Potential role of glutamate neurotransmission in the pathogenesis of ischemic brain damage and of depression. Effects of L-kynurenine on the survival of the hippocampal neurons and on the corticocerebral blood flow in ischemic animal models

Summary of Ph.D. thesis

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LIST OF ABBREVIATIONS

AMPA: α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid,

BBB: blood-brain barrier,

BDNF: brain-derived nerve growth factor,

cCBF: cortical cerebral blood flow,

EAA: extracellular amino acid,

fEPSPs: field excitatory postsynaptic potentials,

FJ-B: Fluoro Jade B,

FJ+: Fluoro Jade positive

GABA: gamma-aminobutyric acid

Glu: glutamate,

Gly: glycine,

HFS: high-frequency stimulation,

HR: heart rate,

IDO: indolamine 2,3-dioxygenase,

IO curves: input-output curves,

KYN: kynurenine,

KYNA: kynurenic acid,

MABP: mean arterial blood pressure,

L-NAME: nitro-L-arginine methyl ester,

LTP: long-term potentiation,

NA: noradrenaline,

NeuN: anti-neuronal nuclei,

NMDA: *N*-methyl-D-aspartate,

NO: nitric oxide,

NOS: nitric oxide synthase,

PSD: post-stroke depression,

PROB: probenecid,

QUIN: quinolinic acid,

SC: sham control,

SSRI: selective serotonin reuptake inhibitor,

TRP: tryptophan,

4VO: four-vessel occluded

INTRODUCTION

Kynurenine pathway. Kynurenic acid – a glutamate antagonist. Rationale of therapeutic administration of kynurenic acid in certain experimental conditions. Pathomechanism of depression also involves glutamate hyperactivity.

Kynurenine (KYN) is a central material of the major route for tryptophan (TRP) metabolism. KYN can be metabolized in two separate ways: one branch results in the formation of kynurenic acid (KYNA), while the other leads to quinolinic acid (QUIN), the precursor of NAD. KYNA is the only known endogenous excitatory amino acid (EAA) receptor blockers with a broad spectrum of antagonistic properties in supraphysiological concentrations. On the other hand, QUIN is a specific agonist at the NMDA receptors, and a potent neurotoxin with an additional marked free radical-producing property.

Summary: QUIN may result in excitotoxic neuronal cell death, while in stark contrast, the other KYN pathway metabolite, KYNA, protects neurons. There are some diseases where elevations of QUIN in the brain have been implicated, *e.g.* CNS inflammation, stroke, traumatic brain injury, epilepsy, malignant gliomas, neuropathic pain and certain neurodegenerative disorders. In these states, hyperactivity of glutamate (Glu) receptors in certain areas of the brain occurs, causing excitotoxic cell death. On the other hand, in diseases with cognitive alterations, such as Alzheimer's type dementia and schizophrenia, elevated levels of brain KYNA have

been demonstrated, which could contribute to cognitive defects by influencing NMDA receptors. Pharmacological manipulations of Glu receptors can hold promise for the amelioration of some dramatic consequences of excitotoxicy. KYNA can be a candidate for it. However, the use of KYNA itself as a neuroprotective agent can be difficult because it hardly crosses the blood brain barrier (BBB), whereas L-KYN is easily transported across it. Accordingly, the systemic administration of L- KYN dose-dependently elevates the level of KYNA in the brain.

The pathomechanism of depression is also supposed to involve Glu receptor hyperactivity in the cortico-limbic (hippocampal) pathways. Key role of chronic stress followed by hyperactivity of the glucocorticoid axis in its pathomechanism is unaequivocal. “Vascular depression” is related to various types of cerebrovascular lesions in the cortex or subcortical white matter. The lesions are believed to interrupt cortico-subcortical (cortico-limbic and cortico-striatal) pathways which can cause biochemical changes, leading to alterations in mood (rather than the localized structural damage itself). Vascular depression and “idiosyncratic” depression can share some common features in their pathomechanism. The role of chronic peripheral immune activation and subsequent release of proinflammatory cytokines by macrophages and dendritic cells in the brain, and hence chronic low-grade cerebral inflammation has come into focus in recent years. Cytokines in the brain can further stimulate the hypothalamo-pituitary-adrenal axis, disturb monoaminergic neurotransmission, and induce the enzyme indolamine 2,3-dioxygenase (IDO). This enzyme initiates the catabolism of TRP through the KYN pathway. Heightened TRP catabolism in the KYN pathway toward the QUIN arm was reported. An imbalance between the two arms of the pathway can exert an impact on the Glu-ergic neurotransmission in the brain. It has been suggested that impaired neuroprotection may play an important role in the pathophysiology and treatment resistance of depression. Theoretically, pharmacological inhibition of Glu receptors in (vascular) depression may result in the improvement of depressive symptoms.

