Protective compounds in animal models of trigeminal activation and neurodegeneration

Summary of Ph.D. thesis

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List of abbreviations

AMPA - α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

BBB – blood-brain barrier

bodyweight – bw

cytosine–adenine–guanine - CAG

CamKIIα – calmodulin-dependent protein kinase II alpha

CGRP – calcitonin gene-related peptide

COX - cyclooxigenase

HD – Huntington's disease

HPLC - High performance liquid chromatograpy

i.p. - intraperitoneal

IR - immunoreactive

KYNA – kynurenic acid

L-KYN – L-kynurenine

MRP – multidrug resistance-associated protein

NMDA – N-methyl-D-aspartate

nNOS – neuronal nitric oxide synthase

NO – nitric oxide

NTG - nitroglycerin

s.c. - subcutaneous

TNC – caudal trigeminal nucleus

I. Introduction

Neurological diseases are disorders, which primarily involve the nervous system and the treatment pose a great challenge. In some of these disorders, there are only functional changes in the nervous system, but there are diseases where there is a progressive cell death in the pathomechanism. Sometimes the functional and morphological disorders coexist and distinguishing them can be difficult. In the present work we chose to examine migraine, a disorder with functional abnormalities and Huntington's disease (HD) which involves neurodegeneration.

Migraine headache is one of most common neurological disorders. The prevalence of migraine is up to 12 % among the population. Migraine is a neurovascular disorder with complex interrelationship between neuronal and vascular mechanisms. Despite recent advances of the therapy the appropriate treatment is yet to be achieved. The systemic administration of the nitric oxide (NO) donor nitroglycerin (NTG) triggers a delayed attack without aura in many migraineurs, but not in healthy subjects. In the rat, NTG administration increased the number of *c-fos*- and neuronal nitric oxide synthase (nNOS) – and Ca^{2+}/calmodulin-dependent protein kinase II alpha (CamKIIα) immunoreactivity in neurons of the lower caudal trigeminal nucleus (TNC), where most of the trigeminal nociceptors project. The NTG-induced nNOS increase may be related to the activation of primary trigeminal afferents by NO, leading to a self-amplifying process. In the lower TNC of the rat, NTG causes a decrease in the area covered by calcitonine gene-related peptide (CGRP)-immunoreactive (IR) fibres, probably due to an increased release.

Huntington's disease is an autosomal dominantly inherited neurodegenerative disorder. Although the prevalence of HD is rather low (~ 5/100 000), the disease displays a progressive nature. Despite recent and continuous research advances, the precise mechanism of the neurodegeneration in HD remains unknown. An advance facilitating study of the pathogenesis of disease was the introductions of transgenic mouse models of HD. Transgenic mice (line N171-82Q) expressing exon 1 of the human HD gene with an expanded cytosine–adenine–guanine (CAG) repeat develop a progressive neurological disorder. These transgenic mice have CAG repeat lengths of 82, under the control of the mouse prion protein promoter. The disease starts at the age of 8 weeks. At 12–16 weeks of age, the transgenic mice begin to exhibit an irregular, uncoordinated gait, hypokinesis,

stereotypic movements and tremor. The brain of the transgenic mice is slightly smaller and exhibits striatal atrophy and neuronal intranuclear inclusions. Consequently, these transgenic mice develop neurological symptoms that resemble many of those seen in HD.

The enhanced release of glutamate, which is the main excitatory amino acid in the brain, leads to the prolonged stimulation of its receptors and due to a complex pathomechanism induce the devastation of postsynaptic neurons. Both human and animal studies have demonstrated the important role of glutamate in migraine pathogenesis. Synaptic transmission between first- and second-order trigeminal neurons is partially mediated by glutamatergic mechanisms. The experimental data suggest that N-methyl-D-aspartate (NMDA) receptors are involved in central sensitization of the sensory system. Glutamate excitotoxicity may play an important role in the development of HD, too. This overactivation is due to the impairments in energy metabolism caused by the altered huntingtin gene of HD patients. The selective impairment of medium-sized spiny neurons could be explained in one hand by that they receive a massive glutamaterg input from the cortex and the thalamus.

