PRACA ORYGINALNA

N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) neonatal treatment, feeding behavior and 5-HT$_{1B}$ receptor reactivity in adult rats

Wpływ podania N-(-2-chloroethylo)-N-ethylo-2-bromobenzylaminy (DSP-4) u noworodków szczurzych na zachowania związane z przyjmowaniem pokarmów i reaktywność receptora 5-HT$_{1B}$ u dorosłych szczurów

DSP-4 lesion and reactivity of 5-ht$_{1b}$ receptor
Lezja DSP-4 a reaktywność receptora 5-HT$_{1b}$

Marzena Ferdyn-Drosik[1], Przemysław Nowak[1], Jacek Kasperski[2], Rafał Muchacki[1], Beata Maksym[1], Michał Bałasz[1], Magdalena Beśka[1], Ryszard Brus[1]

ABSTRACT

BACKGROUND:
We investigated the reactivity of 5-HT$_{1B}$ receptor in adult rats administered as neonates with noradrenergic neurotoxin N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4; 50 mg/kg sc x2, P1 and P3).

MATERIAL AND METHODS:
At 8-10 weeks (after lesion), control and DSP-4 rats were divided into two subgroups injected either with saline or 5-hydroxytryptamine (serotonin; 5-HT) synthesis inhibitor (p-chlorophenylalanine; p-CPA; 100 mg/kg ip x 2 every 24h). On the following day food intake was examined after 5-HT$_{1B}$ receptor ligands administration; agonist CP 94,253, 4.0 mg/kg ip; and antagonist, SB 216641, 4.0 mg/kg ip respectively. Additionally, the level of monoamines, their metabolites and 5-hydroxytrptophane (5-HTP) as an indicator of 5-HT synthesis rate were analyzed by HPLC/ED methods.

RESULTS AND CONCLUSIONS:
CP 94,253 in a dose of 4.0 mg/kg ip significantly decreased food intake in control and DSP-4 rats in comparison to respective control (saline). 5-HT$_{1B}$ antagonist (SB 216641) alone did not alter the examined parameter but injected 30 min before CP 94,253 markedly abolished its effect in both tested groups. Similar results were obtained in p-CPA pretreated animals. The level of amines and their metabolites was not different be-

[1] Department of Pharmacology
[2] Department of Prosthetic Dentistry,
Medical University of Silesia,
Zabrze, Poland

ADRES DO KORESPONDENCJI:
Prof. dr hab. n.med. Przemysław Nowak
Katedra i Zakład Farmakologii SUM
41-808 Zabrze, ul. H. Jordana 38,
tel./fax +(48-32)-272-26-83
e-mail: pnowak@sum.edu.pl

Ann.Acad.Med.Siles. 2009, 63, 1, 16-23

ISSN 0208-5607

tween control and DSP-4 treated rats. Administration of p-CPA (2 x 100 mg/kg ip) reduced the concentration of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) in the hypothalamus of control rats by 94.9 % and 96.7 % respectively. In DSP-4 rats the percentage of 5-HT displacement was 92.5 % (5-HT) and 95.7 % (5-HIAA). As it was presented CP 94,253 and SB 216641 alike, administered in doses of 4.0 mg/kg ip, did not alter biogenic amines and their metabolites content in the hypothalamus of both examined groups (control and DSP-4). There were also no differences in the 5-HT synthesis rate in the hypothalamus between control and DSP-4 rats after 5-HT$_{1B}$ receptor ligands injection. We concluded that in contrast to our previous studies, DSP-4 being without effect on hypothalamus noradrenalin (NA) level and 5-HT synthesis rate did not also affect reactivity of terminal 5-HT$_{1B}$ autoreceptors in this brain area. On the basis of this experiments, it should be noted that in contrast to our previous studies which have shown that the consequence of DSP-4 administration is dramatic reduction in the NA contents in the frontal cortex, hippocampus, striatum, etc., with concomitant desensitization of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors (evaluated by behavioral and biochemical methods), in this experiment, there were no such effects. This proves that the noradrenergic fibers in the hypothalamus are much more resistant to the neurotoxic action of DSP-4, furthermore, for the development of 5-HT$_{1B}$ receptors desensitization in the specific brain structure, decrease in NA contents in this part of the brain is required. In other words, a profound dysfunction of the noradrenergic system expresses as noradrenergic fiber damage in the striatum, frontal cortex, hippocampus, etc. does not affect the status of 5-HT$_{1B}$ receptors in the hypothalamus.