Our own studies – our purposes

1. To investigate whether peripheral L-KYN administration has any neuroprotective effect on hippocampal cell survival under global ischemic conditions in the brain.

In our study, we evaluated the effect of L-KYN administration (300 mg/kg i.p.) on the hippocampus of the global ischemic (transiently four-vessel occluded: 4VO) rat brain, administering the L-KYN before and after the ischemic insult. We used the rat 4VO model, mimicking global ischemia, which is a widely recognized model in ischemic studies.

2. How does peripheral L-KYN loading influence the physiological long-term potentiation (LTP) in the ischemic rat hippocampus?

We investigated how KYN (300 mg/kg) treatment influences LTP in the hippocampus in the rat 4VO model. Global ischemia is known to elicit selective, delayed neuronal death. The pyramidal neurons of hippocampal CA1 area are particularly vulnerable. Activity-dependent LTP, a model of synaptic plasticity, also mediated by Glu receptors, may likewise be impaired by global ischemia. This too was addressed in our experiments.

3. Do small doses of peripherally administered L-KYN influence the cortical CBF (cCBF) under normal and chronic ischemic conditions of the brain in the rabbit?

As little amount of information is available on the effects of the KYN system on the CBF, we investigated the effects of low doses (0.3, 1 and 3 mg/kg) of L-KYN on the cCBF under normal and ischemic conditions of the brain in awake rabbits.

4. Do the antidepressant drugs citalopram and fluoxetine have any effects on the cCBF in normal rabbits and in animals with CBF impairement induced by chronic unilateral carotid occlusion?

Antidepressants have proved to be partly effective in vascular depression, but little was eatlier known about their *in vivo* vascular effects. We investigated for the first time the effects of two selective SER reuptake inhibitor antidepressant drugs (SSRIs), citalopram and fluoxetine, on the normal and the carotid ligation-induced ischemic

cCBF, and also on the mean arterial blood pressure (MABP) and the heart rate (HR) in awake rabbits.

EXPERIMENT 1
KYNURENINE DIMINISHES THE ISCHEMIA-INDUCED HISTOLOGICAL AND ELECTROPHYSIOLOGICAL DEFICITS IN THE RAT HIPPOCAMPUS

The aim of our study was to reveal whether KYN can rescue the CA1 neurons in the 4VO model in the rat. KYN was administered together with PROB, an organic acid transporter inhibitor, in order to facilitate the brain penetration of KYN.

Materials and methods

Animals

Adult male Wistar rats (n = 43, 280-300 g) were used.

Four-vessel occlusion and drug treatment

The rats were anesthetized with Nembutal (60 mg/kg, i.p.). Both vertebral arteries were occluded by cauterization. On the following day, the animals were subjected to 10-min forebrain ischemia by bilateral occlusion of the carotid arteries with atraumatic clips under ether anesthesia. Both vertebral arteries were cauterized, and both common carotid arteries were exposed, but not occluded in the sham-operated animals.

The ***rats used for histology*** were divided into 4 groups: sham-operated controls (**SC** group, n = 5), 4VO animals (**4VO** group, n = 7), KYN+PROB-pretreated animals (**KYN+PROB-4VO** group, n = 6) and KYN+PROB-post-treated animals (**4VO-KYN+PROB** group, n = 7).

The ***rats used for electrophysiology*** were divided into 3 groups: sham-operated controls (**SC** group, n = 6), 4VO animals (**4VO** group, n = 6) and KYN+PROB-pretreated animals (**KYN+PROB-4VO** group, n = 6).

