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Assessment of dopamine (DA) synthesis rate in selected parts of the rat brain with central noradrenergic lesion after administration of 5-HT3 receptor ligands

Ocena szybkości syntezy dopaminy (DA) w wybranych częściach mózgu szczurów z lezją ośrodkowego układu noradrenergicznego po podaniu ligandów receptora 5-HT3

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Summary

Introduction:

The study objective was to determine the effect of central noradrenergic system lesions performed in the early extrafetal life period on dopamine synthesis in the rat brain. The content of L-dihydroxyphenylalanine (L-DOPA) was assessed in the frontal lobe, thalamus, hypothalamus and brain stem of rats by high-pressure chromatography with electrochemical detection (HPLC/ED) after administration of 5-HT3 receptor ligands.

Material and Methods:

Adult male Wistar rats which underwent central noradrenergic lesions by DSP-4 administration (50 mg/kg m.c. i.p.) on day 1 and 3 of life received i.p. injections of the aromatic amino acid decarboxylase inhibitor (NSD-1050) in a dose of 100 mg/kg b.w. Next, 30 min after NSD-1050 injection, the animals were decapitated by guillotine. Selected brain structures were dissected and L-DOPA content was determined by HPLC/ED.

Results and Conclusions:

A statistically significant reduction was found in DA synthesis in the group of animals with DSP-4 lesions induced by PBG (1-phenylbiguanide, 7.5 mg/kg b.w. i.p.) and ondansetron (1.0 mg/kg b.w. i.p.). Morphine and PBG had no major effect on DA synthesis in the cerebral cortex of both control animals and in rats with noradrenergic lesions. The assessment of the effect of DSP-4 lesions on L-DOPA content in the brain stem after administration of morphine (7.5 mg/kg b.w. s.c.), PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) separately or jointly showed a statistically significant increase in the synthesis of DA in animals with DSP-4 lesions, as compared to the control group exposed to 0.9% NaCl and morphine. The analysis of the effect of DSP-4 lesions on L-DOPA content in the thalamus and hypothalamus revealed no statistically significant differences between the control groups of rats and those with DSP-4 lesions. As shown by this model, permanent noradrenergic lesions in animals in the early extra-fetal period result in increased reactivity of the central dopamine system.

Keywords:

rats • dopamine • central serotonergic system • lesion of the central noradrenergic system • serotonin 5-HT3 receptor

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INTRODUCTION

The serotonergic system is associated with other neurotransmitter systems and is currently the subject of intensive studies. The modulation of the function of one of the neurotransmitter systems in the brain affects other CNS systems. However, data concerning the effect of 5-HT₃ receptors on the release of at least five neurotransmitters are quite scarce [9,10]. Up to now, they have been found to enable the release of endogenous (dopamine) DA in the limbic system under the effect of stimulation in the ventral tegmental region, stress, ethanol, nicotine and morphine. Moreover, the 5-HT₃ receptor antagonists are known to enhance the release of endogenous 5-HT and CCK, and inhibit the release of endogenous NA and acetylcholine in the cerebral cortex [8,11].

For many years, researchers from the Pharmacology Department in Zabrze have been concerned with developmental neurotoxicology. Pharmacological substances that can be used in such studies include selective neurotoxins, e.g. 6-hydroxydopamine (6-OHDA), 5,7-dihydroxytryptamine (5,7-DHT), N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4) and others [4,6,7]. Long experience with the use of these substances seems to indicate that damage to a specific neurotransmitter system in the brain induces a number of adaptive and compensatory alterations in the injured system and other neurotransmitter systems. It seems that these types of changes are indispensable for the maintenance of functional integrity of the brain. The depth of a lesion depends both on the neurotoxin dose, administration mode (intraventricular, intrastructural, peripheral) and age of animals used in experiments (newborns, adults). The lesion-induced changes may be manifested by compensatory enhancement of the synthesis and release of a neurotransmitter from preserved nerve endings, proliferation of axon terminals or altered density or reactivity of the respective receptors. The lesion of a specific monoaminergic system is frequently accompanied by altered activity of other neurotransmitter systems that remain under the effect of the injured system.

Therefore, the study objective was to assess the effect of central noradrenergic system lesions in newborn rats on L-DOPA content in selected structures of the brain.

