FINAL REPORT

on the research grant of the Hungarian Research and Development Fund, entitled

„The role and cellular mechanism of action of P2X7 receptors in animal models of neuropsychiatric disorders"

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Introduction and objectives

Major depression (MD) is the most common psychiatric disorder with 10-15% lifetime prevalence in developed countries. The incidence of depression is continuously elevating, and is expected to be the second major cause of disability by 2020 (WHO, 2001). Schizophrenia is the second most common psychiatric disorder affecting about 1% of the population worldwide. Although symptomatic treatment exists for both of them, depression and schizophrenia are still devastating conditions that represent a huge economic and social burden. Moreover, relatively high proportion of patients do not respond to existing medications, which urges the discovery and validation of new potential therapeutic targets.

According to current knowledge, psychiatric disorders are caused by complex interactions between genes, developmental and environmental factors involving a multiplicity of neurotransmitters and signalling pathways. The previous data of our group and others suggest that genetic deletion or inhibition of P2X7 receptors leads to specific changes in behavior in animal models of depression and schizophrenia. However, it is not understood yet how the activity of P2X7 receptors leads to changes in mood and behavior.

The aim of this project was to understand cellular and subcellullar mechanisms underlying behavioural changes mediated by P2X7 receptors and to further validate P2X7 receptors as a new therapeutic target in a variety of neuropsychiatric disorders.

Because it is well accepted that these disorders are characterized by the reduction of neuronal plasticity, we primarily focused on plastic structural and functional changes in the brain, which are potentially under the regulation of P2X7 receptors. We examined two CNS areas, which are instrumental for cognitive function, mood and behavior, i.e. the hippocampus and the prefrontal cortex. In the hippocampus, the level of brain derived neurotrophic factor (BDNF), adult neurogenesis, and spine synapse plasticity were examined in parallel with behavioural studies addressing the role of P2X7 receptors in lipopolysaccharide (LPS)-induced anhedonia response. We also explored the changes in the synthesis, release and uptake of transmitters (glutamate and 5-HT), which probably mediate changes on neuroplasticity. In the prefrontal cortex, developmental changes in the expression and functionality of P2X7 receptors were explored as well as changes in gene expression patterns, which are under the regulation of P2X7 receptors, with a further consolidation of the role of P2X7 receptors in an animal model of schizophrenia. In addition to the two major psychiatric disorders, we

have clarified the role of P2X7 and other P2 receptors in animal models of neuropathic pain, which is a comorbidity of depression, and of other acute and chronic pain syndromes.

Results

The following studies were performed by the support of the grant:

1.) Neurochemical Changes in the Mouse Hippocampus Underlying the Antidepressant Effect of Genetic Deletion of P2X7 Receptors.

Recent investigations have revealed that the genetic deletion of P2X7 receptors (P2rx7) results in an antidepressant phenotype in mice. However, the link between the deficiency of P2rx7 and changes in behavior has not been explored yet. In the present study, we studied the effect of genetic deletion of P2rx7 on neurochemical changes in the hippocampus that might underlie the antidepressant phenotype. P2X7 receptor deficient mice (P2rx7-/-) displayed decreased immobility in the tail suspension test (TST) and an attenuated anhedonia response in the sucrose preference test (SPT) following bacterial endotoxin (LPS) challenge. The attenuated anhedonia was reproduced through systemic treatments with P2rx7 antagonists, such as Brilliant blue G and AZ-10606120.
The activation of P2rx7 resulted in the concentration-dependent release of endogenous and [^{3}H]glutamate in P2rx7+/+ but not P2rx7-/- mice. NR2B subunit mRNA and protein was also upregulated in the hippocampus of P2rx7-/- mice.
The brain-derived neurotrophic factor (BDNF) expression was higher in saline but not LPS-treated P2rx7-/- mice; the P2rx7 antagonist Brilliant blue G elevated and the P2rx7 agonist benzoylbenzoyl ATP (BzATP) reduced BDNF level. This effect was dependent on the activation of NR2B subunit containing NMDA receptors and non-NMDA receptors but not on Group I metabotropic glutamate receptors (mGluR1,5).
An increased 5-bromo-2-deoxyuridine (BrdU) incorporation was also observed in the dentate gyrus derived from P2rx7-/- mice.
Basal level of 5-HT was increased, whereas the 5HIAA/5-HT ratio was lower in the hippocampus of P2rx7-/- mice, which accompanied the increased uptake of [^{3}H]5-HT and an elevated number of [^{3}H]citalopram binding sites. The LPS-induced elevation of 5-HT level was absent in P2rx7-/- mice. [^{3}H]-citalopram/[^{3}H]-nisoxetine/[^{3}H]-dihydroalprenolol binding sites were not changed by genetic deletion of P2rx7 receptors.
In conclusion, the most likely potential underlying mechanisms responsible for the antidepressant phenotype of P2rx7-/- mice is the absence of P2rx7-mediated glutamate release, NR2B receptor activation and the consequently elevated basal BDNF production, enhanced neurogenesis and increased 5-HT bioavailability in the hippocampus.
These results are published (Csölle C, Baranyi M, Zsilla G, Kittel A, Gölöncsér F, Illes P, Papp E, Vizi ES, **Sperlágh B**, PLoS One. 2013 8(6):e66547).