KYN (300 mg/kg, i.p.) and PROB (200 mg/kg, i.p.) were administered daily for 5 days: in the ***pretreated group***, the first KYN+PROB administration preceded the 10-min carotid occlusion by 2 h; then the animals were treated at the same time on the next 4 days. In the ***post-treated group,*** the animals received the first KYN+PROB injection at the start of reperfusion. The remaining 4 injections were given at the same time on the next 4 days.

Histological procedures

Ten days after 4VO, the rats were anesthetized with urethane (2.2 g/kg, i.p.), and perfused transcardially with 0.1 M phosphate-buffered saline, and then with 4% paraformaldehyde. The brains were removed from the cranium, post-fixed in formalin, cryopreserved in 20% sucrose and sectioned at 32 μm with a cryostat microtome. The degenerating cells were stained with Fluoro Jade B®, while that of viable cells were identified by anti-neuronal nuclei (NeuN) immunohistochemistry. The locations of FJ-B positive (FJ+) cells were observed with a fluorescence microscope (viewed at 4x magnification and the most dorsal part of CA1 was counted at 20x magnification) and fluorescence photomicrographs were obtained.

The labeled cells were calculated for 1 mm^2. The numbers of FJ+ and NeuN-immunopositive neurons/mm^2 were determined in each slide of all animals.

In vitro electrophysiology

The electrophysiological recordings were conducted 10 days after 4VO. The rats (n =3x6) were decapitated, and coronal slices (400 μm) were prepared from the middle part of their hippocampi with a vibratome. The slices were then transferred into a Haas-type recording chamber and incubated. The stimulating electrode was placed in the stratum radiatum near the border of the CA3 region to perform orthodromic stimulation of the Schaffer collateral/ commissural pathway.

Field excitatory postsynaptic potentials (fEPSPs) were recorded in parallel from the stratum radiatum. The test stimulus intensity was adjusted to the 30-60 μA range to evoke approximately 50% of the maximal stimulus intensity that evoked maximal fEPSP response in the SC rats. LTP of the Schaffer collateral-CA1 synaptic response was induced by high-frequency stimulation (HFS) (0.2-ms pulses delivered at 100 Hz for 6 s), then the fEPSPs were recorded for a further period of at least 60 min. Input-output (IO) curves were created to measure the basal Glu-ergic synaptic function.

HPLC-MS/MS analysis of KYN and KYNA levels in the plasma and brain tissue

The vertebral arteries were occluded 24 h before treatments with vehicle or KYN and PROB. Two hours later, the rats were anesthetized with pentobarbital (60 mg/kg i.p.). No bilateral carotid artery occlusion was performed. Blood was taken from the aorta, and centrifuged. After decapitation, tissue samples were obtained from the hippocampus and cerebral cortex, and weighed. The plasma and brain tissue was stored at about -70 ^{o}C until analysis. Upon thawing, the brain tissue was homogenized in a potter. The HPLC-MS/MS system was used to measure the KYN and KYNA content of the samples.

Statistical analysis

Neuronal cell counts are presented as means ± SEM, and were analyzed by using one-way ANOVA followed by the Bonferroni test for multiple.comparisons. A $p \leq 0.05$ was considered significant. A nonparametric test on two independent samples was chosen for electrophysiological data (Mann-Whitney U-test).

Results

Histology

In animals subjected to 10-min 4VO, severe neuronal damage was observed in the CA1 area of the hippocampus in both hemispheres 10 days after the intervention. In this injured region, numerous of the pyramidal cells in the CA1 region were FJ+ in each of the coronal sections, while those in the CA3 region and the dentate gyrus were not labeled.

In accordance with this, the NeuN immunohistochemistry indicated a lack of intact cells in the CA1 region in the 4VO animals, but an intact CA3 region and dentate gyrus.

KYN administration

KYN (300 mg/kg, i.p.) administered together with PROB (200 mg/kg, i.p.) caused a marked reduction in the number of damaged neurons. In the KYN-pretreated animals, injured neurons stained with FJ-B could be observed only sporadically in the CA1 area of the hippocampus (the same was true for the CA3 region and the dentate gyrus).