Some prostaglandins act as important inflammatory mediators that contribute to the progression of inflammation. Prostaglandins are thought to play a role in many neurological functions, including nociceptive processing. It is known that non steroid anti-inflammatory drugs, such as acetylsalicylic acid (Aspirin®), are effective in the treatment of acute migraine headache. This effect could be due to their inhibitory action on cyclooxigenase-2 (COX-2) and prostaglandins in the spinal trigeminal complex. Since these substances can play an important role in the process of trigeminal activation during migraine.

Though less well characterized than in Huntington's, inflammatory cytokines are present and increase with disease stage in these disorders as well. Recent study suggests that elevated levels of cytokines are present in blood and spinal fluid of HD patients as early as 16 years before expected symptom onset, and that these levels increase with the stage of disease, both before and after symptom development.

II. Aims

The aims of our studies were to

(i) study the effects of the glutamate-induced excitotoxicity in the animal model of migraine. We examined effects of kynurenic acid (KYNA) which is one of the few known endogenous broad spectrum antagonists of excitatory amino acid receptors. KYNA penetrates the blood-brain barrier (BBB) poorly, which hampers its therapeutic use. Peripheral treatment with L-kynurenine (L-KYN) combined with probenecid, a known inhibitor of the transport of organic acid from the cerebrospinal fluid, dose-dependently increases the concentration of the neuroprotective KYNA in the brain. In our experiments we tested the effects of the coadministration of L-KYN and probenecid and a novel synthetized derivative of KYNA (2-(2-N,N-dimethylaminoethylamine-1-carbonyl)-1H-quinolin-4-one hydrochloride) on the NTG-induced nNOS expression in the rat TNC.

(ii) to examine the effects of probenecid in the animal models of trigeminal activation and neurodegeneration. It is well known that probenecid is a known non-selective inhibitor of multidrug resistance-associated proteins (MRPs) and organic anion transporters. It has been demonstrated that inhibition by probenecid of the ability of the organic acid transporters to cross the BBB can raise the level of KYNA in the brain dose-dependently. The modulation of the probenecid-sensitive transporters can alter the concentration of inflammatory products such as prostaglandins in the central nervous system.

To acquire further data on the effectivity of probenecid

- in trigeminal pain processing, we studied the effects of probenecid on the NTG-induced expressions of CGRP, nNOS and CamKIIα in the rat TNC.
- in the N171-82Q transgenic mouse model of HD, we studied the effects of probenecid on the survival, behaviour and immunohistochemical changes.

III. Materials and methods

III.1. Animals

The procedures utilized in this study followed the guidelines of the International Association for the Study of Pain and the European Communities Council (86/609/ECC).

They were approved by the Ethics Committee of the Faculty of Medicine, University of Szeged. Adult male Sprague-Dawley rats (weighting between 200 and 250 g) were used. The animals were raised and maintained under standard laboratory conditions, with tap water and regular rat chow available *ad libitium* on a 12-h dark 12-h light cycle.

Drugs:

L-KYN sulphate and probenecid were obtained from Sigma (Steinheim, Germany), while the new compound – is covered by Patent reference number #P0900281 - was synthetized in the Department of Pharmaceutical Chemistry, University of Szeged. The doses of L-KYN and probenecid that were chosen were based on earlier works.

III.1.1. Pretreatment of L-KYN combined with probenecid or a novel derivative of KYNA

We used three experimental set-ups, for immunohistochemistry, Western blotting and chromatography, respectively. Thirty minutes before the NTG treatment, the rats were with L-KYN combined with probenecid or the novel KYNA derivative subcutaneously (s.c.). We compared the effect of these drugs on the nNOS induction of NTG in the TNC with immunohistochemistry and Western blotting. For the high-performance liquid chromatography (HPLC) measurements, the rats in the first group, were injected with vehicle solution. In the second group the rats received an i.p. injection of L-KYN and probenecid.