KEY WORDS:
noradrenergic system, lesion, 5-HT$_{1B}$ receptor, food intake, rats

STRESZCZENIE

WSTĘP:
W niniejszej pracy zbadano reaktywność ośrodkowego receptora serotoninergicznego 5-HT$_{1B}$ u dorosłych szczurów, które w okresie noworodkowym otrzymały neurotoksynę N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4; 50 mg/kg sc x 2, 1-go i 3-go dnia).

MATERIAŁ I METODY:
W 8-10-tym tygodniu życia zwierzęta kontrolne i z lezją podzielono na dwie podgrupy, jedna otrzymała sól fizjologiczną, druga inhibitor syntezy serotoniny (5-hydroksytryptaminy; 5-HT) - *p*-chlorofenyloalaninę (*p*-CPA). Następnie po podaniu ligandów receptora 5-HT$_{1B}$ (agonisty; CP 94253 4.0 mg/kg ip i/lub antagonisty; SB 216641 4.0 mg/kg ip) oceniono ilość spożywanej karmy. Dodatkowo zbadano również zawartość amin biogennych po podaniu ligandów receptora 5-HT$_{1B}$ oraz oceniono szybkość syntezy serotoniny w podwzgórzu.

WYNIKI I WNIOSKI:
CP 94253 stosowany zarówno u szczurów kontrolnych jak i DSP-4 zmniejszał ilość spożywanej karmy w porównaniu do odpowiednich grup kontrolnych (po podaniu soli fizjologicznej). Podobne efekty zaobserwowano u zwierząt, które wcześniej otrzymały *p*-CPA. Nie stwierdzono zmian zawartości amin biogennych po podaniu DSP-4. Również CP 94253 oraz SB 216641 nie wpływały na stężenie amin biogennych i ich metabolitów w badanej strukturze mózgu. Po podaniu *p*-CPA (2 x 100 mg/kg ip) obserwowano spadek zawartości 5-HT oraz 5-HIAA w podwzgórzu u kontroli odpowiednio o 94.9% i 96.7%, natomiast w grupie DSP-4 o 92.5 % (5-HT) i 95.7 % (5-HIAA). Szybkość syntezy 5-HT nie różniła się pomiędzy kontrolą a grupą DSP-4, również CP 94253 4.0 mg/kg ip i/lub SB 216641 4.0 mg/kg ip pozostawały bez wpływu na badany parametr. Na podstawie przeprowadzonych eksperymentów stwierdzić należy, że w przeciwieństwie do naszych poprzednich badań, w których wykazaliśmy, że konsekwencją podania DSP-4 jest spadek zawartości NA w korze czołowej, hipokampie i prążkowiu, które wywołuje zjawisko de-

sensytyzacji receptorów 5-HT_{1A} i 5-HT_{1B} (oceniane metodami behawioralnymi i biochemicznymi), w niniejszym doświadczeniu nie stwierdzono takich efektów. Powyższe przemawia za tym, iż zakończenia noradrenergiczne w podwzgórzu są znacznie bardziej oporne na neurotoksyczne działanie DSP-4 oraz, że dla rozwoju desensytyzacji receptorów 5-HT_{1B} w określonej strukturze mózgu konieczny jest spadek zawartości NA w tej części mózgu. Innymi słowy głęboka dysfunkcja układu noradrenergicznego pod postacią zniszczenia zakończeń noradrenergicznych w korze czołowej, prążkowiu oraz hipokampie pozostaje bez wpływu na „status" receptorów 5-HT_{1B} w podwzgórzu.