Accordingly, NeuN immunohistochemistry gave the impression of a non-injured CA1 region (like the CA3 region and the dentate gyrus) in KYN-pretreated 4VO animals.

KYN administration considerably decreased the number of injured neurons in the CA1 region. However, the decrease in the number of injured neurons was highly significant only in the pretreated group. The animals in the post-treated group also exhibited a tendency to a reduction in the number of injured neurons, but this change was not significant. The NeuN immunohistochemistry supplemented these results: the number of non-injured cells was highest in the SC group, and lowest in the 4VO animals. Post-treatment with KYN had minimal effect, while in the 4VO animals which received KYN before ischemia the number of intact cells was comparable to the control level.

We also investigated the change in the numbers of injured cells in the cortex. We obtained similar results, i.e. a significant neuroprotective effect of L-KYN treatment. The only difference was that post-treatment also proved to be significant in the cortex. In short, the KYN+PROB pretreatment was able to reduce the proportion of damaged cells to 52% relative to the damaged cells induced by 4VO without KYN+PROB treatment. The KYN+PROB treatment after the 4VO intervention did not prove to be effective in the hippocampus.

In vitro electrophysiology

The input-output (IO) curves of SC, 4VO and 4VO animals which received KYN+PROB treatment were established by plotting the fEPSP amplitudes against various test pulse intensities from 0 to 100 μA. No significant difference was found between the IO curves in the three groups, implying that the basal functions of the pyramidal cells and synapses were not affected by complete ischemia.

LTP was induced by HFS of the Schaffer collateral-CA1 synapses. In the SC group, the HFS caused a robust increase (170-180%) in the slope of the fEPSPs and this increase in slope (and in amplitude) remained at the elevated level during the 1-h registration period. The same conditioning protocol did not induce a significant, lasting increase of the fEPSPs in the majority of the 4VO animals. In this group, the elevation of the amplitudes was only transient; no LTP was observed. At the end of the registration period, the slopes had returned to the control level, or decreased below the baseline.

The administration of KYN and PROB protected slices from the 4VO-induced LTP impairment. KYN restored the fEPSP slopes to

the control level, and these parameters were stable until 60 min after HFS.

Plasma and brain KYN and KYNA concentrations

Treatment with KYN+PROB considerably increased both the KYN and KYNA concentrations in the plasma and brain, and also altered their proportions within the compartments studied, but this was probably due to the relatively large scatter of the data. In the sham-operated rats, KYN concentration was approximately 3-fold higher in the plasma than in the cortex and hippocampus. On the other hand, the KYNA concentration was higher in the cortex and hippocampus than in the plasma ($p < 0.001$), and higher in the cortex than in the hippocampus. The KYN concentration increased 37-fold in the plasma and approximately 70-fold in the hippocampus and cortex, while the KYNA concentration was elevated roughly 300-fold in the plasma and 50-fold in the hippocampus and cortex.

EXPERIMENT 2
EFFECTS OF SYSEMIC ADMINISTRATION OF L-KYN ON THE CORTICOCEREBRAL BLOOD FLOW UNDER NORMAL AND ISCHEMIC CONDITIONS OF THE BRAIN IN AWAKE RABBITS

The aim of the present study was to examine whether peripherally administered L-KYN can influence the normal and the unilateral carotid artery occlusion induced ischemic cCBF in awake rabbits.

Materials and Methods

Animals

Altogether 60 New-Zealand white rabbits weighing 2.5-3 kg were used

Surgical preparation

Introduction of electrodes into the cerebral cortex. Rabbits were anesthetized with an intravenous solution of diazepam (20 mg/kg) and ketamine (20 mg/kg) in physiological saline solution. Six hydrogen-sensitive electrodes, consisting of glass-insulated (O.D. 0.5 mm) platinum wire (diameter 0.1 mm) with a bare tip length of 1 mm, were inserted stereotaxically into the parietal cortex. After a 3-5-day recovery period, the animals were in good physical condition.