III.1.2. Pretreatment of probenecid

For immunohistochemistry, the animals were divided into two groups. In the first group, the animals were injected i.p. with probenecid. The animals in the second group were treated with an i.p. injection of the solvent of probenecid. One hour later, half of the animals in both groups received a s.c. injection of NTG, and the other half of the rats received a s.c. injection of the vehicle of NTG. For HPLC measurements, the animals were divided into two groups. The rats in the first group were injected with a solution of the vehicle of probenecid. In the second group the rats received an i.p. injection of probenecid.

Data evaluation

nNOS- and CamKIIα-IR cells were counted in laminae I-II of the cervical spinal cord, in three different series of sections in each animal. The individual sections in these series were taken at 0.5 mm intervals along the rostrocaudal axis.

CGRP-IR fibres in laminae I-II of the cervical dorsal horns were determined by video imaging by means of Image Pro Plus® 6.2 image analysis software.

Group values are given as means ± SEM. Statistical comparisons between the control and NTG-treated groups, Western blot optical densities and HPLC measurements were carried out by using analysis of variance (ANOVA) followed by the Scheffe test. Both analyses were implemented in SPSS, with $p < 0.05$ taken as statistically significant.

III.2. Transgenic mouse model of HD

Animals

All animal experiments were carried out in accordance with the European Union Guide for the Care and Use of Laboratory Animals and were approved by the local animal care committee. Male transgenic mice of the N171-82Q strain were originally obtained from Jackson Laboratories (Bar Harbor, Maine, USA) and were maintained on the B6C3F1 background and bred locally. The offsprings were genotyped by using a PCR assay on the tail DNA at the age of 4 weeks. The mice of mixed genotype were housed together, with same-sex animals per cage, under standard laboratory conditions with ad libitum access to tap-water and regular mouse food. They were kept under natural light in 12-h cycles.

Survival test

Eleven animals received i.p. injections of probenecid (Sigma, Steinheim, Germany) at a dose of 150 mg/bw kg 3 times a week starting at 6 weeks of age until death; 19 animals received i.p. injections of the vehicle of probenecid in the same volume at the same times.

Behaviour testing

Drug treatment

A separate set of 6-week-old transgenic- and wild-type mice were used for behavioural, immunohistochemical studies. The transgenic mice were divided into two groups. The

animals in the first group received probenecid. In the second group, the transgenic mice received the vehicle of probenecid. The wild-type mice in the control group received the vehicle of probenecid. The same experimental protocol and drug administration were used as above for immunohistochemistry. In the HPLC measurements, the animals were divided into two groups. The group of the wild-type control mice was injected with vehicle solution. The second group of the wild-type mice received an i.p. injection of probenecid daily for 1 week.

Open-field test

The Conducta system and programme were used to detect and evaluate the changes in spontaneous motor activity in the open-field test. Tests were performed once a week for 10 weeks at the same day and the same time of day to avoid alterations due to the diurnal rhythm. The tests for behaviour were carried out the following day of the probenecid or vehicle injection.

Statistical analysis

Kaplan–Meier analysis and the Mantel-Cox log rank test were used to determine the survival differences between groups. One-way ANOVA followed by Fisher's LPSD test was used to determine significant differences between groups in the behaviour tests and to compare the level of KYNA between the probenecid-treated wild-type mice and the vehicle-treated wild-type mice. Huntingtin-IR cells in the striatum and the cortex and the cresyl violet-stained neurons in the striatum were counted by an observer blinded to the procedures in five different series of sections in each animal. The independent Student t-test was used to detect the difference between the probenecid-treated transgenic mice and the vehicle-treated transgenic mice in case of huntingtin aggregates. All data were expressed as means ± standard error of the mean (x ± SEM). Statistical significance was taken as $p<0.05$.

IV. Results

The HPLC measurements clearly indicated a significantly increased KYNA level in the TNC half hour after L-KYN and probenecid administration ($p<0.001$).

The transverse sections of the cervical spinal cord demonstrated numerous nNOS-IR neurons in the superficial laminae of the dorsal horns. There was no significant difference in the numbers of NOS-positive cells at different levels of the C1–C2 region. In non- pretreated rats, NTG produced a significant increase in the number of nNOS-IR neurons in the superficial layers of the TNC. The administration of L-KYN-probenecid ($p<0.001$) or of the new KYNA analogue ($p<0.01$) significantly attenuated the effect of NTG on the number of nNOS-IR cells in the TNC.

