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Research report

Effects of single and simultaneous lesions of serotonergic and noradrenergic pathways on open-space and bright-space anxiety-like behavior in two animal models

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ABSTRACT

The objective of the present study is to investigate the effects of single and simultaneous lesions of the noradrenergic and serotonergic pathways (NA-X, 5-HT-X and XX, respectively) by intracerebroventricular administration of selective neurotoxins *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine-HCl (DSP-4) and 5,7-dihydroxytryptamine (5,7-DHT) on anxiety-like behavior in rats. To evaluate the effects of the various lesions, animals were tested in elevated plus-maze (EPM) and light-dark (LD) paradigms. In EPM, single lesions produced strong, statistically significant increase ($p < 0.001$) of both time spent in the open arms (OT) and number of entries into the open arms (OE) compared to sham-lesioned animals. Simultaneous lesion further strengthened this anxiolytic effect causing an approximate 500% elevation of OT compared to sham-lesioned animals. In LD, 5-HT lesion caused a significant ($p < 0.05$) increase in both light movement time and light horizontal activity parameters compared to intact, sham, and NA-lesioned groups. Neither of the lesions caused any change in the spontaneous locomotor activity of the animals up to 15 min as measured in activity meter. These findings suggest that single and simultaneous lesions of 5-HT- and NA-pathways modify anxiety-related state of experimental animals to different extents and these modifications alter the behavior of animals differently in the two models used: NA-X and 5-HT-X reduce open space anxiety-like behavior and XX further strengthens this effect in the EPM, while only 5-HT-X is resulting in reduced bright-space anxiety-like behavior leaving the performance of NA-X and XX animals unchanged.

1. Introduction

Noradrenaline (NA) and serotonin (5-HT) are involved in various physiological and pathophysiological processes. Their important role in the regulation of anxiety-related behavior is inevitable, but not fully understood yet, however, it is known that the physiological integrity and proper communication of these two monoamine neurotransmitter systems are prerequisite for normal CNS-functioning. A sensitive balance exists between NA and 5-HT, the cessation of which may lead to the development of psychiatric illnesses. A widely used method to investigate the mode of action, effects and relations to other modulators of these monoamine transmitters is the selective chemical lesion (depletion) of their central pathways by specific neurotoxins [15,28,42,53,54]. *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine-HCl (DSP-4) selectively destroys NA-ergic pathways, whilst 5,7-dihydroxytryptamine (5,7-DHT) is the agent harmful for the serotonergic neurons. These two neurotoxins act promptly by destroying projections ascending from the locus coeruleus and raphe nuclei and their axon-terminals, causing transmitter-depletion and inhibiting re-uptake mechanisms [16,23]. Sensitivity to their harm differs throughout the various brain-areas, and the damage caused by them vary with the age of the animal, route of administration, and dosage [51,52].

Results obtained from behavioral tests measuring anxiolytic-like activity following selective lesion of the central serotonergic and noradrenergic pathways with these neurotoxins are ramifying and often controversial, and seem to highly depend on the route of administration, dosage of the chemicals and selection of models applied. Nevertheless, the lesions achieved by these neurotoxins might alter the impulsivity of the animals, further contributing to the controversy of the results. It is also likely, that for compensatory mechanisms activated right after lesions, the major trigger is the

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shifted balance of NA and 5-HT systems rather than total absence of the transmitters [20,48].

Selective lesion of the noradrenergic pathways with DSP-4 administered intraperitoneally proved to be anxiolytic in the elevated plus-maze in group-reared rats [27], while showed pro-conflict effect in the conditioned suppression of drinking test [14], induced neophobia [22] and reduced time spent with social interaction [25]. Our previous finding is that when DSP-4 was administered icv, the consecutive 5-HT-dominance abolished NMDA-receptor mediated shock-induced behavioral deficit in social avoidance with exerting no effect on the social investigation behavior [48]. Regarding locomotor activity, DSP-4 either reduced this phenomenon [25], or had no effect on it [46,48].

In the literature, the selective lesion of the ascending serotonergic terminals by 5,7-DHT has most often been reported to be anxiolytic. Regarding elevated plus-maze (EPM), selective 5-HT depletion was shown to be anxiolytic both when the toxin was administered icv [8,20] and when administered into the median raphe nucleus in restrained rats [2]. Anxiogenic response has been shown when 5,7-DHT was administered into the medial prefrontal cortex [38] and medial raphe nucleus of chronically restrained animals [33]. No effect has been reported when the toxin was administered into the dorsal [40] or median raphe nucleus for non-restrained rats [2,33], or into amygdala [44], entorhinal- or occipital cortex [38]. In light-dark transitions (LD), 5,7-DHT administered icv showed no influence on parameters related to anxiety [9], nor did it cause increase of time spent in the lit compartment in restrained rats [2]. In social interaction model, 5,7-DHT lesion was proved to be anxiolytic when administered into the dorsal raphe nucleus [13] while left social interaction of rats intact when administered icv [48]. In the Vogel conflict model, effect of 5,7-DHT lesion is proved to be indirect, and dependent on intact adrenocortical functions [37,45,47]. However, anxiolytic effects of 5-HT depletion have not always been observed in this model [43,50].

The controversial findings with the selective lesion of 5-HT pathways in models designed for measuring one behavioral aspect of anxiety-like behavior might be explained by the theory that different parts of the 5-HT system are responsible for conducting fear and anxiety [17,18]. The lesions may affect these parts to different extents, and the behavioral paradigms with divergent ethological background may reflect to the altered functioning of these parts to a greater or lesser extent.