Occlusion of the carotid artery. In the ischemic group of animals, permanent surgical occlusion was accomplished with ligatures on the left external and internal carotid arteries. The operation was performed under the same general anesthesia as above.

Measurements

Measurement of cCBF. The rabbits were allowed to recover for 3 days after surgery, and the cCBF was determined at 6 sites in their brain by means of the hydrogen clearance technique. The basal and the unilateral carotid occlusion-induced impaired cCBF, and the flow changes following drug treatment were determined. One of the groups consisting of 6 awake rabbits without drug treatment was used to measure the effect of physiological saline solution as control, on the cCBF.

Measurement of MABP, HR and blood gas parameters. The MABP was measured in conscious rabbits via a catheter inserted into the left or right central ear artery, connected through a pressure transducer, at the same time, the ECG was continuously recorded by a radiotelemetry system in order to monitor the HR. Arterial pH, pCO_2, pO_2 and O_2 saturation were measured at intervals, utilizing a blood gas analyzer.

Drug treatment

The marginal ear vein was cannulated and used for the administration of L-KYN and other agents.

L-KYN free base was freshly dissolved in physiological saline solution each day and was administered intravenously in a volume of 1.5-2 ml. First the baseline values were registered, and then the effects of L-KYN on the cCBF, MABP and HR were followed for 240 min.

As vasodilation in the cerebral vessels can be mediated via acethylcholine and/or nitric oxide (NO), we gave their selective inhibitors to investigate the potential mechanisms of L-KYN on cerebral vessels.

Atropine sulfate (1 mg/kg) was given intravenously in aqueous solution in a volume of 2-3 ml 5 min before the administration of L-KYN (1 mg/kg), and the cCBF, MABP and HR values and the L-KYN-induced responses were registered.

Nitro-L-arginine methyl ester (L-NAME) (40 mg/kg) was administered intravenously in aqueous solution in a volume of 1 ml

after registration of the basal cCBF values. Thereafter, the measurements were repeated; 45 min later L-KYN (1 mg/kg) was administered and the changes in cCBF, MABP and HR were determined.

Statistical analysis

The results were expressed as means ± SEM. Data were collected in pairs from the same measuring sites (electrodes) before and after the experimental intervention or the administration of agents. The statistical significance of the observed differences was calculated by Student's paired *t*-test and repeated measures analysis of variance (ANOVA). Multiple comparison of different time points and groups was carried out by means of one-way ANOVA. p values < 0.05, when obtained with both statistical tests, were considered significant.

Significance of reductions in mean basal cCBF values in the controls and in animals with unilateral carotid occlusions treated with 0.3, 1 and 3 mg/kg L-KYN (Group "1", "2" and "3") were calculated by means of the unpaired Student's *t*-test. p values < 0.05 were considered significant.

Results

Administration of L-KYN resulted in a significant increase in the normal cCBF. This effect was particularly obvious after the administration of 1 mg/kg L-KYN. The cCBF was enhanced by all three applied doses of L-KYN within 30 min, attained its maximum values soon thereafter, and remained high even around the 240th min, *i.e.* at the end of the recording period.

Treatment with physiological saline solution did not change the cCBF significantly.

Unilateral carotid occlusion caused a significant reduction in cCBF from 117±15 to 57±11 ("1"), from 90±12 to 52±8 ("2") and from 113±10 to 58±11 ("3") ml/min/100 g tissue.

Following the administration of L-KYN, there was an immediate increase in the cCBF in the animals with carotid occlusion. The L-KYN-induced maximal percentage increases in the cCBF in the ischemic animals were more pronounced than the cCBF decreases caused by unilateral occlusion (92-94% /at 1 mg/kg dose/ vs 42-51%). This effect was of long duration, and peak values were recorded at 60-240 min after L-KYN injection.

Pretreatment with atropine or L-NAME prevented the cCBF-increasing effect of 1 mg/kg L-KYN in the control rabbits and also in those with carotid occlusion.

L-KYN did not alter the MABP or HR, whereas L-NAME caused a small increase in the MABP as did atropine in the HR. The duration of the latter effects was relatively short (< 60 min).

No significant changes in the arterial blood gas parameters or pH were noted after the administration of L-KYN, atropine or L-NAME.