2.) Characterization of simultaneous 5-HT and glutamate release in response to a behaviorally relevant optogenetic stimulation of median raphe afferents and its modulation by P2X7 receptors

Serotonergic neurotransmission has been implicated in the etiology of disorders of emotion regulation such as anxiety disorders and depression, and it is a primary target for psychotherapeutic drugs. The present study has examined several characteristics of the release of [^{3}H]5-HT from the median raphe nucleus (MRN) and hippocampus in terms of its dependence of nerve impulse. We used electrical stimulation and the sodium channel opener veratridine, which excite all of the neuronal processes in the stimulation field, and optogenetics to selectively stimulate those terminals which express channelrhodopsin-2 (ChR2) and compared 5-HT release evoked by electrical and chemical depolarization and by light. Because the diffusion of ChR2 throughout the cell and populating the membrane can take one to two months to see adequate expression in the terminals of axons, we used animals 4 and 8 weeks after virus injection.

Adult C57/Bl6 wild-type mice were anesthetized with ketamine-xylazine mixture, placed in a stereotaxic apparatus, and given 40 nL injections of AAV5.hSyn. hChR2(H134R)FYFP.WPRE.hGH (Penn Vector Core) into the MRN and hippocampus. Stereotaxic coordinates were chosen for each target area based on The Mouse Brain in Stereotaxic Coordinates. The position of injection site and expression of the virus construct by the target areas were post hoc verified by fluorescent microscopy. 4 or 8 weeks after the injection, acute 300-µm-thick coronal brain slices containing the MRN or hippocampus were prepared using a vibratom. Brain slices were then incubated with [^{3}H]5-HT dissolved in Krebs-bicarbonate buffer for 60 min at 37 °C. At the end of the loading period, the tissues were transferred into low volume superfusion chambers and superfused with aerated (95% O_2/5% CO_2) and preheated (37 °C) Krebs-bicarbonate buffer by using a peristaltic pump. The superfusate is discarded for the first 60-min period of the experiments then a series of 3- or 1-min fractions were collected by a fraction collector. Blue light (473 nm, (10, 20, 50, 100 Hz) was delivered using a Grass S88 REV K Stimulator-controlled DPSS laser (Ike-Cool) coupled to a 100-µm fiber optic probe; electrical stimulation (10, 20, 50, 100 Hz) was delivered by bipolar electrode and chemical depolarization was applied by veratridine (20 µM) to stimulate the efflux of [^{3}H]5-HT from superfused MRN and hippocampal slices.