A reduced content of L-DOPA may indicate a decrease in dopamine synthesis and suggest compensatory changes in this neurotransmitter system. Up to now, no data have been published on the correlations between the neurotransmitter systems investigated in the present study in accordance with the designed model. Thus, the current research seems fully justified as its results may contribute to the understanding of mutual relationships between the neurotransmitter systems of the brain, and may elucidate the pathomechanisms of mental disorders, neurodegenerative disturbances and other CNS diseases in humans.

MATERIAL AND METHODS

The study used male Wistar rat newborns and adults aged 8-10 weeks. The animals were kept in a room at a constant temperature of about 22°C and 12-hour artificial light day/night cycle: 12 h/12 h (light from 7:00 to 19:00). Throughout the experiment the animals had free access to water and a standard diet. On days 1 and 3, the newborns received s.c. injection of neurotoxin DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine] in a dose of 50 mg/kg x 2 to induce permanent damage to the central noradrenergic system. Control animals were given 0.9% NaCl solution (1.0 ml/kg s.c.). Adult male rats in both groups were injected with morphine (7.5 mg/kg b.w., s.c.), ondansetron (1.0 mg/kg b.w., i.p.), PBG (1-phenylbiguanide, 7.5 mg/kg b.w., i.p.), and ondansetron combined with PBG in doses as above. After 30 min, the aromatic amino acid decarboxylase inhibitor (NSD-1050) was injected in a dose of 100 mg/kg b.w., i.p. Half an hour after the injection of NSD-1050, the animals were decapitated with a guillotine. Then, the skin and covering cranial bones were removed, and the brain taken out and placed on a glass plate with ice at 0°C. Next, the frontal lobe, brain stem, and thalamus with hypothalamus were dissected and frozen on solidified CO₂. On the day when the amino acids were determined, the tissue was homogenized at 0°C (in a water bath) in a solution of 0.1 M perchloric acid (HClO₄ – Fluka) with addition of 25 mg/l ascorbic acid for approximately 10 s. The frontal lobe, the brain stem and the thalamus with hypothalamus were homogenized in 1.0 ml of the solution. Next, the homogenate was centrifuged at 4°C at the speed of 15 000 rpm for 20 min. Following centrifugation, the supernatant was centrifuged again, this time with the use of a cellulose filter with pore diameter of 0.2 mm, for 10 min, at 4°C, 10,000

rpm, and afterwards the supernatant was frozen at -18°C for 24 h. After that time, the sample was defrosted and subjected to high-pressure chromatography with electrochemical detection. The intensity of DA synthesis was measured indirectly, by means of chromatographic measurement of L-DOPA (DA precursor) content. Each group contained 6 animals. Data were analyzed by a licensed version of the computer program STATISTICA 6.0 (StatSoft, Tulsa OK, USA).

RESULTS

DA synthesis intensity was measured by an indirect chromatographic method assessing L-DOPA (DA precursor) level in the frontal lobe, brain stem and thalamus with hypothalamus.

The assessment of the effect of the DSP-4 lesion on L-DOPA content in the brain stem after the administration of morphine (7.5 mg/kg b.w. s.c.), PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) separately or jointly showed a statistically significant decrease in DA synthesis in animals with DSP-4 lesions caused by administration of PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.). There was no significant effect of morphine or PBG on DA synthesis rate in the cerebral cortex of the control animals and those with noradrenergic lesions, as compared to the respective group exposed to 0.9% NaCl (Fig. 1).

The analysis of the effect of DSP-4 lesion on L-DOPA content in the brain stem after administration of morphine

(7.5 mg/kg b.w. s.c.), PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) separately or jointly showed a statistically significant increase in DA synthesis in animals with DSP-4 lesions as compared to the control group exposed to 0.9% NaCl and morphine (Fig. 2).

The analysis of the effect of DSP-4 lesion on L-DOPA content in the thalamus and hypothalamus after administration of morphine (7.5 mg/kg b.w. s.c.), PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) to rats separately or jointly revealed no statistically significant differences between the control groups of rats and those with DSP-4 lesions (Fig. 3).