EXPERIMENT 3
EFFECTS OF CITALOPRAM AND FLUOXETINE ON THE CORTICOCEREBRAL BLOOD FLOW IN AWAKE RABBITS

In our study, we investigated for the first time the effects of two SSRI antidepressants, citalopram and fluoxetine, on the normal and the carotid ligation-induced ischemic cCBF, and also on MABP and HR in awake rabbits.

Materials and Methods

Male and female New Zealand White rabbits (altogether *n*=64) weighing 2.5-3.0 kg were used.

Surgical preparation

Introduction of electrodes into the cerebral cortex, occlusion of the carotid artery: This was performed on the same way as described in Experiment 2.

Measurements

Measurement of cCBF, MABP and HR: This was also the same as described previously.

Drug treatment

The marginal ear vein was cannulated and used for the administration of drugs, which were freshly dissolved in physiological saline solution each day. The effects of each of the individual doses (0.1, 0.3 and 1.0 mg/kg) of fluoxetine or citalopram were investigated separately and each animal was treated with only one dose of drug. The amounts of drugs applied in the study correspond to the therapeutically meaningful (antidepressant) human doses.

Fluoxetine was administered intravenously as a bolus, in a total volume of 1 ml.

Citalopram hydrochloride was infused continuously for 60 min (total volume 10 ml).

The basal and the unilateral carotid occlusion-induced impaired cCBF and the cCBF changes following fluoxetine or citalopram treatment were determined at 10 min and every 30 min during 150 min after drug administration.

Statistical analysis

The results were expressed as means ± SEM. Student's paired *t*-test and for multiple comparisons of different time points and groups one-way ANOVA were used. Only p values < 0.05 with both statistical tests were considered significant.

Results

Fluoxetine and citalopram caused only minor, insignificant changes in the normal cCBF. Unilateral carotid occlusion produced a significant decrease in cCBF in all 6 control groups (90±17→48±16; 88±16→49±14; 87±14→41±16) and 92±18→48±17; 97±14→56±15; 98±16→55±17 ml/min/100g tissue). Fluoxetine did not influence the cCBF significantly at any dose in the ischemic animals; there was only a very slight tendency to an increase. In contrast, citalopram (0.1, 0.3 or 1 mg/kg in infusion) improved the unilateral carotid occlusion-induced impaired cCBF response as compared with the basal occluded values. The effect was dose-dependent; the most pronounced change was seen in response to 1 mg/kg citalopram, *i.e.* the highest applied dose of this drug. The average maximal changes were: 48±17→58±19, 56±15→72±19 and 55±17→82±21 ml/min/100g tissue at 0.1, 0.3 and 1.0 mg/kg citalopram, respectively.

The cCBF-increasing effects appeared toward the end of the 150-min recording period: the peak values were obtained at 90-120 min.

Administration of 10 ml physiological saline solution did not alter the cCBF significantly in either the control or the ischemic group.

Both fluoxetine and citalopram decreased the HR as compared with the baseline value. Fluoxetine administration caused a slight, but significant dose-dependent reduction in HR in both the normal and the ischemic group. At the highest dose (i.e. 1 mg/kg), the HR was reduced by 13-14%. The treatment with citalopram affected the

HR significantly only at the 0.3 and 1 mg/kg doses of the drug, and the percentage changes were lower relative to those observed after fluoxetine administration (3-5%).

No significant changes in MABP were noted in the fluoxetine-treated groups. However, significant reductions in MABP were observed in the groups treated with 0.3 or 1 mg/kg citalopram under either normal or unilateral carotid occlusion-induced ischemic conditions.

SUMMARY AND CONCLUSIONS

L-KYN (300 mg/kg) + PROB (200 mg/kg) i.p. pretreatment caused significant neuroprotection in a global ischemic rat model, while its effect as post-treatment was moderate. L-KYN (0.1, 1 and 3 mg/kg i.v.) led to an increase in cCBF in both the control rabbits and the animals with chronic cerebral ischemia induced by chronic unilateral carotid artery occlusion. While the hippocampal neuron rescue effect of L-KYN could be due to the Glu antagonistic property of its immediate derivative, the mechanism of its cCBF-enhancing effect remains to be elucidated. Involvement of the cholinergic pathways of the cranial parasympathetic nervous system could be assumed, in which vasodilation is related to the release of NO, as atropine, an anticholinergic drug, or L-NAME, a NOS inhibitory drug, attenuated the cCBF-increasing effect. In any case, this effect could have an additional beneficial impact on the disease outcome under ischemic conditions in the brain.