Whereas optical stimulation at various frequencies (10, 20, 50, 100 Hz) elicited only a negligible increase in 5-HT release either from the hippocampus or from the MRN in mice 4-weeks after virus injection, when slices stimulated 8-weeks after virus injection light stimulation elicited a substantial and reproducible efflux of 5-HT, which was comparable to the effect of electrical field stimulation and veratridine. The light stimulation evoked [^{3}H]5-HT release in the MRN was frequency- and $[Ca^{2+}]_o$- dependent as well as sensitive to the sodium channel blocker tetrodotoxin (TTX, 10 µM) and to NMDA/AMPA receptor antagonist CNQX/AP-5. Light stimulation with identical parameters also released [^{3}H]glutamate from MRN slices. Consequently [^{3}H]5-HT from MRN terminals is released in response to the action of glutamate originated from neighboring terminals, and synapsing onto local serotonergic cell bodies. In contrast, in

hippocampal slices light stimulation evoked [^{3}H]5-HT efflux was [Ca^{2+}]$_o$- dependent, but insensitive to the action of either TTX or glutamate receptor antagonists implying that light stimulation in this case directly releases 5-HT from serotonergic terminals. Ongoing experiments clarify the potential modulatory role of P2rx7 receptors. The effect of optical stimulation on 5-HT efflux was also demonstrated in vivo, in microdialysis experiments and on the behaviour of the animals.

The above results demonstrate for the first time the effect of optogenetic stimulation on simultaneous 5-HT and glutamate release in the brain, measured directly by a neurochemical method. The results are published at conferences and the manuscript is ready for submission (F. Gölöncsér, M. Baranyi, R. D. Andó, D. Balázsfi, D. Zelena, K. Demeter, J. Haller, G. Nyíri, T. F. F. Freund and **B. Sperlágh**, Characterization of simultaneous 5-HT and glutamate release in response to a behaviorally relevant optogenetic stimulation of median raphe afferents).

3.) <u>Hippocampal spine synapse plasticity in the absence and presence of P2rx7 receptors in the learned helplessness model of depression</u>

Recent studies revealed that in the learned helplessness animal model of depression in parallel with the depression-like behavior, a loss in spine synapses was observed in the hippocampus, and both changes could be reversed by antidepressant administration (Hajszán et al., 2009). The mechanism underlying P2X7 receptor action in depression is not fully explored yet, but the existing data indicates links with neuronal plasticity. Therefore, to identify cellular actions that mediate the effect of P2x7 receptor on mood-related behavior, we have chosen the hippocampus as the target area of our present study and performed a quantitative analysis of spine synapses in the dentate gyrus (DG) and measured the alterations of synaptic protein levels in the learned helplessness paradigm.
In naïve animals the number of spine synapses was slightly but significantly lower in P2rx7-/- mice and similar effect was detected after subacute treatment of the animals with the P2X7 receptor antagonist Brilliant blue G (50 mg/kg/day i.p.).
Then, we have set up the learned helplessness behavioural paradigm: wild type C57Bl/6 and P2rx7 knock out male mice (8-10 weeks old) were exposed to inescapable footshocks in shuttle boxes during training days (n=19-26). They received 2x180 scrambled footshocks (2 s, 0.15 mA, with 1-15 s random intertrial intervals, ITI) on two consecutive days. On the 3rd day learned helplessness was tested (30 trials, 10 s footshock, 0.15 mA, 30 s average ITI) when the aversive stimuli could be avoided, but helpless animals failed to escape. Control animals were also placed in shuttle boxes but did not receive footshocks until the test day. Number of escape failures and the latency to escape was measured automatically to determine helpless behaviour. In line with literature data series of inescapable footshocks lead to learned helplessness behaviour in wild-type mice and a significant decrease in the number of spine synapses in the DG. In parallel with these changes a time-dependent upregulation of P2rx7 mRNA

expression, and a downregulation of the synaptic protein synaptopodin was also observed in wild-type, but not in P2rx7-/- mice.
In P2rx7 deficient mice no changes were observed either in behavior or in spine synapse density in response to inescapable footshocks, i.e. they did not develop the learned helplessness behaviour.
In conclusion, we have confirmed that genetic deletion of P2X7 receptor leads to an antidepressant phenotype with a more complex animal model of depression than the previously used behavior tests. Whereas in wild type animals learned helplessness caused a decrease in spine synapse density, this change was absent in P2X7 receptor knockout animals, which is a potential underlying mechanism of the antidepressant effect. Therefore our initial hypothesis was confirmed that activation of P2rx7 probably leads to changes in hippocampal plasticity and thereby regulate stress-induced and mood related behavior.