Western blot analysis of the C1 and C2 region confirmed the results obtained by immunohistochemistry. A band characteristic of the nNOS protein was identified at ~155 kDa. Densitometric analyses confirmed that the nNOS band in the Western blots was significantly enhanced in segments C1 and C2 after NTG administration ($p<0.05$).

Transverse sections of the cervical spinal segments revealed abundant CGRP-positive fibres in the superficial layers of the caudal trigeminal nucleus. The areas covered by these fibres were not significantly different at the various rostro-caudal levels, nor on the different sides of the C1-C2 segments. The CGRP-innervated area for the NTG-treated group was significantly smaller than that for the placebo-treated group ($p<0.01$). This decrease was successfully attenuated by probenecid pretreatment ($p<0.05$).

Numerous nNOS-IR neurons have been demonstrated in the superficial layers of the cervical spinal cord. There was no significant difference in the number of nNOS-positive cells at the different levels of the C1-C2 region. In the non-pretreated rats, NTG produced a significant increase in the number of nNOS-IR neurons in the superficial layers of the TNC. The administration of probenecid significantly attenuated the effect of NTG on the number of nNOS-IR cells in the TNC ($p<0.001$).

On microscopic examination of immunostained transverse sections, CamKIIα immunoreactivity was found in the neurons of the TNC and in the neuropil of lamina II. CamKIIα-IR cells were abundant in the superficial layers of the TNC. The numbers of cells were not significantly different at the various rostro-caudal levels, nor on the different sides of the TNC. NTG induced a significant increase in the number of CamKIIα-positive cells in the superficial layers of the TNC in the non-pretreated rats. Probenecid attenuated the CamKIIα increase ($p<0.05$).

Even 1 week of administration of probenecid resulted in an increased level of KYNA in the wild-type mouse cortex ($p<0.05$) over that in the control group but not in the striatum.

The mean duration of survival of the vehicle-treated N171- 82Q mice was 118.1 days. Probenecid administration produced a highly significant increase in the survival of 35% (159.6 days).

Our earlier experiments showed that chronic probenecid treatment did not cause significant motor activity changes between the probenecid-treated wild-type mice and the vehicle-treated control wild-type mice. The transgenic mice treated with probenecid moved significantly more than the controls by the age of 14 weeks ($p<0.05$; $p<0.01$). The probenecid-treated group spent significantly less time in the same place compared with the vehicle-treated transgenic group ($p<0.05$; $p<0.01$). The probenecid treatment induced a slight, but not significant increase in the mean velocity. The frequency of rearing was significantly lower in the control transgenic mice than in the probenecid-treated group ($p<0.01$).

Probenecid treatment also ameliorated the striatal neuronal loss in these animals. Our quantitative analysis demonstrated that the probenecid-treated transgenic animals had a significantly higher number of surviving striatal neurons relative to the vehicle-treated group ($p<0.05$).

Huntingtin-IR aggregates were significantly more numerous in the outer lamina of the pyriform cortex (layer II), which is an important area of the N171-82Q transgenic mice as concern the EM48 positivity, and within the lateral striatum. The aggregates were much more prominent within the cortex as compared with the neostriatum. In the probenecid-treated group, fewer EM48 positive neurons were detected in both areas than the vehicle-treated transgenic group. Probenecid treatment significantly reduced the numbers of striatal ($p<0.01$) and cortical ($p<0.05$) positive neurons.

V. Discussion

Our HPLC data confirm earlier findings and observations, we found that the administration of L-KYN in combination with probenecid caused a robust increase in the level of KYNA in the rat TNC. In the present study, this combination or a novel KYNA derivative attenuated the NTG-induced enhancement of nNOS in the most caudal portion of the rat TNC.