In another study, we investigated whether the SSRI drugs fluoxetine and citalopram (0.1, 0.3 and 1 mg/kg) influence the cCBF in doses corresponding to that used in clinical settings. We found that only citalopram had a significant effect on the cCBF: it improved the unilateral carotid occlusion-induced impaired cCBF response. Besides the possibility of the blocking of Ca^{2+} and/or Na^{+} channels on cerebral arteries by SSRI drugs, several authors have proposed that these drugs can exert a secondary (inhibitory) action on the Glu - NMDA receptors in addition to their primary inhibitory action at the SER transporter. Taken together, the cation channel-inhibitory or

NMDA receptor antagonistic properties of citalopram may have played a role in the beneficial effect of the drug in the animals with an impaired cCBF induced by unilateral carotid occlusion in our experiments. Both fluoxetine and citalopram slightly decreased the HR, whereas higher doses of citalopram reduced the MABP under either normal or unilateral carotid occlusion-induced ischemic conditions. However, it is not likely that the mild alterations in the peripheral circulatory parameters could efficiently influence the cCBF. On the basis of our findings, citalopram may have advantages in the treatment of PSD and other types of vascular depression, but further studies of the exact mechanism by which the drug improves the cCBF are clearly necessary.

Details of our results can be itemized as follows:

1. In our 4VO rat ischemia model, there was severe neuronal damage in the CA1 area of the hippocampus in both hemispheres 10 days after the intervention, as proved by FJ-B staining and NeuN immunohistochemistry. The CA3 area and the dentate gyrus remained intact. KYN (300 mg/kg, i.p.) administered together with PROB (200 mg/kg, i.p.) considerably decreased the number of injured neurons in the CA1 region. However, the decrease in the number of injured neurons was highly significant only in the pretreated group (to 52% relative to the damaged cells induced by 4VO without KYN+PROB treatment). The animals in the post-treated group also exhibited a tendency to a reduction in the number of injured neurons, but this change was not significant. The NeuN immunohistochemistry results were in accord with those observed with FJ-B staining: in the 4VO model, post-treatment with KYN had hardly any effect, while KYN pretreatment proved to be highly neuroprotective: the number of intact cells was comparable to the control level.
2. Our *in vitro* electrophysiological studies indicated that in 4VO rats, there was no significant difference between the IO curves in the three groups, implying that the basal functions of the registered pyramidal cells and synapses were not affected by complete ischemia. In the majority of the 4VO animals, no LTP was observed. The administration of KYN and PROB protected the brain slices from the 4VO-induced LTP impairment. KYN restored the fEPSP slopes to

the control level, and these parameters were stable until 60 min after HFS.

3. Administration of KYN (300 mg/kg) + PROB (200 mg/kg) to 4VO rats considerably increased both the KYN and KYNA concentrations in the plasma and brain, and also altered their proportions within the brain compartments studied. The KYN concentration increased 37-fold in the plasma and approximately 70-fold in the hippocampus and cortex, while the KYNA concentration was elevated roughly 300-fold in the plasma and 50-fold in the hippocampus and cortex.

4. Chronic unilateral carotid occlusion caused a significant reduction in cCBF in awake rabbits (from 117±15 to 57±11 ("1"), from 90±12 to 52±8 ("2") and from 113±10 to 58±11 ("3") ml/min/100 g tissue). L-KYN administered intravenously in single doses of 0.3, 1 and 3 mg/kg resulted in a significant increase in the cCBF in the control rabbits, and also in the animals with unilateral carotid occlusion. The effect was particularly obvious after the administration of 1 mg/kg L-KYN.

