhaps there is a need to adjust the doses of analgetics applied to patients with noradrenergic system dysfunction (e.g. depression and/or anxiety disorders).

**KEY WORDS**
DSP-4 lesion, morphine, paracetamol, nefopam, analgesia, rats

## STRESZCZENIE

**WSTĘP**
W niniejszym eksperymencie oceniono wpływ lezji ośrodkowego układu noradrenergicznego wykonanej u noworodków szczurzych na przeciwbólowe efekty morfiny, paracetamolu i nefopamu u dorosłych szczurów.

**MATERIAŁ I METODY**
1-go i 3-go dnia życia noworodkom szczurzym podano neurotoksynę DSP-4 (50 mg/kg sc). Zwierzęta kontrolne otrzymały 0.9% roztwór NaCl (1.0 ml/kg sc). Po osiągnięciu wieku 10-ciu tygodni wykonano test imersji ogona oraz test wycofania łapy. Ponadto posługując się metodą HPLC/ED oznaczono zawartość amin biogennych i ich metabolitów w wybranych częściach mózgu badanych zwierząt.

**WYNIKI I WNIOSKI**
Nie stwierdzono różnicy w przeciwbólowym działaniu morfiny (5.0 mg/kg sc) i paracetamolu (100 mg/kg ip) w teście imersji ogona pomiędzy grupą kontrolną i DSP-4, natomiast nefopam 20 mg/kg ip wywoływał słabszą analgezję u zwierząt DSP-4 w porównaniu do kontroli. Działanie przeciwbólowe morfiny i paracetamolu w teście wycofania łapy było silniej wyrażone u szczurów kontrolnych w porównaniu do grupy DSP-4. Nie stwierdzono natomiast różnic w przeciwbólowych efektach nefopamu pomiędzy grupą kontrolną a DSP-4. DSP-4 stosowany 1 i 3-go dnia życia pozapłodowego spowodował istotny spadek zawartości NA w korze przedczołowej (do 10.4 %, p<0.005), wzgórzu z podwzgórzem (do 54.4 %, p<0.005) oraz w rdzeniu kręgowym (12.3 %, p<0.005) w porównaniu do kontroli. W móżdżku oraz w pniu mózgu u szczurów DSP-4 obserwowano istotny wzrost zawartości NA (odpowiednio do 171.2 % i 123.5 % wartości NA u szczurów kontrolnych, p<0.005; p<0.05). W prążkowiu nie stwierdzono zmian zawartości NA. Podanie DSP-4 nie miało istotnego wpływu na zawartość 5-HT i jej metabolitu 5-HIAA oraz DA i jej metabolitów DOPAC oraz HVA we wszystkich badanych strukturach mózgu poza rdzeniem kręgowym. Na podstawie przeprowadzonych badań wyciągnięto wnioski, iż zniszczenie ośrodkowego układu noradrenergicznego zmienia przeciwbólowe efekty badanych analgetyków. Powyższe może pośrednio wskazywać na konieczność odpowiedniego dostosowania dawek tych leków u pacjentów z dysfunkcją ośrodkowego układu noradrenergicznego (np. u chorych z depresją lub zaburzeniami lękowymi).

**SŁOWA KLUCZOWE**
DSP-4 lezja, morfina, paracetamol, nefopam, analgezja, szczury

## INTRODUCTION

There is considerable evidence to suggest that the central 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 $\alpha_2$-adrenergic as well as 5-$HT_1$ and 5-$HT_2$ serotonin mediated mechanisms [1, 2]. Obviously, the most vivid evidence of noradrenergic neurons impact on pain modulation includes opioid withdrawal syndrome. Abrupt cessation of opioid intake or acute opioid antagonists administration precipitate opioid withdrawal, which produces several aversive responses and symptoms, including an abnormal increase in pain sensitivity (hyperalgesia) [3]. It was demonstrated that the alternation in noradrenaline (NA) exocytosis in the thalamus, brain stem and other nuclei alters the output of nociceptive information to the higher brain center from projection neurons [3-6]. Furthermore, locus ceruleus (LC) stimulation which increases NA release in the spinal cord inhibits the nociceptive transmission in the dorsal horn through $\alpha_2$-adrenergic receptors. Also, NA depletion with 6-hydroxydopamine or intrathecal administration of $\alpha_2$-adrenergic receptor antagonist diminished the antinociception evoked by electrical or chemical stimulation of the LC [7, 8]. Altogether, these and other studies imply that stimulation of LC neurons which activates the descending noradrenergic system inhibits nociceptive transmission through $\alpha_2$-adrenergic receptors in the spinal cord [9]. Acute intraperitoneal (ip) administration of the selective noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine (DSP-4) to newborn rats (on the 1 st and 3 rd day) produces marked NA terminals destruction with following neurotransmission perturbation observed in adulthoods [10]. Also other neurotransmitter systems in the brain e.g., serotoninergic [11] and GABA-ergic [12, 13] are functionally affected by DSP-4 treatment. In fact, Dąbrowska et al. [11] found that chemical lesioning of noradrenergic neurons with DSP-4 results in desensitization of serotoninergic 5-$HT_{1A}$ autoreceptors [11]. Conversely, Bortel et al. [12] demonstrated that administration of vigabatrine (GABA transaminase inhibitor) causes a greater increase in GABA concentration 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 (dorsal and medial), 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 is no literature data on the effect of DSP-4 treatment (in neonates) and antinociceptive effect of paracetamol (COX inhibitor) and nefopam (serotonin uptake inhibitor). Therefore, the goal of this study was to determine whether DSP-4 administration to newborn rats alters antinociceptive effects of morphine, paracetamol and nefopam.

