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Clinical Neurosciences

THE *IN VIVO* AND *IN VITRO* EFFECTS OF LESIONS OF BRAIN NORADRENERGIC AND SEROTONERGIC SYSTEMS IN RATS

Ph.D. Theses

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INTRODUCTION

Noradrenaline and serotonin are biogenic amines belonging to the classical neurotransmitters. Discovery of them is an important scientific result of the 20th century. They can be found both in the central nervous system and periphery, and take part in numerous physiological processes. These two systems are in close interaction, that manifests in many different mechanisms. Disruption of their balance plays significant role in the ethiology of several neuropsychiatric diseases, such as anxiety related disorders, and progressive symptoms with cognitive disturbances, i.e. dementias. Extensive research is carried out on the neurobiological background and medication possibilities of these diseases nowadays, as anxiety disorders belong to the most frequent psychiatric illnesses worldwide: their prevalence per year is between 12.6-17.2 % in the world. It is even higher in Hungary: 17.7%. Moreover, the prevalence and incidence of dementia-syndromes increase exponentially with the raising absolute and relative numbers of elderly people.

An important tool of this research is the chemical lesion of neurotransmitter-systems with selective neurotoxins, modelling this way the neuron degeneration, which accompanies physiological ageing and pathophysiological processes. In the international scientific literature, several reports have been published on the examination of the effects of targeted lesions of noradrenergic and serotonergic systems in *in vivo* behavioral - and *in vitro* slice - techniques in the last few years. The results are often contradictory, especially on the areas of anxiety and cognitive disturbances. However, the different dosage and way of administration of the neurotoxins must be considered. Although, some publications reported on results with double lesions achieved by simultaneous administration of selective neurotoxins, there was no paper available on the parallel lesion of these two systems at the time of our experiments.

At EGIS Pharmaceuticals, we have tested several potential compounds for anxiolytic efficacy during the preclinical research. It is well known, that utmost anxiolytic substances display their effects via the noradrenergic and/or serotonergic systems. We have used the serotonergic and noradrenergic lesion to discover the way of action of one of our putative anxiolytic agent. Our hypothesis was, that if the anxiolytic effect of the molecule would decrease or even disappear in the lesioned animals, that would support the role of the lesioned system in the effect of the compound. The anxiolytic efficacy was tested on the elevated plus maze paradigm. Unexpectedly, the

serotonergic and noradrenergic lesions resulted in decreased anxiety of the animals without further treatments. At this point we decided to examine the effect of the parallel lesion of the two systems. No similar data in the literature were published that time.

This dissertation summarizes the results of our research on the anxiolytic and cognitive effects of NA-erg and 5-HT-erg monolesions, and the simultaneous lesions of the two transmitter-systems (latter performed and published by our group as novelty). We also tried to reveal the interaction between the two neurotransmitter systems by the lesions. For these purposes *in vivo* behavioral paradigms, and *in vitro* slice-techniques were performed.

AIMS

1. performance of single, and, as a novelty, simultaneous lesions of the noradrenergic and serotonergic transmitter-systems by i.c.v. administration of selective neurotoxins

2. investigation of the effects of the single- and simultaneous lesions on anxiety by performing the following tests
    - elevated plus maze (EPM)
    - light-dark (LD) - similar to EPM regarding ethological background
    - stress-induced social avoidance – different from EPM regarding ethological background

3. investigation of the effects of the single- and simultaneous lesions on cognition by performing the following paradigms
    - passive avoidance and novel object recognition *in vivo*
    - population spike – long term potentiation (PS-LTP) *in vitro*

4. investigation of the interaction of the noradrenergic and serotonergic transmitter-systems by superfusion release technique on the hippocampal slices of the lesioned animals, *in vitro*

METHODS

Animals and housing

Male Sprague-Dawley rats with normal diurnal rhythm, weighing 270-300 g at the time of stereotaxic operation were used in our experiments. Behavioral testing started after a minimum 7 day long recovery period. The *in vitro* experiments on the time-dependent effects of the lesions were performed 1, 6, 7, 14 or 30 days after lesions.