DISCUSSION

Chemical damage to the central noradrenergic system induced by DSP-4 administration to rat newborns has been found to cause a considerable modification of the central dopamine and serotonin transmission, and may lead to disorders in the GABA-ergic system in these animals [2,3,6,7,9].

DSP-4 causes total damage to noradrenergic neurons in the cerebral cortex, hippocampus and hypothalamus. On the other hand, the cerebellum and brain stem show a rise in NA content, probably associated with increased noradrenergic innervation [1].

The increase in the activity of the noradrenergic system within the locus coeruleus (i.e. the brain stem, where descending antinociceptive pathways originate) may

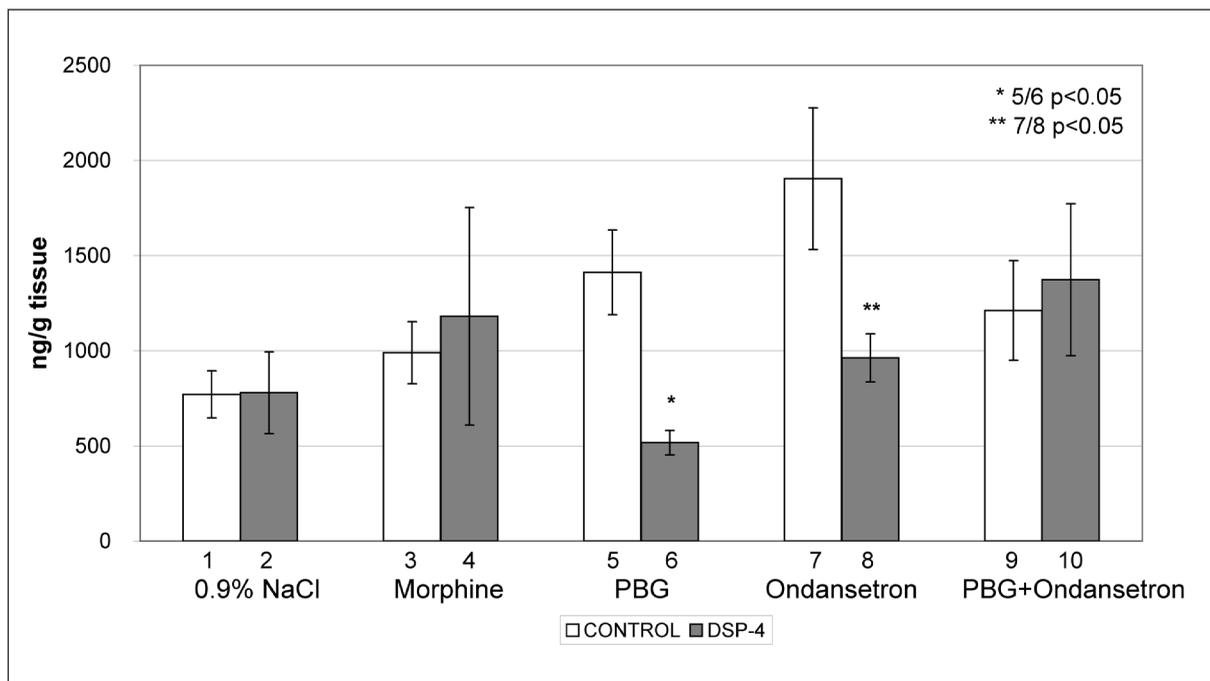


Fig. 1. Effect of DSP-4 lesion on L-DOPA content in the cerebral cortex after administration of morphine (7.5 mg/kg b.w. s.c.), PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) in rats ($\bar{x} \pm SEM$; n = 10)