## MATERIAL AND METHODS

### ANIMALS AND TREATMENT

Male Wistar rats (University Animal Department; Katowice, Poland) were used. The animals were housed in plastic cages and kept in a temperature-controlled room (22±1°C) with a 12 h : 12 h light–dark cycle (lights off at 20 : 00 h). They had free access to standard food and water. 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 serotoninergic system destruction. Rats continued to be housed as above until 8-10 weeks, for further experimentation. Procedures involving animals and their care are conducted in conformity with the institutional guidelines that are in compliance with the principles and guidelines described in the NIH booklet *Care and Use of Laboratory Animals*. All procedures were reviewed and approved by the Local Bioethical Committee for Animal Care. Experiments were carried out in the morning and the animals were used only once.

### THERMAL STIMULUS: TAIL IMMERSION TEST [14]

Each rat was placed in a cone restrainer, and the end of the tail was immersed 5 cm in a 58°C water bath (58.5°C). 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:

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

Where: $T_x$ – is 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 30, 60, 90 and 120 min after morphine (5.0 mg/kg ip), paracetamol (100 mg/kg ip) and nefopam (20 mg/kg ip) injection.

**Mechanical stimulus: paw pressure test [15]**

Nociceptive thresholds expressed in grams (g), determined by a modification of the Randall-Selitto method were measured using an Ugo Basil analgesimeter (probe tip diameter 1 mm; weight 25 g) by applying increasing pressure to the right dorsal hindpaw until a flexion response was elicited. In brief, a constantly increasing pressure was applied to the right hindpaw 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. Mechanical threshold was always assessed three times before drug administration to yield a mean value. A 750-g cutoff value was used to prevent tissue damage. 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 30, 60, 90, 120 min after drug treatment.

The experiments were performed in a quiet room by the same investigator blinded as to the treatment used.

Assay of biogenic amines and their metabolites

Animals were decapitated. The medial prefrontal cortex, striatum, thalamus, brain stem, cerebellum and spinal cord were rapidly dissected and placed on dry ice, weighed and stored at –70$^0$C, pending assay. 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. Levels of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and NA were assayed [16, 17]. 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$^o$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). The obtained results of catecholamine assay were present in ng per gram of wet tissue (ng/g).

### DATA ANALYSIS

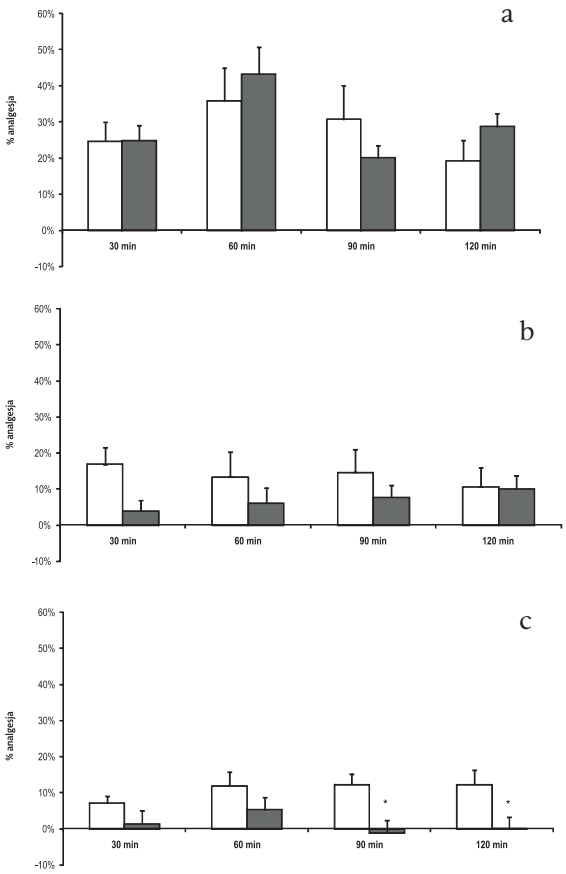
Group differences were assessed by an analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls. A P value <0.01 and <0.05 was taken as the level of significant difference.