The mechanisms through which NA and 5-HT control anxiety-related behavior are not well characterised yet. According to our previous findings [48] and other studies [17,18,31,52] the two monoaminergic systems play an indirect neuromodulatory role on these processes instead of exerting direct and critical effects on them. The role of peptidergic systems seems to be important as well, since impact of lesions of either the NA- or 5-HT-projections on the functionality of hypothalamic or extrahypothalamic CRF, CCK, NPY or neurotensine systems is already demonstrated [11,21,25,26], however, the results are often controversial.

Despite the widespread use of single lesions of either NA- or 5-HT-pathways, the effect of their simultaneous lesion was not investigated up to now. The selective neurotoxins DSP-4 and 5,7-DHT were first administered simultaneously by icv administration, and their common effects on the behavior were first examined by our group [48]. The aim of this study was to investigate the effects of parallel depletion of the NA- and 5-HT-pathways on anxiety-like behavior in rats using the EPM and LD paradigms. To show that behavioral results obtained were not due to potential impact of lesions on the locomotor system, spontaneous locomotor activity of the animals was measured as well.

2. Experimental procedures

2.1. Animals and housing

Male Sprague Dawley rats (EGIS Pharmaceuticals Plc., Hungary) weighing 270–300 g at the time of operation were used. Before surgical intervention, animals were kept in polycarbonate cages (Macrolon, Lignifer, Hungary), 5 rats/cage in a thermostatically controlled room (temperature: 23 ± 2 °C, relative humidity: 60 ± 10%). The room was artificially illuminated from 6 a.m. to 6 p.m. Rats received commercial pellet rat-mouse feed (Altromin, LATI, Hungary) autoclaved at 105 °C, and filtered tap water, ad libitum. After stereotaxic surgery, animals were housed individually and handled daily by the experimenters.

2.2. Ethics

All studies were carried out in accordance with NIH Principles of laboratory animal care (publication no. 86-23, revised in 1985) and the European Communities Council Directive of 24 November 1986 (86/609/EEC); and, were reviewed and approved by the Animal Welfare Committee of EGIS Pharmaceuticals Ltd as well. During performance of experiments, all efforts were made to minimize animal suffering, to reduce the number of animals used, and to use alternatives to *in vivo* techniques if available.

2.3. Drugs

Equithesin was made from chloral-hydrate (4.2 g), magnesium-sulfate-7-hydrate (2.12 g), nembutal (pentobarbital-Na) liquid (16.2 ml), 1,2-propylenglycol (40.0 ml), ethanol 96 m/m % (10.0 ml) and distilled water (30.0 ml). These components were purchased from Sigma–Aldrich Plc., Hungary, and Reanal Plc., Hungary. The selective serotonergic neurotoxin 5,7-dihydroxytryptamine (5,7-DHT) and the selective noradrenergic neurotoxin *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine-HCl (DSP-4) were purchased from Sigma–Aldrich Plc., Hungary. Fluoxetine–HCl and Desipramine–HCl were obtained from EGIS Pharmaceuticals Plc., Hungary. Methyl-cellulose was purchased from DOW Chemical Company 1131 Building, Midland, MI, USA. Chemicals used in HPLC measurement were purchased from Sigma–Aldrich Plc., Hungary.

2.4. Stereotaxic surgery and the chemical destruction of noradrenergic and serotonergic neurons

Rats were anaesthetized with equithesin (3 ml/kg *ip*), and were placed in a Kopf stereotaxic apparatus. The selective 5-HT neurotoxin 5,7-dihydroxytriptamine (5,7-DHT; 300 μg), and/or the selective NA-neurotoxin *N*-(2-chloroethyl)-thyl-2-bromobenzylamine-HCl (DSP-4; 400 μg) were administered intracerebroventricularly into the right lateral ventricle (stereotaxic coordinates (according to the atlas of Paxinos and Watson [35]): antero-posterior: 0.9; medio-lateral: 1.4; dorso-ventral: 3.4 from skull surface). The compounds were dissolved and administered in 10 μl saline containing 0.1% ascorbic acid. The solutions were infused over 6 min (flow rate: 1.7 μl/min). Sham-operated controls were administered vehicle (saline + ascorbic acid). To lower unspecific damage, rats lesioned with 5,7-DHT were pre-treated with Desipramine–HCl (20 mg/kg), whereas rats treated with DSP-4 were pre-treated with Fluoxetine–HCl (10 mg/kg) to protect noradrenergic and serotonergic neurons, respectively.

2.5. HPLC measurement of NA- and 5-HT-contents in the hippocampus

After brain sampling, the hippocampus was quickly removed and stored at −70 °C until analysis. Biochemical assessments were done by the method of Adams and Marsden [1]. Briefly, brains were homogenized in perchloric acid that contained antioxidant and EDTA. Homogenates were centrifuged and the supernatant was assessed for serotonin and noradrenaline content by means of reverse phase high-performance liquid chromatography with electrochemical detection. A Beckman System Gold HPLC (column: C-18 ESA Cathecolamine HR-80) and ESA Coulochem ll electrochemical detector was used. The mobile phase consisted of NaH_2PO_4, octane-sulphonic acid (as ionpair reagent), EDTA (pH 3.1) and acetonitrile. The working electrode potential was set to +250 mV. Monoamine content was expressed as ng/g wet weight.

2.6. Experimental design

All procedures were carried out in sound-proof, air-conditioned rooms between 08:00 and 13:00 h at an ambient temperature of 23 ± 2 °C. Behavioral procedures started 1 week after surgery, allowing time for recovery. The following operated groups were formed in behavioral testing: sham-operated (Sh), NA-lesioned (NA-X), 5-HT-lesioned (5-HT-X), and double (NA + 5-HT)-lesioned (XX). Intact rats (Int) were also used in the tests to exclude potential behavioral effects of surgical procedures.