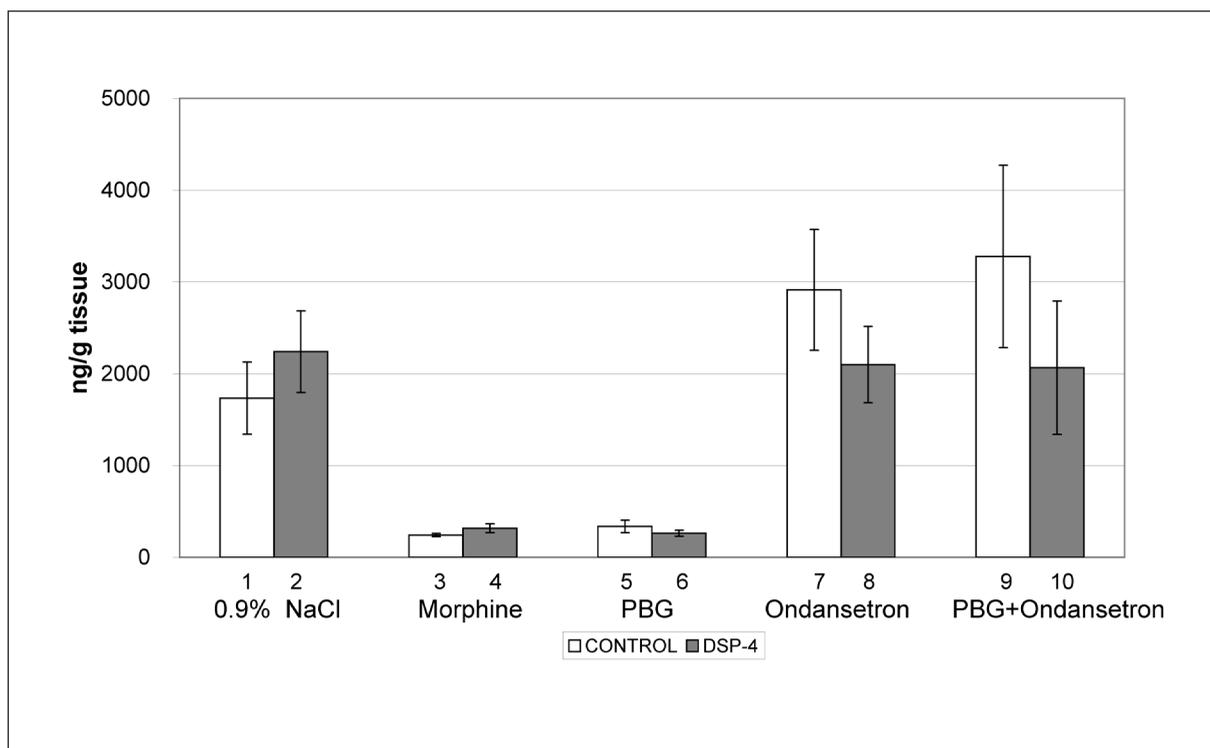


Fig. 2. Effect of DSP-4 lesion on L-DOPA content in the brain stem after administration of morphine (7.5 mg/kg b.w. s.c.), PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) in rats ($\bar{X} \pm SEM$; n= 10)