## RESULTS

### TAIL IMMERSION TEST

There were no differences between antinociceptive effect evoked by morphine (5.0 mg/kg sc) and paracetamol (100 mg/kg ip) administration between control and DSP-4

rats (Fig.1a and 1b). Nefopam (20 mg/kg ip) elicited only slight analgesia in control rats (~ 17 %), this effect was not longer observed in the DSP-4 treated group; differences were statistically significant at 90 and 120 min of this test (Fig. 1c).




**Rycina 1.** Efekt podania DSP-4 (50 mg/kg *sc* w pierwszym i trzecim dniu życia pozapłodowego) 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 imersji ogona u szczurów (n=10).

**Figure 1.** Effect of neonatal DSP-4 (50 mg/kg *sc* on the 1st and 3rd day of postnatal life) treatment on analgesia assessed in tail-immersion 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) in adult rats (n=10).

Objaśnienia (Explanations):

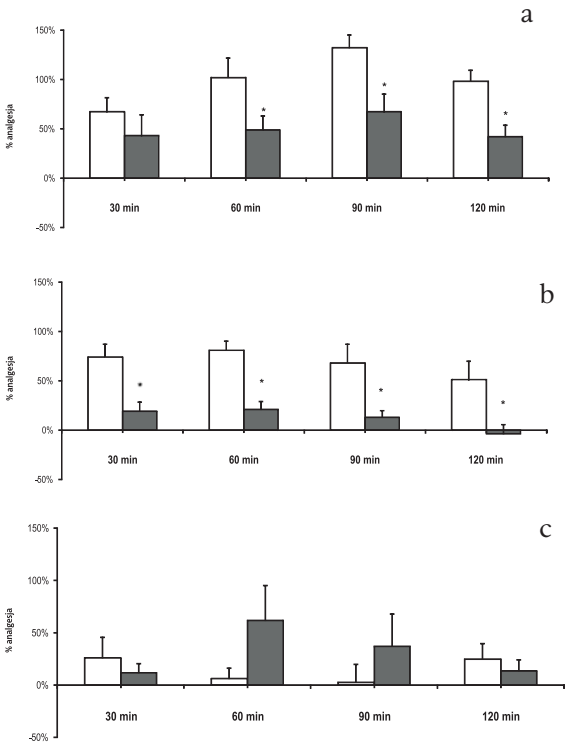
□ Kontrola (Control)

▨ DSP-4

* $p < 0.05$ Kontrola (Control) *vs.* DSP-4

### PAW PRESSURE TEST

Before drugs injection, withdrawal thresholds of intact and DSP-4 rats were 144 ±24.7 g and 154 ±23.5 g respectively (means from all measurements n=30 for each examined group). Morphine (5.0 mg/kg sc) elicited lower analgesia in DSP-4 rats in comparison to control rats and the effect was significant at 60, 90 and 120 min of the observation (Fig. 2a). The antinociceptive effect of paracetamol (100 mg/kg ip) was also greatly diminished in the DSP-4 group and significant in all tested intervals (Fig. 2b). Nefopam (20 mg/kg ip) produced only slight analgesia in both tested groups (Fig. 2c).




**Rycina 2.** Efekt podania DSP-4 (50 mg/kg *sc* w pierwszym i trzecim dniu życia pozapłodowego) 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 wycofania łapy u szczurów (n=10).

**Figure 2.** Effect of neonatal DSP-4 (50 mg/kg *sc* on the 1st and 3rd day of postnatal life) treatment on analgesia assessed in paw pressure 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) in adult rats (n=10).