SŁOWA KLUCZOWE:
układ noradrenergiczny, lezja, receptor 5-HT_{1B}, przyjmowanie karmy, szczury

INTRODUCTION

It is well established that 5-hydroxytryptamine (serotonin; 5-HT) controls feeding behavior, as shown by the potent anorectic properties of some 5-HT releasers and uptake inhibitors (e.g. fenfluramine). Pharmacological studies have clearly demonstrated that the 5-HT_1 and 5-HT_2 receptors play a key role in regulating food intake in mammals. It was shown that the 5-HT_{1B} and 5-$HT_{2A/2c}$ receptor agonists produce hypophagia [1] whereas 8-OH-DPAT (5-HT_{1A} agonist) stimulates food intake [2]. Also other monoamine systems that use dopamine (DA) or noradrenaline (NA) as neurotransmitters participate in feeding behavior, energy balance and maintenance of body weight. It was demonstrated (both in preclinical and clinical studies) that drugs that enhance the release of monoamines from neuronal stores (e.g. the amphetamines, phentermine, ephedrine, etc.) reduced feeding and weight gain [for review see 3]. It is worth knowing that sibutramine (BTS 54524) - the compound which is metabolized in vivo to produce metabolites that have varying degrees of inhibition of NE, 5-HT, and/or DA uptake has been currently approved for the long-term control of obesity [4, 5].

As shown there is extensive evidence for functional interactions between central noradrenergic and serotonergic neurons. The locus coeruleus (LC), the major NA brainstem nucleus, sends projections to the dorsal raphe nuclei (DRN), while the DRN projects to the LC, so there is ample opportunity for cross-modulation [6, 7]. Dąbrowska et al. [8] demonstrated that rats in which noradrenergic nerves were largely destroyed shortly after birth with the neurotoxin N-(-2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) developed 5-HT_{1A} autoreceptors desensitisation. In HPLC/ED assay R-(+)-8-OH-DPAT (0.03 mg/kg) significantly decreased 5-HT synthesis rate in striatum, hypothalamus and frontal cortex of control, being without effect in DSP-4-lesioned adult rats (10-12 weeks old). Also R-(+)-8-OH-DPAT (0.015 mg/kg) caused hyperphagia of control rats, but did not change feeding of DSP-4 treated rats. More recently we also found that the terminal 5-HT_{1B} autoreceptors in the medial prefrontal cortex and striatum also underwent desensitization in DSP-4 treated rats [9, 10].

Therefore the primary goal of this study was to investigate whether the neonatal noradrenergic lesion could modify 5-HT_{1B} receptors agonist-evoked hypophagic response in adult rats.

MATERIAL AND METHODS

ANIMALS AND TREATMENT

Male Wistar rats (University Animal Department; Katowice, Poland) were housed in cages with free access to water and chow pellets in a light-controlled (12 h on/12 h off; lights off at 20:00 h) and temperature-controlled (20–22°C) environment. Offspring rats were weaned at 21days, and segregated by sex.

The central noradrenergic system of newborn rats was destroyed with DSP-4 (Sigma, St. Louis, MO, USA). Rats were injected on the 1 st and 3 rd 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 0.9% NaCl immediately before injection. 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, in all the experiments the animals were used once.

FOOD INTAKE

The feeding procedure followed exactly the same as the one used by Dąbrowska et al. [8]. 24 h before the experiments, rats were housed singly with ad libitum access to standard food and tap water. On the testing day, the remaining of food was removed, the cages carefully cleaned. Rats from both tested groups (control and DSP-4) received one of the following paired injections: (1) saline (1.0 ml/kg ip) twice, 30 min interval, (2) saline (1.0 ml/kg ip) 30 min before CP 94,253 (4.0 mg/kg ip), (3) SB 216641 (4.0 mg/kg ip) 30 min before saline (1.0 ml/kg ip), (4) SB 216641 (4.0 mg/kg ip) 30 min before CP 94,253 (4.0 mg/kg ip). 30 min later a new portion of food (10 g) was placed into the cage. After the 30 min period of observation food was briefly removed and weighed. The same procedure was performed on animals (control and DSP-4) that formerly were pretreated with 5-HT synthesis inhibitor, p-chlorphenylalanine (p-CPA) 100 mg/kg ip x2 every 24 h.