The most probable way for NTG to enhance nNOS expression is via the peripheral afferents, causing a self-amplifying process in the TNC, where glutamate may also play a role. It has been shown that the activation of NMDA receptors is pivotal for the development of central sensitization in the dorsal horn. Earlier data have revealed that NTG can also enhance the level of CamKIIα in the TNC of the rat, which is in line with other data, since phosphorylation of the NMDA receptors initiated prolonged increases in the excitability of the spinal cord neurons. The central nervous system uptake of KYN and its metabolism to KYNA may act to reduce NMDA receptor-mediated central sensitization. Besides the NMDA antagonistic effect, KYNA is able to act on the AMPA / kainate receptors. A synthetic NMDA receptor antagonist, MK-801, and a synthetic AMPA receptor antagonist, GYKI-52466, effectively block trigeminovascular nociception. Indeed, the mixed AMPA/kainate receptor antagonist LY293558 has been shown to be effective in the acute treatment of migraine. Thus, glutamate plays a key role in trigeminal activation and sensitization and modulation of the glutamate receptors appears crucial in the pathogenesis of migraine.

In our experiments, in the N171-82Q transgenic mouse model of HD the chronic administration of a novel analogue of KYNA improved the survival time, motor activity changes as compared with that in the control transgenic mice (data not shown). Thus, our results have shown that the KYNA derivative treatment in N171-82Q mouse model of HD results the delay of symptom development and lessening of symptom severity.

Since KYN metabolites may modulate different neuronal targets in head pain conditions and HD, the side-effect profile and hence the possible indications and contraindications would be different relative to the drugs already in use. Taken together, these observations suggest the involvement of metabolites of the kynurenine pathway on various sites of nociception and neurodegeneration. One strategy with the aim of increasing the therapeutic potential of KYNA is to develop synthetic analogues which can readily penetrate the BBB, and act as glutamate receptor antagonists. Our results show that L-KYN (metabolized to KYNA) and a novel KYNA derivative exert a modulating effect on the trigeminal activation in the NTG model of migraine and the neurodegenerative processes in the transgenic mouse model of HD, possibly via the glutamate receptors. Thus, KYNA derivatives may afford novel therapeutic opportunities in the management of these neurological disorders.

As a non-selective inhibitor of MRPs and organic acid transporters in the BBB, probenecid can inhibit the elimination of the excitatory amino acid receptor antagonist KYNA produced in the brain. Although probenecid treatment increased the KYNA level in our results (data not shown), it did not attain the concentrations needed to block NMDA receptors. Overall, these data suggest that the modulatory effects of probenecid are only marginally linked to the above mechanisms.

It has been suggested that probenecid is able to inhibit MRP4, which can transport prostaglandins E1 and E2 with higher affinity than for other MRPs. MRP4 catalyses the uptake of the key inflammatory mediators prostaglandins E1 and E2 in a time- and ATP-dependent manner. Several lines of evidence indicate that prostaglandins induce the release of neurotransmitters such as excitatory amino acids, CGRP and NO. Conversely, glutamate, CGRP, cytokines and NO enhance prostaglandin release.

CGRP is a key transmitter in primary nociceptive afferents, and both basic research and clinical studies have provided evidence of its role in the pathomechanism of migraine. Peripheral sensitization of meningeal trigeminal nociceptors and the increased release of CGRP are thought to activate second-order neurons that mediate central sensitization. The decrease in CGRP immunoreactivity after systemic NTG administration is probably due to the activation of primary trigeminal Aδ and C fibres and their interactions with second-order trigeminal neurons. The release of neuropeptides such as CGRP from peripheral nerve endings induces a painful state of local neurogenic inflammation in the cerebral dura. In our experiments, probenecid attenuated the NTG-induced depletion of CGRP in the rat TNC, inhibiting CGRP release from the sensory nerve endings by blocking the inflammation process, which plays a key role in trigeminal activation.

The most likely explanation for the NTG-induced nNOS increase is the secondary activation of the peripheral afferents interacting with second-order trigeminal neurons, leading to a self-amplifying process in the TNC. In migraine patients, early activation of the L-arginine/NO pathway and a late rise in the synthesis of prostanoids has been demonstrated after the onset of headache and increases in the release of prostaglandin E2 and in the NO production of monocytes have been found in migraineurs without aura, suggesting an interaction. Moreover, acetylsalicylic acid and a selective COX-2 inhibitor, NS398, inhibited the NTG-induced nNOS expression changes in the rat, which indicates that prostanoids are involved in this process. Probenecid, which inhibits MRP4, may block

the sensitization process via the prostanoids at the level of the TNC.