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

### BIOGENIC AMINES AND THEIR METABOLITES CONTENT

In HPLC/ED assay we demonstrated that in DSP-4 treated rats there was a marked decrease in NA level in the prefrontal cortex (to 10.4

%, p<0.01), thalamus with hypothalamus (to 54.4 %, p<0.05) and spinal cord (to 12.3 %, p<0.01) in comparison to the control group. Conversely, in the cerebellum and brain stem of DSP-4 lesioned rats there was a significant increase in the NA content versus control (respectively to 171.2 % and 123.5 %; p<0.05; p<0.05) . We did not observe any changes in NA level in the striatum between both examined groups of rats. Also the levels of 5-HT and its metabolite 5-HIAA were not altered by DSP-4 treatment in all tested structures with the exception of the spinal cord (approx. 40% decrease) and the level of DOPAC (also 40% reduction) (Tab. 1).

| | Structures | NA (ng/g wet tissue) | 5-HT (ng/g wet tissue) | 5-HIAA (ng/g wet tissue) | DA (ng/g wet tissue) | DOPAC (ng/g wet tissue) | HVA (ng/g wet tissue) |
|---|---|---|---|---|---|---|---|
| CONTROL | Prefrontal cortex | 245.9 ± 24,9 | 250.3 ± 25.5 | 139.8 ± 14.5 | 47.7 ± 5.1 | 16.8 ± 1.5 | 27.6 ± 4.1 |
| | Striatum | 87.2 ± 4.3 | 442.4 ± 32.4 | 715.6 ± 64.7 | 9597.2 ± 1167.1 | 1212.9 ± 137.4 | 451.8 ± 83.5 |
| | Thalamus with hypothalamus | 2158.2 ± 132.8 | 821.9 ± 50.2 | 724.7 ± 39.7 | 484.9 ± 34.4 | 74.6 ± 7.5 | ---- |
| | Brain stem | 518.0 ± 38.1 | 678.3 ± 35.7 | 690.0 ± 49.6 | 49.8 ± 5.1 | 17.8 ± 1.8 | ---- |
| | Cerebellum | 184.0 ± 12.2 | 48.2 ± 3.8 | 99.6 ± 7.7 | 3.8 ± 0.4 | 3.0 ± 0.4 | ---- |
| | Spinal cord | 383.3 ± 19.9 | 529.8 ± 32.0 | 480.3 ± 36.9 | 21.5 ± 1.9 | 3.2 ± 0.3 | ---- |
| DSP-4 | Prefrontal cortex | 25.7 ± 3.5 ** | 169.9 ± 55.4 | 102.1 ± 26.4 | 45.5 ± 5.0 | 15.5 ± 2.2 | 33.7 ± 7.8 |
| | Striatum | 65.8 ± 10.8 | 521.2 ± 21.3 | 691.5 ± 48.0 | 6553.4 ± 283.1 | 922.2 ± 40.3 | 276.2 ± 24.2 |
| | Thalamus with hypothalamus | 1178.5 ± 99.9* | 879.5 ± 81.5 | 684.2 ± 63.8 | 403.2 ± 37.9 | 56.8 ± 6.0 | ---- |
| | Brain stem | 640.3 ± 32.3* | 622.8 ± 50.1 | 674.0 ± 62.7 | 53.0 ± 4.5 | 14.3 ± 1.8 | ---- |
| | Cerebellum | 315.1 ± 31.0* | 59.5 ± 10.1 | 96.5 ± 8.3 | 6.8 ± 1.0 | 3.0 ± 0.7 | ---- |
| | Spinal cord | 47.2 ± 7.8** | 285.1 ± 48.7* | 276.2 ± 50.6* | 21.2 ± 3.3 | 1.9 ± 0.3* | ---- |

**Tabela 1.** Efekt podania DSP-4 (50 mg/kg *sc* w pierwszym i trzecim dniu życia pozapłodowego) na zawartość amin biogennych w korze przedczołowej, prążkowiu, wzgórzu, pniu mózgu, móżdżku oraz rdzeniu kręgowym (x ± SEM; n = 6)

**Table 1.** Effect of DSP-4 (50 mg/kg *sc* on the 1st and 3rd day of postnatal life) treatment on biogenic amine level in the prefrontal cortex, striatum, thalamus, brain stem, cerebellum and spinal cord (x ± SEM; n = 6)
*p < 0.05; ** p < 0.01