HPLC/ED ANALYSIS FOR BIOGENIC AMINES AND THEIR METABOLITES

Control and DSP-4 rats were divided into four subgroups receiving CP 94,253 (4.0 mg/kg ip) and/or SB 216641 (4.0 mg/kg ip) according to the same paradigm as described in the section "Food intake". 30 min after the last injection, unanesthetized rats were decapitated, and their brains were rapidly removed. The hypothalamus was dissected, weighed and stored at –70^{0}C, pending assay. Determination of DA, 5-HT, NA and metabolites, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxyindoleacetic acid (5-HIAA) was conducted by HPLC/ED analyses according to Magnusson et al. [11] with slight modification [12]. In brief, samples were first homogenized for 15-20 sec in ice-cold trichloroacetic acid (0.1 M) containing 0.05 mM ascorbic acid, then centrifuged (5,000g, 5 min). Supernatants were filtered through 0.2 µm cellulose membranes (Titan MSF Microspin filters, Scientific Resources Inc., Eatontown GB). The analytes were separated on a C_{18}, 3µm column (Hypersil BDS C18, 250x4.6 mm; ThermoQuest GB) with precolumn Hypersil BDS C_{18}, 10x4 mm, 3 µm (ThermoQuest GB). The mobile phase with final pH of 3 (adjusted with phosphoric acid) (Fluka) consisted 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). The flow rate was maintained at 0.7 ml/min, at a temperature of 22°C, and the oxidation potential was set at +700 mV, 10 nA/V sensitivity. The instrumentation included an electrochemical detector model 141 with flow cell, piston pump model 302 with head 5SC, manometric module model 802 (Gilson, France) and thermostat for STH 595 column (Dionex, Germany),

5-HTP ACCUMULATION ASSAY AS AN INDICATOR OF 5-HT SYNTHESIS RATE

The rate of 5-HT synthesis was estimated by measuring the accumulation of 5-hydroxytryptophane (5-HTP) after administration of 100 mg/kg, ip of the aromatic L-amino acid decarboxylase inhibitor NSD-1015 [13].

Rats from control and DSP-4 groups were divided into 4 subgroups receiving CP 94,253 (4.0 mg/kg ip) and/or SB 216641 (4.0 mg/kg ip) according to the same paradigm as described in "Food intake". 30 min after the last injection and 30 min after the administration of NSD-1015 rats were killed by decapitation and hypothalamus was dissected on ice, and analyzed by HPLC/ED for 5-HTP assay as described above.

STATISTICAL ANALYSIS

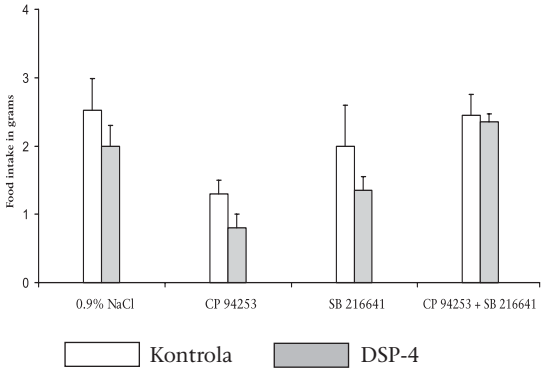
Data were analyzed by analysis of variance (ANOVA) and the post-ANOVA test of Newman-Keuls. A P value <0.05 was taken as the level of significant difference.

RESULTS

FOOD INTAKE

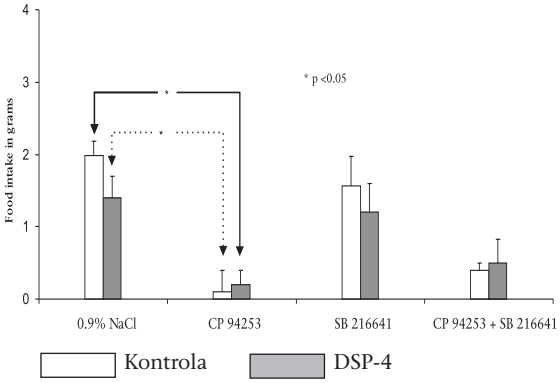
The were no differences in food intake between control and DSP-4 rats after saline injection. CP 94,253 in a dose of 4.0 mg/kg ip significantly decreased food intake during 30 min period of observation in control and DSP-4 rats in comparison to respective control (saline). 5-HT_{1B} antagonist (SB 216641) alone did not alter the examined parameter but injected 30 min before CP 94,253 markedly abolished its effect in both tested groups (Fig. 1).