Statistical data analysis

Mean ± SEM values were calculated from the data of all measured parameters. Treatment effects of multiple groups were assessed by one-factor- or repeated measures- ANOVA, and Fisher LSD multiple comparison post hoc analysis. Independent data samples were compared with Student t test.

Surgical intervention design

Toxins were administered into the right lateral ventricle of anaesthetized animals (stereotaxic coordinates: antero-posterior: 0.9 mm; medio-lateral: 1.4 mm; dorso-ventral: 3.4 mm from bregma).
Solvent of the neurotoxic compounds: saline containing 0.1% ascorbic acid .

NA-ergic lesion (NA-X)
pretreatment: fluoxetine 10 mg/kg i.p., administered 30 min before surgery (for sparing the 5-HT-system)
neurotoxin: DSP-4 (*N* -(2-chloroethyl)-ethyl-2-bromobenzylamine–HCl) 400 μg /10 μl solvent + 10 μl solvent i.c.v.

5-HT-ergic lesion (5-HT-X)
pretreatment: desipramine 20 mg/kg i.p., administered 30 min before surgery (for sparing the NA-system)

neurotoxin: 5,7-DHT (5,7-dihydroxy-tryptamine) 300 μg /10 μl solvent + 10 μl solvent i.c.v.

Double (NA+ 5-HT) lesion (XX)

pretreatment: solvent 2 ml/kg i.p., administered 30 min before surgery

neurotoxins: 5,7-DHT 300 μg/10 μl solvent + DSP-4 400 μg/10 μl solvent i.c.v.

Sham operation (Sh)

pretreatment: solvent 2 ml/kg i.p., administered 30 min before surgery

20 μl solvent i.c.v.

Intact control (Int)

Intact animals did not undergo any drug administration or lesion procedure before testing

HPLC measuerment of NA- and 5-HT- contents in the hippocampus

The hippocampal content of the two monoamines and the 5-HT metabolite 5-HIAA of the lesioned animals was detected by HPLC method.

Anxiolytic tests

Elevated plus maze test

non-conditioned exploratory model, based on the conflict of innate fear and contradictory exploratory drive of rodents

Light-dark test

unconditioned exploratory model, similar to EPM paradigm

Stress-induced social avoidance test

subchronic generalized anxiety model, based on conditioned fear

Complementary experiments

to test our hypothesis, that shock displays effect on the development of social avoidance behavior, intact rats were administered with NMDA-antagonist MK-801 (0.1 mg/kg i.p.), before and after shocking procedure of the social avoidance test

Cognitive tests

Passive avoidance test

Associative memory model, based on aversive conditioning

PS-LTP test

in vitro method of the cell-dependent mechanisms of memory-formation

Novel object recognition test

unconditioned model, measuring visual recognition memory processes

Investigation of effects on locomotion in the spontaneous locomotor activity test

unconditioned model for detecting sedative or stimulating effects of the lesions on the central nervous system

Detecting radioactive transmitter-release with superfusion technique

in vitro test measuring radioactivity from the hippocampal slices of the lesioned animals

RESULTS AND DISCUSSION

HPLC measuerment of NA- and 5-HT- contents in the hippocampus

I.c.v. infusion of DSP-4 (400 µg) caused a strongly significant, dramatical decrease by about 70% in NA-concentration of hippocampal tissue of the NA-X- and by about 80 % of the XX-groups, compared to sham-lesioned animals. I.c.v. infusion of 5,7-DHT (300 µg) resulted in even a greater reduction by about 90% of the 5-HT concentration of hippocampal tissue in the 5-HT-X and XX-groups, compared to sham-lesioned animals. However, reduction of 5-HT-concentration was detected in the NA-X group, and reduction of NA-concentration of the 5-HT-X group compared to sham NA- and 5-HT-levels, but these decreases were significantly smaller than reductions occurred in the NA-X,5-HT-X, and XX groups, respectively. Despite these parallel changes in the two systems, the lesion of serotonergic neurons caused a prevailing noradrenergic dominance, whereas the lesion of noradrenergic neurons shifted the ratio towards serotonergic dominance.