5. Pretreatment with atropine (1 mg/kg) or L-NAME (40 mg/kg) prevented the cCBF-increasing effect of 1 mg/kg L-KYN in control rabbits and also in those with chronic unilateral carotid occlusion.

6. Both SSRI drugs fluoxetine and citalopram (in individual doses of 0.1, 0.3 and 1.0 mg/kg, which correspond to the therapeutic human doses) caused only minor, insignificant changes in the normal cCBF in rabbits.

7. Citalopram (0.1, 0.3 or 1 mg/kg in infusion) improved the unilateral carotid occlusion-induced impaired cCBF response as compared with the basal occluded values. The effect was dose-dependent; the most pronounced change was seen in response to 1 mg/kg citalopram, *i.e.* the highest applied dose of the drug.

8. Both fluoxetine and citalopram decreased the HR as compared with the baseline value.

9. Significant reductions in MABP were observed in the groups treated with 0.3 or 1 mg/kg citalopram under either normal or unilateral carotid occlusion-induced ischemic conditions.

KYNA-like agents – as potential antiglutamatergic drugs for the future

Glu is a predominant excitatory transmitter in the central nervous system. Glu-ergic neurotransmission plays an important role in many physiological processes, such as learning, development of the nervous system, moving, sensation and mood control. Under particular circumstances, its extreme release may cause irreversible damage to the neurons through their overexcitation. This is termed excitotoxicity and is involved in the pathogenesis of many diseases which are apparently different in nature (stroke, neurodegenerative and mood disorders, *etc.*). In these cases, a therapeutic elevation of the brain KYNA content may hold promise to diminish the consequences of excitotoxicity. On the other hand, it must be mentioned that in diseases with cognitive alterations, such as Alzheimer's dementia and schizophrenia, elevated brain KYNA levels have been reported which could contribute to the cognitive defects by interfering with the NMDA receptor function.

Our experiments demonstrated that KYN treatment abates neuronal cell loss in a rat global ischemia model, in the pathogenesis of which excitotoxicity is assumed. In addition, low doses of L-KYN enhance the cCBF in rabbits under both normal and ischemic conditions of the brain. Citalopram, an SSRI drug, increased the cCBF only in rabbits with unilateral carotid occlusion. Its precise mechanism is still unclear, but an NMDA antagonistic effect of the drug may be involved. Our findings strengthen the data which indicate that KYNA or one of its derivatives with better bioavailability may be fruitful in the treatment of certain acute or chronic neurological diseases involving Glu receptor hyperactivity in their pathomechanism.

POTENTIAL CLINICAL RELEVANCE OF THE RESULTS

The pathological overactivation of Glu receptors plays a role in the pathogenesis of numerous neurological and psychiatric diseases. Abatement of the excitotoxic process can retard the disease course and diminish its damaging consequences.

In our experiments, L-KYN, an NMDA receptor antagonist prodrug that is produced within the body, proved to be neuroprotective in a transient global ischemic animal model. Although NMDA receptor antagonists failed in most acute stroke

trials, as they suspend even the physiological NMDA receptor function and thereby cause severe side-effects, smaller doses of L-KYN administered for a longer period of time may theoretically halt the neurodegenerative process, and (as this is an endogenous substance) may lack toxic side-effects.

In the event of therapeutic administration, the additional CBF-increasing effect of L-KYN under chronic cerebral ischemic conditions may provide further benefit.

Hyperactivity of the NMDA receptors in the cortico-hippocampal pathways in depression may be presumed, a precise understanding of which may hold further therapeutic perspectives. Citalopram, an SSRI antidepressant drug, dose-dependently increased the CBF in animals, a feature in favor of its use in PSD.

SCIENTIFIC PUBLICATIONS OF THE AUTHOR OF THE PH.D. THESIS

Original papers directly related to the thesis

I. **Sas K**, Robotka H, Rózsa É, Ágoston M, Szénási G, Gigler G, Marosi M, Kis Zs, Farkas T, Vécsei L, Toldi J. Kynurenine diminishes the ischemia-induced histological and electrophysiological deficits in the rat hippocampus. *Neurobiology of Disease,* accepted for publication, 2008 (Impact factor: 4.377)

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Total impact factor: 6.735

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