In p-CPA pretreated animals CP 94,253 also significantly diminished food intake in control and DSP-4 groups (in comparison to saline injection). SB 216641 alone was without effect as well as did not abolish the effect of CP 94,253 (Fig. 2).



Rycina 1. Wpływ CP 9425 4.0 mg/kg ip oraz SB 216641 4.0 mg/kg ip na ilość spożywanej karmy u szczurów kontrolnych oraz z lezją DSP-4 (50 mg/kg sc) (n=8-10).

Figure 1. Effect of CP 9425 4.0 mg/kg ip and SB 216641 4.0 mg/kg ip on food intake in control and DSP-4 (50 mg/kg sc) lesioned rats (n=8-10).



Rycina 2. Wpływ CP 9425 4.0 mg/kg ip oraz SB 216641 4.0 mg/kg ip na ilość spożywanej karmy u szczurów kontrolnych oraz z lezją DSP-4 (50 mg/kg sc), które uprzednio otrzymały p-CPA (100 mg/kg ip x 2 co 24h) (n=8-10).

Figure 2. Effect of CP 9425 4.0 mg/kg ip and SB 216641 4.0 mg/kg ip on food intake in control and DSP-4 (50 mg/kg sc) lesioned rats pretreated with p-CPA (100 mg/kg ip x 2 every 24 h) (n=8-10).

TISSUE ASSAY

The level of NA, DA, 5-HT and their metabolites was not different between control and DSP-4 treated rats. As it was presented CP 94,253 and SB 216641 alike, administered in doses of 4.0 mg/kg ip, did not alter NA, DA, DOPAC, HVA, 5-HT and 5-HIAA contents in the hypothalamus of both examined groups (control and DSP-4) (Tab. 1).

Administration of p-CPA (2 x 100 mg/kg ip) reduced the concentration of 5-HT and 5-HIAA in the hypothalamus of control rats by 94.9 % and 96.7 % respectively. In DSP-4 rats the percentage of 5-HT displacement was 92.5 % (5-HT) and 95.7 % (5-HIAA). As shown p-CPA (to a much lesser extent) also affected NA, DA and their metabolites content in the examined brain structure (the drops varied between 10 – 60%) (Tab. 2).

Groups	Substances	NA	5-HT	5-HIAA	DA	DOPAC	HVA
Control	0.9 % NaCl	2435,7 ±365,9	722,8 ±82,1	286,8 ±85,0	434,9 ±67,7	97,8 ±14,5	42,7 ±12,2
	CP 94253	2699,0 ±219,4	803,1 ±104,7	212,3 ±36,7	613,0 ±87,2	114,0 ± 16,6	30,8 ±3,6
	SB 216641	2216,2 ±239,6	624,8 ±95,5	295,0 ±42,3	517,2 ±47,5	104,5 ±12,4	42,9 ±7,0
	CP 94253 + SB 216641	2040,7 ±268,9	582,2 ±68,7	174,4 ±40,7	397,3 ±39,3	72,4 ±8,9	28,3 ±6,4

Groups	Substances	NA	5-HT	5-HIAA	DA	DOPAC	HVA
DSP-4	0.9 % NaCl	2178,9 ±334,4	716,1 ±141,5	270,8 ±54,5	460,3 ±63,7	109,0 ±16,0	33,6 ±12,8
	CP 94253	2415,4 ±378,4	680,5 ±88,5	200,9 ±37,1	411,9 ±67,7	91,0 ±14,3	23,0 ±3,7
	SB 216641	2258,2 ±244,7	635,5 ±78,1	252,0 ±43,7	497,0 ±47,2	94,1 ±16,8	42,8 ±9,9
	CP 94253 + SB 216641	1786,3 ±489,2	505,2 ±118,2	170,8 ±49,0	451,1 ±66,4	105,8 ±20,8	27,7 ±5,7