Effects of the lesions on anxiety

Effects of the lesions on anxiety were not uniform in the three paradigms used. The noradrenergic lesion proved to be anxiolytic in the elevated plus maze and stress-induced social avoidance tests, but did not affect the behavioral parameters related to anxiety in the light-dark test. The serotonergic deficit decreased the anxiety of the animals in the elevated plus maze and the light-dark tests, but it strengthened the avoidance behavior caused by electric shocks. The simultaneous lesion of the two systems produced a significantly stronger anxiolytic effect in the elevated plus maze test than the single lesions. However, these parameters of double lesioned animals did not differ from those of sham operated group in the light-dark-, and the stress-induced social avoidance tests. These differences can be attributed to the fact, that these three tests in question model different types of anxiety-related behavior. Elevated plus maze models open space-, whilst light-dark paradigm bright space anxiety. In turn, in the stress-induced social avoidance one can measure effects on conditioned anxiety. The role of the two systems is different in these three types of anxiety.

Effects of the lesions on cognition

The serotonergic and the simultaneous lesion of the two systems produced negative effect on cognition in the *in vivo* conditioned cognitive paradigm (passive avoidance test), but these effects were not detected when we examined the visual memory in the object recognition test and the spatial memory processes of hippocampal slices in the PS-LTP paradigm *in vitro*. The time-dependent procognitive effect of the noradrenergic deficit was only detectable in the PS-LTP test. It did not strengthen itself the passive avoidance behavior, and did not antagonize the response-attenuating effect of the serotonin-deficit. It did not exhibit any effect on the visual memory of the animals, either. According to the synaptic transmission-elevating effect of the noradrenergic deficit, an improvement in spatial cognitive processes of the NA-lesioned animals may be predicted *in vivo*. Thus, trials to detect the procognitive effect of the NA-ergic lesion in special tests for hippocampus-dependent spatial learning/memory processes (Morris water maze, eight-arm maze) could be successful. Despite the similar molecular mechanisms in the background of LTP and spatial memory, there are a lot of differences in the processes of *in vitro* events and *in vivo* hippocampal memory-formation, therefore the results of PS-LTP-test cannot be extended to *in vivo* paradigms.

Effects of the lesions on spontaneous locomotor activity

There was no statistically significant difference between groups regarding locomotor activity up to 15 min. Therefore it can be stated, that neither of the lesions, nor the sham operation displayed stimulating or sedating effect on the central nervous system, therefore the results achieved on the behavioral paradigms are not the consequences of locomotory changes, but are due to the effect of the lesions on anxiety and cognition.

Effects of the lesions on [^{3}H]transmitter-release

[^{3}H]NA-release

During the experiments on the effects of lesions on tritiated NA-release, an unexpected result occurred, as, 14 days after lesions, the [^{3}H]NA-efflux from the NA-X slices not only was retained, but was increased at both stimulations. This increase in response to the first stimulation was significant compared to the other groups. Neither 5-HT-X nor XX altered the radioactive efflux of the slices. The reasons for these unexpected results may be direct or indirect compensatory mechanisms. The exact way of them requires further investigation. The shapes of the fractional release curves of [^{3}H]NA-efflux from lesioned slices, plotted on 7th and 14th days, were identical. The surgical intervention itself did not affect the hippocampal radioactive transmitter release in the case of NA.

[^{3}H]5-HT-release

Effect of desipramine on the release of [^{3}H]5-HT in intact slices

DMI, a TCA antidepressant, which is a selective inhibitor of the NAT, was administered to hippocampal slices of intact animals to test our hypothesis, i.e. NAT influences the [^{3}H]5-HT-efflux similarly as 5-HTT modifies the [^{3}H]NA release. This hypothesis was not justified, as DMI did not affect the released quantity of radioactivity from intact tissues, neither when administered from tritium-load, nor when administered from the incubation, till the end of experiment, in the cc. of 1 µM.