The results are published at conferences and the manuscript from the results is ready for submission (Otrokocsi L, F. Gölöncsér, Á. Kittel and **B. Sperlágh**, P2X7 receptors drive spine synpase plasticity in the learned helplessness model of depression).

4.) <u>The role P2X7 receptors in a rodent PCP-induced schizophrenia model</u>

In this study the expression and function of central P2X7 receptors was examined in a phencyclidine (PCP)-induced schizophrenia model in mice. In young adult P2rx7+/+ mice, PCP (2 and 5 mg/kg i.p.) induced hyperlocomotion, stereotype behavior, ataxia and decreased social interactions, mimicking positive and negative symptoms of schizophrenia. In mice, genetically deficient in P2X7 receptors (P2rx7-/-), the social interactions were increased, whereas the PCP induced hyperlocomotion and stereotype behavior were alleviated. Acute treatment with the selective and brain permeable P2X7 receptor antagonist JNJ47965567 (30 mg/kg i.p.) largely replicated the effect of gene deficiency on PCP-induced behavioral changes and counteracted PCP-induced social withdrawal.
mRNA encoding P2X7 receptors displayed slight developmental changes and upregulated in response to the lower (2 mg/kg i.p.) and higher (5 mg/kg i.p.) dose of PCP in the prefrontal cortex and the hippocampus, respectively.
PCP also induced changes in the transcription of various other proteins in the prefrontal cortex, including increased mRNA expression encoding neuregulin 1, NR2A and NR2B and decreased mRNA expression of NR1and, GABA α1 subunits in young adult (56 days) but not in juvenile (18 days) animals. The above PCP-induced transcriptional alterations were absent in mice genetically deficient in P2X7 receptors.
P2X7 receptor activation released [^{3}H]glutamate, but not [^{3}H]dopamine from acute prefrontal cortex slices, which was enhanced by preceeding in vivo PCP treatment. The amplitude of NMDA evoked currents (1-1000 μM) recorded from layer V pyramidal neurons of cortical slices were slightly alleviated by genetic deletion and pharmacological blockade (JNJ47965567, 100 nM) of P2X7 receptors.

In summary, we report here for the first time the alleviation of PCP induced behavioral changes by the inhibition of P2X7 receptors. Changes in the balance of glutamate receptor subunits and other pre- and postsynaptic proteins as well as functional alterations of glutamatergic transmission in the prefrontal cortex might underlie these changes.
The manuscript prepared form the results are ready for submission (B. Koványi, C. Csölle, S Calovi, A. Hanuska, E. Kató, L. Köles, A. Bhattacharya, J. Haller and **B. Sperlágh**, The role P2X7 receptors in a rodent PCP-induced schizophrenia model, to be submitted to Biological Psychiatry).

5.) <u>The role of P2X7 and non-P2X7 purinergic receptors in the modulation of action potential threshold and sEPSCs in the neonatal rat PFC</u>

Maternal immune activation during pregnancy is a risk factor for neurodevelopmental disorders, such as schizophrenia and autism, however, a full mechanistic understanding has yet to be established. The activity of a transient cell population, the subplate neurons (SPNs), is critical for the development of cortical inhibition and functional thalamocortical connections. Sensitivity of these cells to factors released during inflammation, therefore, may offer a link between maternal immune activation and the aberrant cortical development underlying some neuropsychiatric disorders. An elevated extracellular concentration of ATP is associated with inflammation. Furthermore, ATP has been shown to have an effect on neuronal activity. Here, we investigated the effect of ATP on the electrophysiological properties of subplate neurons.
High concentrations of ATP and the P2X7 receptor agonist BzATP increased action potential threshold in SPNs. Exogenous ATP also increased amplitude and frequency of spontaneous post-synaptic currents at a concentration of 100µM. Whilst the effect of ATP on action potentials proved to be P2X7 receptor-dependent, the effect of ATP on excitatory post-synaptic currents was suramin and PPADS-sensitive, but P2X7 receptor-independent. Further, ATP released by astrocytes activated by the PAR-1 agonist, TFLLR-NH2, also increased the amplitude and frequency of sEPSCs in subplate neurons. The electrophysiological properties of subplate neurons recorded from PFC slices from neonatal rats were also disrupted in a maternal immune activation rat model of schizophrenia, with a suramin-sensitive increase in frequency and amplitude of sEPSCs. An alternative neurodevelopmental rat model of schizophrenia, MAM-E17, which did not rely on maternal immune activation, however, showed no change in subplate neuron activity. Both models were validated with behavioural assays, showing schizophrenia-like endophenotypes in young adulthood. The purinergic modulation of subplate neuron activity offers a potential explanatory link between maternal immune activation and disruptions in cortical development that lead to the emergence of neuropsychiatric disorders.