NTG can also enhance CamKIIα in the TNC of the rat by similar mechanisms. Research findings suggest that CamKIIα has an important role in nociceptive processing and contributes to central sensitization. A CamKII inhibitor dose-dependently mitigates inflammation-induced thermal hyperalgesia and mechanical allodynia, while pretreatment with acetylsalicylic acid and the selective COX-2 inhibitor NS398 attenuates the NTG-induced changes in CamKIIα immunoreactivity; accordingly, the inhibition of MRP4 by probenecid, which affects prostanoid transport, may have a key role in the modulatory process.

It has been well known that excitotoxicity, energy deficit, oxidative stress, protein aggregation and inflammatory process also play an important role in the pathogenesis of HD. In this study, we used the N171-82Q transgenic mouse model of HD to determine the chronic effects of probenecid on the survival time, motor activity changes neuronal loss and on the formation of the mutant huntingtin aggregates. The administration of probenecid significantly improved the observed parameters in the N171-82Q mice as compared with that in the control transgenic mice. Thus, our results have shown that probenecid treatment results in the delay of symptom development and lessening of symptom severity. These improvements may be accounted for by the protection of striatal cells observed in probenecid-treated transgenic group. The N-terminal fragments of mutant huntingtin accumulate in the nucleus of affected neurons and form intranuclear aggregates. The role of the intranuclear aggregates is still controversial, but the effect on the neuronal loss may be hypothesized. In our results, there were significant decreases in EM48 immunoreactivity in the striatum and in the pyriform cortex of the probenecid-treated N171-82Q mice relative to the control transgenic group. Our findings suggest that a decrease in nuclear aggregates may decelerate the progress of the disease which is in accordance with other experiments. Inhibition of MRP4 by probenecid may contribute to the protection of neurons in this transgenic animal model of HD by blocking inflammatory signals.

On the other hand, recent studies have demonstrated that probenecid inhibits the pannexin-1 channel, which is largely expressed in the spinal cord and may play an important role in neuronal inflammatory processes and hence in pain processing. P2X7 receptors, which are closely related to pannexin-1, are present in the sensory ganglia and

associated with neuron-glia communication and involved in nociception. Moreover, the pannexin-1-related P2X7 activation that occurs in the spinal dorsal horns is implicated in the development of chronic neuropathic pain, allodynia and the sensitization process. Its inhibition may be responsible for the attenuation of NTG-induced CGRP, nNOS and CamKIIα expression-changes and for the abolition of the peripheral trigeminal activation and the central sensitization process in the TNC.

Pannexin-1 can be activated not only by inflammatory process but apoptotic signals. Pannexin-1 is a potential candidate protein of ATP release during high extracellular potassium-induced cell death. The pathomechanism of HD involves neuronal atrophy and cell death and thus probenecid may also exert protective effect on this process. Interestingly, extracellular ATP has been recently reported to elicit neuronal death through stimulation of P2X7 receptors. Recent findings demonstrate that alteration in P2X7-mediated Ca^{2+} permeability may contribute to HD synaptic dysfunction and increased neuronal apoptosis. In cultured neurons expressing mutant huntingtin showed increased susceptibility to apoptosis triggered by P2X7-receptor stimulation. Furthermore, *in vivo* data strongly suggest that altered P2X7-receptor level and function contribute to HD pathogenesis and highlight the therapeutic potential of P2X7 receptor antagonists.

Overall, inhibition by probenecid of the ability of the organic acid transporters to cross the BBB can raise the excitatory amino acid antagonist KYNA level of the brain dose-dependently. In this model, the relatively slight KYNA concentration increase lead us to suggest that the glutamatergic modulatory effects of probenecid in the trigeminal complex and the striatum may be only marginal, and the effects of probenecid on prostanoids and pannexin-1 could play a more significant role. The fact that probenecid-influenced mechanisms are involved in this self-amplifying process in the trigeminal area and in the neurodegenerative process in HD as well may furnish further details about the pathophysiology of migraine and HD. Despite these encouraging results, further studies are clearly needed to elucidate the exact mechanism of the modulatory effect of probenecid in these neurological disorders.