## DISCUSSION

As has been noted DSP-4 administered to rats in their early stage of postnatal life results in persistent destruction of the noradrenergic system. Almost complete NA-denervation in the prefrontal cortex and spinal cord with concomitant severe damage in the thalamus was observed (prefrontal cortex ~ 89.6%, spinal cord ~ 87.7% and thalamus ~ 45.6% reduction in NA content). At the same time significant increase in NA content in the cerebellum occurred (Tab. 1), probably due to noradrenergic fiber hyperinnervation. These findings confirmed that DSP-4 has a demolishing effect on the central noradrenergic system in rats with the accompanying, profound "anatomical reorganization" as has been proved in the present work. Conversely, NA is involved in the regulation of attention, arousal, anxiety and as mentioned in the introduction in the nociception processes, all of which are potential targets for the action of opioids and other analgesic drugs action [18, 19]. It must be added that considerable but insignificant reduction in 5-HT and 5-HIAA content was observed in prefrontal cortex of DSP-4 treated rats. It is likely that the dose of zimelidine (10 mg/kg sc) applied to newborn rats (30 min before DSP-4) for prevention serotoninergic system destruc-

tion was to low to completely protect 5-HT fibers. Similar drop was observed in DA and its metabolites in the striatum, it is difficult to find an explanation for this findings. In the present study we found that DSP-4 treatment affected (diminished) morphine (5.0 mg/kg sc) analgesia in the paw pressure test being without effect in thermal assay (Fig. 1a and 1b). The last finding is contrary to Zhong et al. [20] who found that in rats pretreated with DSP-4 (injected intrathecally), the analgesic effect of morphine given either icv or ip and assessed in a tail-flick test was significantly attenuated. These observations are in accordance with others who employed mice in their experiments [21]. It must be recognized that the cited authors administered DSP-4 intrathecally, destroying only spinal cord's noradrenergic system and at the same time being without effect on brain NA phenotypes. The above may, at least in part, explain the discrepancy with our results. Conversely, we demonstrated diminished morphine (5.0 mg/kg sc) evoked analgesia in paw pressure test and as mentioned above no changes in thermal analgesia assay. Although obtained results indicate on the NA involvement in the antinociceptive action of morphine, a different conclusion might have been reached with the type of noxious stimulation used in a specific study and in other species. For example, it was reported that 6-OHDA suppressed the antinociception effects of morphine in the tail-pinch test but not in the hot-plate and tail-flick tests, conversely 5,6-dihydroxytryptamine attenuated the effects of morphine in the hot-plate test but not in the tail-pinch and tail-flick tests [22]. We also demonstrated that the DSP-4 treatment markedly altered the antinociceptive effect of paracetamol (100 mg/kg ip) assessed in the paw pressure test (diminution) and no changes in the tail flick test (Fig 1b and 2b). To the best of our knowledge this is the first report showing that disruption of NA neurotransmission (by neonatal DSP-4 treatment) affects the perception of analgesia elicited by paracetamol. It is worth knowing that Fiebich et al. [23] found that the fixed combination of aspirin, paracetamol and caffeine (APC) produced a significant reduction in extracellular DA and a dramatic increase in NA release from the striatal slices suggesting that the mechanism of this commonly used analgesic combination is based on the modulation of catecholaminergic neurotransmission. Others demonstrated that 45 min after paracetamol administration (200-400 mg/kg po) significant increase in 5-HT and NA was observed with concomitant decrease in both the levels of the DA and its metabolites in the posterior cortex, hypothalamus, striatum, hippocampus and brain stem, but not spinal cord [24]. These and our results indicate that paracetamol affects central monoaminergic neurotransmission, thereby suggesting that monoamines (including NA) might participate in its analgesic action. In the present work, nefopam used in a dose of 20 mg/kg ip elicited only slight analgesia, the effect observed in two different tests (employing thermal and mechanical stimulus) and in both examined groups. Differences between control and DSP-4 group were noted only in the tail immersion test (Fig. 1c). The obtained results are contrary to our expectation because it is generally accepted that the descending serotonergic and noradrenergic pathways are involved in nefopam-induced antinociception [25, 26]. However, Esposito et al. [27] demonstrated that pretreatment with reserpine (2.0 mg/kg) which "switches off" noradrenergic, serotoninergic and dopaminergic systems, significantly reduced the antinociceptive action of nefopam (40 mg/kg), indicating that the interaction of this drug with the monoaminergic systems is important for its biological effects. At the same time they ruled out a role for 5-HT or NE because the selective depletion of 5-HT (using 5,7-dihydroxytryptamine) or NE (using DSP-4 or FLA-63) did not affect nefopam antinociception. In conclusion the obtained results showed that neonatal DSP-4 treatment alters the antinociceptive effects of morphine, nefopam, paracetamol (each of them with different mechanism of action). These data lead to the proposal that perhaps there is a need to adjust the doses of analgesics applied to patients with dysfunction of the noradrenergic system (e.g. depression and/or anxiety disorders).

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