The manuscript prepared form the results are ready for submission (Beamer, E, Illes P and **Sperlágh, B**, ATP released from astrocytes modulates action potential threshold and

sEPSCs through different purinergic receptors in the neonatal rat PFC to be submitted to The Journal of Neuroscience).

6.) The role of glutamate release mediated by extrasynaptic P2X7 receptors in animal models of neuropathic pain.

The expression of the purinergic ligand gated ion channel P2X7 receptor (P2rx7) has been described on nerve terminals as well as in non-neuronal cells, such as astrocytes and microglia. The activation of P2rx7s results in $Ca(^{2+})$ influx and increased transmitter release in the brain. P2rx7s previously suggested having a pivotal role in different pain modalities, including neuropathic pain. We investigated here, whether the activation of P2rx7 leads to increased glutamate release from the spinal cord in an experimental model of neuropathic pain (partial nerve ligation of the sciatic nerve, PNL). One week after surgery, we studied the effects of PNL on tactile allodynia using aesthesiometry, in parallel with the in vitro release of $[(^{3})H]$glutamate from lumbar spinal cord slices. The observed allodynia in wild-type (P2rx7+/+) mice one week after PNL surgery was lower that was observed in P2rx7 deficient (P2rx7-/-) animals. Perfusion of spinal cord slices with ATP (10mM) elicited $[(^{3})H]$glutamate release in both sham operated and neuropathic P2rx7+/+ animals. The ATP-induced $[(^{3})H]$glutamate release was absent in P2rx7-/- mice. Electrically evoked release of $[(^{3})H]$glutamate from spinal cord slices was not significantly altered in PNL animals and in P2rx7-/- mice. The results suggest that activation of P2rx7 by ATP releases glutamate in the spinal cord, which might contribute to mechanical allodynia following PNL. On the other hand, this release does not contribute to glutamate efflux evoked by conventional neuronal activity, which is consistent with the idea that P2X7 receptors are either extrasynaptic or expressed on non-neuronal cells.
The results are published (Andó RD, **Sperlágh B.**, Brain Res Bull 2013 93:80-5.).

7.) Astrocyte-neuron interaction in the substantia gelatinosa of the spinal cord dorsal horn via P2X7 receptor-mediated release of glutamate and reactive oxygen species

In collaboration with the German partners we have explored P2rx7 receptors expressed by astrocytes, nerve terminals in acute spinal cord slices utilizing the patch clamp experiments and the microelectrode biosensor technique. The substantia gelatinosa (SG) of the spinal cord processes incoming painful information to ascending projection neurons. Whole-cell patch clamp recordings from SG spinal cord slices documented that in a low Ca(2+) /no Mg(2+) (low X(2+)) external medium adenosine triphosphate (ATP)/dibenzoyl-ATP, Bz-ATP) caused inward current responses, much larger in amplitude than those recorded in a normal X(2+) -containing bath medium. The effect of Bz-ATP was antagonized by the selective P2X7 receptor antagonist A-438079. Neuronal, but not astrocytic Bz-ATP currents were strongly inhibited by a combination of the ionotropic glutamate receptor antagonists AP-5 and CNQX. In fact, all neurons and some