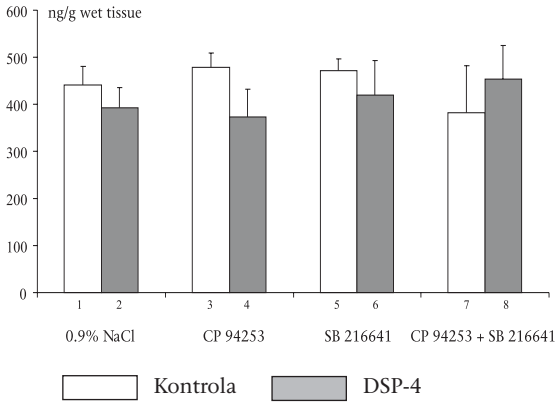
Tabela 1. Wpływ CP 9425 4.0 mg/kg ip oraz SB 216641 4.0 mg/kg ip na zawartość amin biogennych i ich metabolitów w podwzgórzu u szczurów u szczurów kontrolnych oraz z lezją DSP-4 (50 mg/kg sc) (n=5-6).

Table 1. Effect of CP 9425 4.0 mg/kg ip and SB 216641 4.0 mg/kg ip on biogenic amines and their metabolites level in the hypothalamus in control and DSP-4 (50 mg/kg sc) lesioned rats (n=5-6).

Groups	NA	5-HT	5-HIAA	DA	DOPAC	HVA
Control (0.9% NaCl 1.0 ml/kg *IP*)	2435,7 ±365,9	722,8 ±82,1	286,8 ±85,0	434,9 ±67,7	97,8 ±14,5	42,7 ±12,2
Control (*p*-CPA 100 mg/kg x2 *IP*)	1831,1* ±230,8	36,9* ±4,1	9,3* ±1,2	233,0* ±12,7	58,2* ±5,7	16,2* ±2,6
DSP-4 (0.9% NaCl 1.0 ml/kg *IP*)	2178,9 ±334,4	716,1 ±141,5	270,8 ±54,5	460,3 ±63,7	109,0 ±16,0	33,6 ±12,8
DSP-4 (*p*-CPA 100 mg/kg x2 *IP*)	1678,1* ±161,7	54,1* ±11,7	11,6* ±1,7	333,6* ±44,3	55,6* ±8,9	10,6* ±1,7

Tabela 2. Wpływ p-chlorofenyloalaniny (p-CPA) 100 mg/kg ip x 2 co 24h na zawartość amin biogennych i ich metabolitów w podwzgórzu u szczurów u szczurów kontrolnych oraz z lezją DSP-4 (50 mg/kg sc) (n=5-6).

Table 2. Effect of p-chlorophenylalanine (p-CPA) 100 mg/kg ip x2 every 24h on biogenic amines and their metabolites level in the hypothalamus in control and DSP-4 (50 mg/kg sc) lesioned rats (n=5-6).
* $p < 0.05$ (control *vs* p-CPA control; DSP-4 *vs* p-CPA DSP-4)



Rycina 3. Wpływ CP 9425 4.0 mg/kg ip oraz SB 216641 4.0 mg/kg ip na zawartość 5-hydroksytryptofanu (5-HTP) w podwzgórzu u szczurów u szczurów kontrolnych oraz z lezją DSP-4 (50 mg/kg sc) (n=5-6).

Figure 3. Effect of CP 9425 4.0 mg/kg ip and SB 216641 4.0 mg/kg ip on 5-hydroxytryptophan (5-HTP) content in the hypothalamus in control and DSP-4 (50 mg/kg sc) lesioned rats (n=5-6).

There were no differences in the 5-HT synthesis rate in the hypothalamus between control and DSP-4 rats (after saline injection). Also CP 94,253 administered in a dose of 4.0 mg/kg ip did not significantly alter 5-HT synthesis rate in control and DSP-4 treated animals. SB 216641 did not influence the examined parameter as well (Fig. 3).

DISCUSSION

This is the first study attempting to evaluate the role of DSP-4 treatment on 5-HT$_{1B}$ receptor mediated feeding behavior examined in adult rats. Previously we established that DSP-4 administered to newborn rats (50 mg/kg sc x2, P1 and P3) results in serious noradrenergic system damage in adulthood; endogenous NA level of the medial prefrontal frontal cortex

was reduced by approx. 96%, hippocampus by 98% and striatum by 50% without noticeable impairment of dopaminergic and serotoninergic neurons [10, 14]. Conversely, in the current study no significant changes in NA and other biogenic amines content in the hypothalamus were found after neonatal DSP-4 treatment (Tab. 1). These data contrast with Jaffer et al. [15]. Because they administered DSP-4 (60 mg/kg) to adult rats 10 days before testing it makes comparison difficult. Furthermore, it is worth knowing that LC neurons which survive the lesion exhibit a vigorous regenerative response, even in those animals in which cell loss exceeds 60-70%. This regenerative process leads progressively to restoration of the NE innervation pattern in the forebrain, with some regions becoming even markedly hyperinnervated [16, 17].

