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Original research article

The effect of central noradrenergic system lesion on dopamine (DA) and serotonin (5-HT) synthesis rate following administration of 5-HT_3 receptor ligands in chosen parts of the rat brain

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ABSTRACT

Introduction: Since little has been known about the effect of the central noradrenergic system on the reactivity of serotonin 5-HT_3 receptors, the aim of the current study was to find out whether this reactivity could be altered by chemical damage to the system in adult rats in early developmental stage.
Materials and methods: Adult male Wistar rats with central noradrenergic lesion induced by DSP-4 on day 1 and 3 of life were injected with analgesic model substance – morphine, serotoninergic 5-HT_3 receptor agonist (1-phenylbiguanide, PBG), 5-HT_3 receptor antagonist (ondansetron) or both compounds jointly followed by decarboxylase inhibitor of aromatic amino acids (NSD-1050). After 30 min following NSD-1050 injection, the animals were decapitated using a guillotine. Chosen cerebral structures were dissected, and the contents of 5-hydroxytryptofan (5-HTP) and L-dihydroxyphenylalanine (L-DOPA) were determined using high-pressure liquid chromatography with electrochemical detection (HPLC/ED).
Results: Neither PBG nor morphine affected L-DOPA contents in the hippocampus in control rats; however, DSP-4 lesion caused a significant decrease in the synthesis rate of DA in this structure. Hippocampal contents of 5-HTP increased after morphine or PBG administration, and central noradrenergic lesion attenuated this effect. Morphine or PBG decreased cerebellar DA synthesis rate in control rats and DSP-4 lesion did not modify it. Cerebellar levels of 5-HTP increased after morphine or PBG challenge in control rats. DSP-4 lesion intensified the effect of morphine and attenuated that of PBG. Ondansetron abolished the effects mediated by PBG. We did not observe any impact of PBG or ondansetron on DA and 5-HT synthesis in the striatum.
Conclusion: Damage to the central noradrenergic system in rat newborns, through altered reactivity of central 5-HT_3 receptors, results in permanent disorders in serotoninergic transmission in hippocampus and cerebellum as well as dopaminergic transmission in hippocampus, which may attenuate the activity of the descending pathways that derive from these structures.

Introduction

Functional regulation of one neurotransmission system in the brain may affect the action of other systems of the CNS. The serotonergic and noradrenergic systems and their connections with other neurotransmission systems are currently an object of numerous studies.

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The central noradrenergic system mediates pain sensation inhibition, especially chronic pain [1]. Many literature data also indicate its involvement in the mechanism of anxiety and depression which may be accompanied by pain [2–6].

It has been shown that the peripheral 5-HT_3 receptors take part in the generation of pain induced by inflammatory processes, but not by mechanical or thermal stimuli. The 5-HT released in tissues, by affecting the peripheral 5-HT_3 receptors sensitizes neurons that are responsible for pain sensation to the action of bradykinin (pronociceptive effect) [7,8].

On the other hand, centrally released 5-HT exerts an antinociceptive effect, by stimulating *e.g.* 5-HT_3 receptors. This effect can be blocked by a central 5-HT_3 receptor antagonist [9].

As it has been reported, the serotonergic and noradrenergic systems cooperate in pain modulation at the level of the spinal cord, although the central interactions of these neurotransmitters are poorly known [1].

The analgesic action of tricyclic antidepressants (inhibiting the reuptake of NA and 5-HT synaptic cleft) as well as serotonin-norepinephrine reuptake inhibitors and to the less extent – selective serotonin reuptake inhibitors in some types of chronic pain needs to be explained.

The 5-HT_3 receptors are known to affect the release of certain neurotransmitters or modulatory substances such as dopamine (DA) in the limbic system. Agonists of the 5-HT_3 receptor enhance the release of endogenous 5-HT and CCK, but inhibit the secretion of endogenous NA and acetylcholine in the cerebral cortex [10,11]. Data concerning the effect of 5-HT_3 receptors on the release of neurotransmitters still remain quite scarce and the knowledge of mutual interactions of the respective types of receptors is extremely important to elucidate the functioning of the given system.

Behavioral studies conducted in the last decade have shown that the administration of 5-HT_3 ligands to animals does not basically alter their behavior, yet it affects the action of other substances in the animal models of anxiety, psychoses and drug addictions [12,13].

We used a model of central noradrenergic lesion induced by DSP-4, designed in the Department of Pharmacology in Zabrze. DSP-4 is a neurotoxin which easily permeates through the blood–brain barrier and can be administered peripherally (*sc*, *ip*). The neurotoxin causes permanent inhibition of NA reuptake in the CNS pathways and a decrease in the content of endogenous NA in the central and peripheral nervous system in rats. The reduction in NA content in the peripheral noradrenergic system is temporary and approximately 80% of neurons regain the ability to synthesize NA 4–6 weeks after DSP-4 injection. This substance does not affect the content of catecholamines in the adrenal medulla [14]. In turn, dysfunction of the adrenergic neurons is permanent and the action of DSP-4 on nerve cells of the dorsal pathway is stronger than in the ventral one [15].