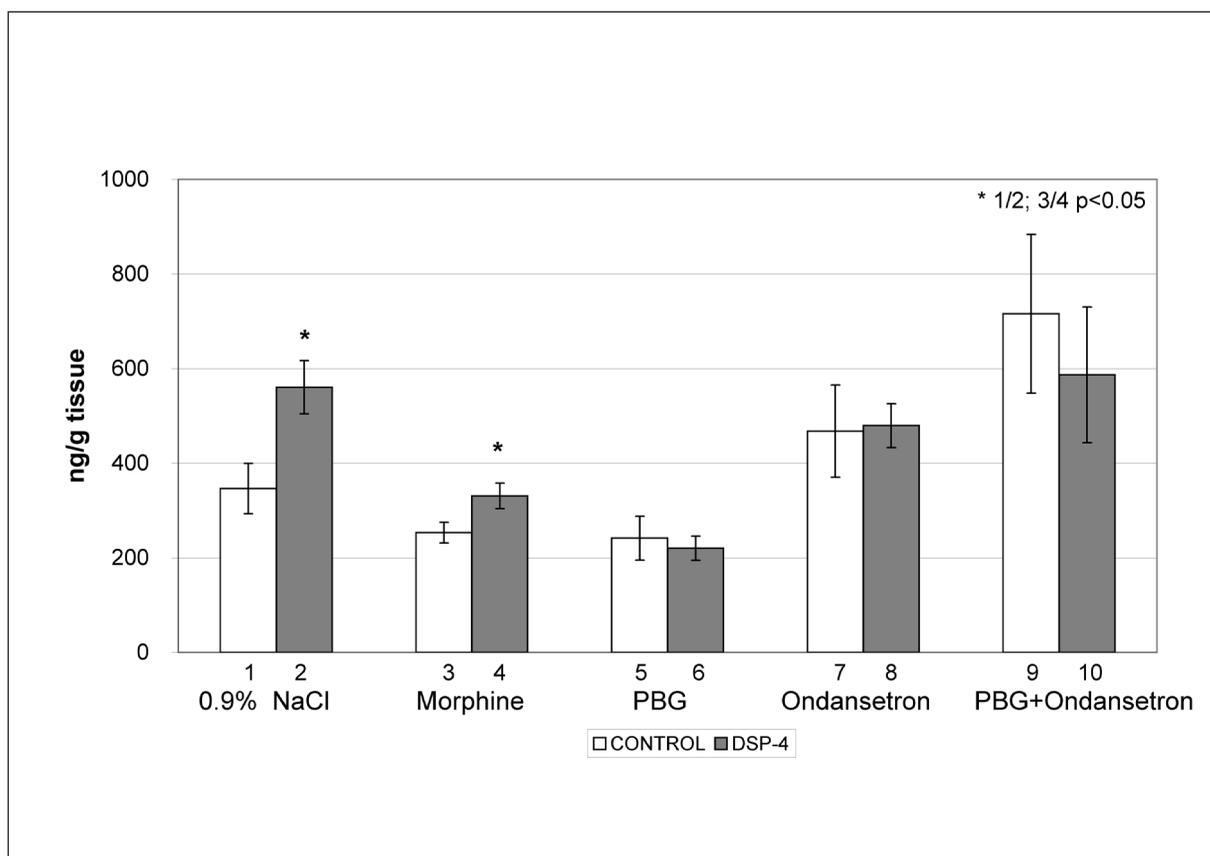


Fig. 3. Effect of DSP-4 lesion on L-DOPA content in the thalamus and hypothalamus after administration of morphine (7.5 mg/kg b.w. s.c.), PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) in rats ($\bar{X} \pm SEM$; n= 10)

compensate for impaired physiological functions of the spinal cord, although it is still unknown why this does not apply to higher cerebral structures. The serotonergic and adrenergic systems cooperate to modulate pain sensation at the spinal cord level. A disturbance in the noradrenergic transmission induced by DSP-4 administration to newborn rats does not affect sensation of pain stimuli mediated by serotonergic 5-HT₃ receptors at the spinal cord level but does at higher levels of the CNS [2,3,5,10]. Administration of DSP-4 to newborn rats influences the intensity of convulsions induced by such GABA agonists as bicuculline and pentylenetetrazol applied to adult rats [2], since lesions to the central noradrenergic system in newborn rats modify the GABA-ergic transmission in certain structures of the developing brain. It has also been revealed that the GABA transaminase inhibitor vigabatrin causes a marked increase in GABA content in the prefrontal cortex of control rats and it is even twice as high in those with DSP-4 lesions compared to the controls [3].

Other experiments have shown that animals with a lesion of the central noradrenergic system sustained in the early extra-fetal period are less sensitive to the sedative and hypnotic effects of phenobarbital and ethanol, and to the anxiolytic action of diazepam; however, no signifi-

cant differences were observed in GABA level in the prefrontal cortex, hippocampus, cerebellum and brain stem [4,5]. Using that model, it has been demonstrated that a permanent noradrenergic lesion in the early extra-fetal period of animals results in increased reactivity of the central dopamine system (D₂ and D₃ receptors) [9]. It also affects the function of the central serotonergic system by inducing desensitization of 5-HT_{1A} autoreceptors [6] and exerts an effect on 5-HT₃ [10].

The current study revealed a decrease in DA synthesis in the cerebral cortex after administration of PBG (7.5 mg/kg b.w. i.p.) or ondansetron (1.0 mg/kg b.w. i.p.) separately or jointly, and a statistically significant increase in DA synthesis in the brain stem in animals with DSP-4 lesions, as compared to the control group receiving 0.9% NaCl and morphine.

CONCLUSIONS

The current study indicates that a chemical lesion to the central noradrenergic system can have a permanent effect on neurotransmission in the rat brain. Moreover, administration of serotonergic 5-HT₃ receptor ligands affects dopamine synthesis in the CNS of animals with an injured noradrenergic system.

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