Effects of the lesions on [^{3}H]5-HT-release

During the experiments on the effects of lesions on tritiated 5-HT-release, we detected, that NA-X decreased hippocampal [^{3}H]5-HT-release at both stimulations. This decrease, in response to the second stimulation, was significant, compared to the Int and Sh groups. However, the efflux from the slices of NA-X lesioned animals was significantly higher than the efflux from the slices of 5-HT-X- and XX-group. 5-HT-X- and XX ceased the release of hippocampal [^{3}H]5-HT almost totally. The shapes of the fractional release curves of [^{3}H]5-HT-efflux from lesioned slices plotted on the 7th and 14th days, were identical. The surgical intervention itself did not affect the hippocampal radioactive transmitter release in the case of serotonin either.

CONCLUSIONS

We have produced in rats successful NA-ergic lesion by injectig 400 µg DSP-4 i.c.v., protecting the serotenergic axon-terminals with desipramine-; and a successful 5-HT-ergic depletion with injection of 300 µg 5,7-DHT i.c.v., protecting the NA-erg neurons with fluoxetine pretreatment. We have performed the double lesion of the two systems with the simultaneous i.c.v. administrations of the two neurotoxins, as a novelty. The reduction of the NA-content of hippocampal tissue by about 70%, and the level of 5-HT by about 90%, as the result of the lesions, prove, that the dosage of the neurotoxins and the stereotaxic coordinates chosen by us were proper. The smaller, but significant depletion of the other transmitter system experienced during the performance of the single lesions, is the consequence of the non-specific effects of the toxins, and the incomplete sparing effects of the administered protective compounds. Coming from these shortcomings of this method, a total selective lesion cannot be performed with it, it can only change the NA/5-HT balance.

On the elevated plus maze and light-dark tests based on non-conditioned spontaneous exploration, the lesions produced different anxiolytic effect-pattern, thus, it can be concluded that the two paradigms model different types of anxiety. The elevated plus maze measures open space-, and the light-dark tests bright-space anxiety-related behaviors. These two types of anxiety are differently affected by the NA- and 5-HT-systems.

Due to the effects of the NMDA antagonist MK-801 (0.1 mg/kg, i.p.) on intact animals, it can be concluded, that the stress–induced subchronic anxiety manifested in the stress-induced social avoidance paradigm, is a result of NMDA-dependent glutamatergic processes. The development of it is modulated by the NA- and 5-HT-systems.

It can be concluded from our data measured in the passive avoidance test, that the 5-HT lesion and the double lesion both caused deficit of the passive avoidance behavior. As this deficit could be detected in the non-shocked control group as well, we cannot exclude, that, besides the cognitive effects, anxiolysis also contributes to its development.

The time-dependent procognitive effect of NA-deficit developed in the PS-LTP test, was only detectable in a tight time interval. It could be detected on the 14th, but not on the 30th day after lesions.

The lesions displayed no effect on visual memory of the animals. This can refer on the one hand, to that, that the two systems do not play important role int he visual memory processes. On the other hand, it is also possible, that at the time of test the adaptive changes caused by the compensating mechanisms had already antagonized the effects of lesions to some extent.

The effect of the lesions on the hippocampal [^{3}H]NA- and [^{3}H]5-HT release *in vitro* was independent of time in the tested period. It could be detected on the 7th day after the lesions, and did not change until the 14th day.

The lesion procedures and sham operation did not alter the spontaneous locomotor activity, nociception, the emotional consequenses of the inavoidable shocking, and the social interaction behavior of the animals. The surgical intervention itself displayed no effect on locomotion, anxiety, cognitive processes, or [^{3}H]NA- and 5-HT-release. Therefore, it can be concluded, that our results represent the real effects of the lesions on anxiety, cognitive processes and radioactive transmitter-efflux.

The results listed hereby, are the consequences of the complex modulating effects of the NA- and 5-HT systems on anxiety, cognitive processes, and release of tritiated transmitters from hippocampal tissue. Deeper understanding of the compensatory and regenerating mechanisms starting right after the lesions requires further examinations.