It is noteworthy that the pre-treatment with the KYNA analogue or L-KYN and probenecid or probenecid alone produced the same effect on the NTG induced nNOS activation in the trigeminal system. This suggests that this modulatory action involves at least two mechanisms one related to glutamate the other to probenecid. The conclusion is

similar to the observations in the HD model. Chronic administration of KYNA derivative or probenecid improved the survival time and the motor activity changes in the transgenic mouse model of HD possibly in two different ways.

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VII. Original publications directly related to the PhD thesis

I. **Vámos E**, Párdutz A, Varga H, Bohár Z, Tajti J, Fülöp F, Toldi J, Vécsei L. (2009) L-kynurenine combined with probenecid and the novel synthetic kynurenic acid derivative attenuate nitroglycerin-induced nNOS in the rat caudal trigeminal nucleus. *Neuropharmacology* **57:**425-9.
IF: 3,383

II. **Vámos E**, Párdutz A, Fejes A, Tajti J, Toldi J, Vécsei L. (2009) Modulatory effects of probenecid ont he nitroglycerin-induced changes in the rat caudal trigeminal nucleus. *European Journal of Pharmacology* (in press)
IF: 2,787

III. **Vámos E**, Vörös K, Zádori, Vécsei L, Klivényi P. (2009) Neuroprotective effects of probenecid in a transgenic animal model of Huntington's disease. *Journal of Neural Transmission* **116:**1079-86.
IF: 2,514

Total impact factor: 8,684

Publications not directly related to the thesis

I. **Vámos E**, Fejes A, Koch J, Tajti J, Fülöp F, Toldi J, Párdutz Á and Vécsei L. (2009) Kynurenate derivative attenuates the nitroglycerin-induced CamKIIα and CGRP expression changes. *Headache* (in press)
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II. **Vámos E**, Vörös K, Vécsei L, Klivényi P. (2009) Neuroprotective effects of L-carnitine in a transgenic animal model of Huntington's disease. *Biomedicine and Pharmacotherapy* (in press)
IF: 2,198

III. **Vámos E,** Párdutz Á, Klivényi P, Toldi J, Vécsei L. (2009) The role of kynurenines in disorders of the central nervous system: possibilities for neuroprotection. *Journal of the Neurological Sciences* **283:** 21-7.
IF: 2,359

IV. Zádori D, Klivényi P, **Vámos E,** Fülöp F, Toldi J and Vécsei L. (2009) Kynurenines in chronic neurodegenerative disorders: future therapeutic strategies. *Journal of Neural Transmission* (in press)
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V. Varga H, Párdutz Á, **Vámos E**, Plangár I, Egyud E, Tajti J, Bari F, Vécsei L. (2007) Cox-2 inhibitor attenuates NO-induced nNOS in rat caudal trigeminal nucleus. *Headache* **47:**1319-25.
IF: 3,081

VI. Varga H, Párdutz Á, **Vámos E**, Bohár Z, Bagó F, Tajti J, Bari F, Vécsei L. (2009) Selective inhibition of cyclooxygenase-2 attenuates nitroglycerin-induced calmodulin-dependent protein kinase II alpha in rat trigeminal nucleus caudalis. *Neuroscience Letters* **451**:170-3.
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IF: 4,94

Total impact factor: 20,373

Other papers

I. **Vámos E,** Csáti A, Vécsei L, Klivényi P. (2009) Effects of valproate on the dopaminergic system in mice. *Neurological Research* **31:**217-9.
IF: 1,63

II. Zádori D, Geisz A, **Vámos E**, Vécsei L, Klivényi P. (2009) Valproate ameliorates the survival and the motor performance in a transgenic mouse model of Huntington's disease. *Pharmacology Biochemistry and Behavior* (in press)
IF: 2,751

Total impact factor: 4,381

Patent

Use of kynurenic acid and its analogues in the treatment of headaches. (It is covered by Patent reference number #P0900281)

Cumulative impact factor: 33,43