Feeding behavior is deeply affected by serotonergic neurotransmission. This regulatory activity is mediated mainly by specific 5-HT_1 and 5-HT_2 receptors, and the paraventricular nucleus of the hypothalamus plays a key role in this phenomenon [1 – 3]. It is worth knowing that the 5-HT_{1B}-immunoreactive neurons are widely distributed in the hypothalamus. Accumulations of 5-HT_{1B} neurons occurred in magnocellular nuclei, supraoptic nucleus, paraventricular nucleus and accessory periformical, circular and retrochiasmatic nuclei [18]. The selective 5-HT_{1B} agonists injected locally into the hypothalamic paraventricular nucleus or systematically reduced food intake in mildly deprived rats [19, 20]. Similar results were obtained with 5-HT_{2C} agonists [21].

As mentioned in the introduction, in one of our studies we also showed that in neonatally DSP-4-treated rats R-(+)-8-OH-DPAT (0.015 mg/kg) – 5-HT_{1A} receptor agonist caused hyperphagia of control rats, but did not change feeding of DSP-4 treated rats. It was the study which provided the first demonstration that noradrenergic lesion produces a prolonged functional desensitization of terminal 5-HT_{1A} autoreceptors [8]. In the present work we showed that CP 94,253 (4.0 mg/kg ip) significantly decreased food intake during 30 min period of observation both in intact and DSP-4 lesioned rats in comparison to respective control (after saline administration) and this effect was abolished by SB 216641 pretreatment (Fig. 1). Others also found that DSP-4 lesion neither altered water [22] nor food intake [23].

5-HT_{1B} receptors play a crucial role in regulating 5-HT neurotransmission, as they serve as both autoreceptors on 5-HT-containing neurons and heteroreceptors on several neurons that do not contain 5-HT e.g. NA, DA [24 - 26]. For this reason p-CPA was used for functional inactivation of 5-HT_{1B} autoreceptors (present on the terminals of 5-HT neurons). As shown, administration of p-CPA dramatically reduces the concentration of 5-HT and 5-HIAA in rats hypothalamus (Tab. 2).

We also demonstrated that in p-CPA pretreated animals CP 94,253 in the same manner diminished food intake in both tested groups (control and DSP-4 in comparison to saline administration; Fig. 2). It is likely that 5-HT_{1B} agonist eliciting its behavioral response (diminution in feeding) is not directly dependent on serotoninergic system integrity and 5-HT_{1B} heteroreceptors located on different neurons (e.g. dopaminergic, noradrenergic) seems to be more strongly involved in this phenomenon. Other also observed that the hypophagic effect of d-fenfluramine which indirectly acts through at least in part by 5-HT_{1B} receptor was not significantly affected by pretreatment with the inhibitor of 5-HT synthesis (p-CPA) [27].

We concluded that in contrast to our previous studies [9, 10] which have shown that the consequence of DSP-4 administration is dramatic reduction in the NA contents in the frontal cortex, hippocampus, striatum, etc., with concomitant desensitization of 5-HT_{1A} and 5-HT_{1B} receptors (evaluated by behavioral and biochemical methods), in this experiment, there were no such effects. This proves that the noradrenergic fibers in the hypothalamus are much more resistant to the neurotoxic action of DSP-4, furthermore, in opposition to 5-HT_{1A} receptor (see cited above [8]) for the development of 5-HT_{1B} receptors desensitization in the specific brain structure, decrease in NA contents in this part of the brain is required. In other words, a profound dysfunction of the noradrenergic system expresses as noradrenergic fiber damage in the striatum, frontal cortex, hippocampus, etc. does not affect the status of 5-HT_{1B} receptors in the hypothalamus.

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