SUMMARY

Single and simultaneous lesions – performance of the latter is a novelty by our group – of the NA- and 5-HT-systems with selective neurotoxins i.c.v., resulted in different effects regarding anxiety and cognition. NA-depletion decreased both open-space anxiety related behavior on the elevated plus maze, and conditioned social avoidance response in the stress-induced social avoidance paradigm. It had no effect on the bright-space anxiety in the light-dark test. 5-HT-depletion decreased both open- and bright space anxiety, but in turn strenghtened the conditioned avoidance response. The parallel lesion of the monoamines yielded stronger antagonistic effect to open-space anxiety compared to the mono-depletions, but displayed no significant influence on bright-space anxiety and conditioned social aversion. By administering the non-competitive NMDA-antagonist MK-801 to intact animals before and after conditioning, we have demonstrated that the stress-response manifesting in this behavioral deficit, develops under the influence of NMDA-dependent glutamatergic mechanisms. These mechanisms are attenuated by the 5-HT- and increased by the NA systems.

5-HT-ergic and parallel lesions displayed negative effect on cognition in the passive avoidance test *in vivo*, however both were devoid of this effect regarding the object recognition-, and the *in vitro* PS-LTP paradigms. The time-dependent procognitive effect of NA-deficit was developed only in the PS-LTP test, as the lesion neither improved *in vivo* passive avoidance behavior, or antagonized the attenuating effect of 5-HT-depletion, and it had no influence on visual memory of the animals either.

The evoked hippocampal [^{3}H]NA-release for the first stimulation was significantly stronger from NA-lesioned slices compared to the other slices, whilst [^{3}H]5-HT-efflux almost quenched at slices with 5-HT- or both NA- and 5-HT-deficits, and it remained constant in intact slices despite administration of DMI in different time-points of the experiment.

Lesions did not alter the spontaneous locomotor activity, and the surgical intervention itself had no effect on locomotion, anxiety, cognitive processes, or [^{3}H]NA- and 5-HT-release.

Our results listed above reflect the complex modulating influence of NA- and 5-HT on anxiety, cognition and [^{3}H]transmitter-release, just as the multiple effects of the compensating and regenerating mechanisms beginning right after lesions.

LIST OF PUBLICATIONS

Publications related to the dissertation

1. **Sziray N**, Kuki Zs, Nagy KM, Markó B, Kompagne H, Levay G. (2010) Effects of single and simultaneous lesions of serotonergic and noradrenergic pathways on open-space and bright-space anxiety-like behavior in two animal models. Behav Brain Res, 209(1):93-98. IF: 3,171

2. **Sziray N**, Leveleki C, Levay G, Markó B, Hársing LG Jr, Mikics E, Barsy B, Haller J. (2007) Mechanisms underlying the long-term behavioral effects of traumatic experience in rats: the role of serotonin/noradrenaline balance and NMDA receptors. Brain Res Bull, 71(4):376-85. IF: 1,943

3. Leveleki C, **Sziray N**, Levay G, Barsvári B, Soproni K, Mikics E, Haller J. (2006) Pharmacological evaluation of the stress-induced social avoidance model of anxiety. Brain Res Bull, 69(2):153-60. IF: 1,684

Other publications

1. Megyeri K, Marko B, **Sziray N**, Gacsalyi I, Juranyi Z, Levay G, Harsing LG Jr. (2007) Effects of 2,3-benzodiazepine AMPA receptor antagonists on dopamine turnover in the striatum of rats with experimental parkinsonism. Brain Res Bull, 71(5):501-7. IF: 1,943

2. Juranyi Z, **Sziray N**, Marko B, Levay G, Harsing LG Jr. (2004) AMPA receptor blockade potentiates the stimulatory effect of L-DOPA on dopamine release in dopamine-deficient corticostriatal slice preparation. Crit Rev Neurobiol, 16(1-2):129-39. IF: Ø

3. Harsing LG Jr, Gacsalyi I, Szabo G, Schmidt E, **Sziray N**, Sebban C, Tesolin-Decros B, Matyus P, Egyed A, Spedding M, Levay G. (2003) The glycine transporter-1 inhibitors NFPS and Org 24461: a pharmacological study. Pharmacol Biochem Behav, 74(4):811-25. IF: 2,307