The application of the central noradrenergic system lesion model in the developing brain allows for compensatory changes from other neurotransmission systems. Previously, using the same model and 5-HT_3 receptor agonist (1-phenylbiguanide, PBG) and antagonist (ondansetron) we have demonstrated the effect of lesion of the central noradrenergic system on analgesic effects mediated by serotoninergic 5-HT_3 receptors in behavioral experiments as well as during the assessment of DA and 5-HT synthesis rate in chosen parts of rat brain. We have found that in animals with central noradrenergic system lesion, the reactivity of 5-HT_3 receptors is diminished in the main structures involved in nociception: in frontal cortex and thalamus [16–18]. In the current study, we decided to examine other subcortical structures with a potent ability to modify the perception of pain stimuli giving their projections to the thalamus, where 5-HT_3 receptor expression can be found: cerebellum, striatum and hippocampus [19]. As we were particularly interested in the elucidation of the involvement of serotoninergic–noradrenergic central interactions in the context of analgesia we used morphine as a model analgesic reference substance.

Materials and methods

The study used male Wistar rats, newborns and adults aged 8–10 weeks. The animals were kept in a room at a constant temperature of approximately 22 °C and 12 h/12 h day/night artificial light cycle (light from 7:00 to 19:00). Throughout the experiment, the animals had free access to water and standard diet. Rat newborns were injected (*sc*) with the neurotoxin DSP-4 [N-(2-chloroethyl)-N-ethyl-2-bromo-bensylamine] on day 1 and 3 of life at a dose of 50 mg/kg × 2 to induce permanent damage to the central noradrenergic system. The control animals received 0.9% NaCl solution (1.0 ml/kg *sc*). Previously, we demonstrated, that such procedure reduced noradrenaline content in frontal cortex and hippocampus by 96.6% and 95.5%, respectively as compared to the control rats. Conversely, in the cerebellum it increased by 66% suggestive of reactive neuronal sprouting [15]. It has been shown that other neurotransmitter systems are not influenced in this model [20].

Adult male rats were injected with morphine (7.5 mg/kg b.w. *sc*), 5-HT_3 receptor antagonist (ondansetron; 1.0 mg/kg b.w. *ip*), 5-HT_3 receptor agonist (1-phenylbiguanide, PBG; 7.5 mg/kg b.w. *ip*) and jointly ondansetron and PBG in the above mentioned doses. Next, after 30 min decarboxylase inhibitor of aromatic amino acids (NSD-1050) was injected *ip* in a dose of 100 mg/kg b.w. After another 30 min following NSD-1050 injection the animals were decapitated with a guillotine. Then, the skin and cranial vault bones were removed to take out the brain, which was placed on a glass plate with ice at 0 °C. Next, striatum, hippocampus and cerebellum were dissected and frozen on solidified CO_2. On the day when amino acids were determined, the tissue was homogenized at 0 °C (in water bath) in a solution of 0.1 M $HClO_4$ (Fluka, Germany) with addition of 25 mg/l of ascorbic acid for approximately 10 s. Then, the homogenate was centrifuged at 4 °C at 15,000 rotations/min for 20 min. After centrifugation, the supernatant was centrifuged again, this time using a cellulose filter (pore diameter of 0.2 μm) for 10 min at 4 °C at 10,000 rotations per minute, and the supernatant was frozen at −18 °C for 24 h. Next, the sample was defrosted and subjected to chromatography with the use of high-pressure chromatography with electrochemical detection (Gilson, France). The intensity of DA and 5-HT synthesis was measured by an indirect method, determining chromatographically the intensity of accumulation of L-DOPA (DA precursor) and 5-HTP (5-HT precursor). The respective groups consisted of 10 animals.

The current study was approved by the local Bioethics Committee of the Silesian Medical University (consent no 66 of 11 December, 2007).

Statistical analysis

Licensed version of the computer software STATISTICA 6.0 (StatSoft, Tulsa OK, USA) was used for the analysis.

After the analysis of normally distributed data (Kolmogorov–Smirnov test), the analysis of variance (ANOVA univariate and bivariate) and Newman–Keuls *post hoc* test were applied. The Kruskal–Wallis test was used for nonparametric data. The significance criterion were the values of $p < 0.05$.

Results

We showed that in the hippocampus neither morphine (7.5 mg/kg b.w. *sc*) nor 5-HT_3 receptor agonist – PBG (7.5 mg/kg b.w. *ip*)