astrocytes responded to NMDA, AMPA, and muscimol with inward current, demonstrating the presence of the respective receptors. The reactive oxygen species H_2O_2 potentiated the effect of Bz-ATP at neurons but not at astrocytes. Hippocampal CA1 neurons exhibited a behavior similar to, but not identical with SG neurons. Although a combination of AP-5 and CNQX almost abolished the effect of Bz-ATP, H_2O_2 was inactive. A Bz-ATP-dependent and A-438079-antagonizable reactive oxygen species production in SG slices was proven by a microelectrode biosensor. Immunohistochemical investigations showed the colocalization of P2X7-immunoreactivity with microglial (Iba1), but not astrocytic (GFAP, S100β) or neuronal (MAP2) markers in the SG. It is concluded that SG astrocytes possess P2X7 receptors; their activation leads to the release of glutamate, which via NMDA- and AMPA receptor stimulation induces cationic current in the neighboring neurons. In addition, at least under the present experimental conditions, P2X7 receptors have a low density under resting conditions but become functionally upregulated under pathological conditions.
The results are published (Ficker C, Rozmer K, Kató E, Andó RD, Schumann L, Krügel U, Franke H, **Sperlágh B**, Riedel T, Illes P. Glia. 2014 62(10):1671-86.)

8.) <u>Effect of genetic deletion and pharmacological antagonism of P2X7 receptors in a mouse animal model of migraine</u>

Purine receptors participate in peripheral and central sensitization and are associated with migraine headache. However the role of P2X7 receptors have not been explored yet in animal models of migraine. We investigated the role of P2X7 receptor (P2X7) in a nitroglycerin (NTG)-induced mouse model of migraine.
Intraperitoneal NTG injection (15 mg/kg) triggered thermal hyperalgesia in the hindpaws of wild-type C57BL/6J mice, followed by the induction of c-fos in upper cervical spinal cord and trigeminal nucleus caudalis. The effect of genetic deletion of P2X7 and the selective P2X7 antagonist Brilliant Blue G (BBG) were examined on hyperalgesia and c-fos induction. NTG decreased the paw withdrawal threshold in both wild-type and P2X7 knockout mice. Nevertheless, subacute BBG treatment (50 mg/kg/day i.p.) completely prevented the effect of NTG in wild-type, but not in knockout mice. Whereas P2X7 deficiency differentially affected the expression of c-fos, the average number of fos-immuno-reactive neurons in trigeminal nucleus caudalis, but not in upper cervical spinal cord was lower in BBG-treated wild-type mice after NTG treatment.
Our results show that P2X7 receptors might participate in the pathogenesis of migraine, although upregulation of other P2X receptors probably compensate for the loss of its action in knockout mice. The data also suggest the therapeutic potential of P2X7 antagonists for the treatment of migraine.

The results are published (Gölöncsér F. and **Sperlágh B.** J Headache Pain. 2014 May 1;15:24.).

9.) Central P2Y12 receptor blockade alleviates inflammatory and neuropathic pain and cytokine production in rodents.

Among various subtypes of P2X and P2Y receptors, in addition to P2X7 receptor a promising new possibility could be the metabotropic P2Y12 receptor as it is the molecular target of widely used antithrombotic drugs. In this study the role of P2Y12 receptors (P2Y12R) was explored in rodent models of inflammatory and neuropathic pain and in acute thermal nociception. In correlation with their activity to block the recombinant human P2Y12R, the majority of P2Y12R antagonists alleviated mechanical hyperalgesia dose-dependently, following intraplantar CFA injection, and after partial ligation of the sciatic nerve in rats. They also caused an increase in thermal nociceptive threshold in the hot plate test. Among the six P2Y12R antagonists evaluated in the pain studies, the selective P2Y12 receptor antagonist PSB-0739 was most potent upon intrathecal application. P2Y12R mRNA and IL-1β protein were time-dependently overexpressed in the rat hind paw and lumbar spinal cord following intraplantar CFA injection. This was accompanied by the upregulation of TNF-α, IL-6 and IL-10 in the hind paw. PSB-0739 (0.3mg/kg i.t.) attenuated CFA-induced expression of cytokines in the hind paw and of IL-1β in the spinal cord. Subdiaphragmatic vagotomy and the α7 nicotinic acetylcholine receptor antagonist MLA occluded the effect of PSB-0739 (i.t.) on pain behavior and peripheral cytokine induction. Denervation of sympathetic nerves by 6-OHDA pretreatment did not affect the action of PSB-0739. PSB-0739, in an analgesic dose, did not influence motor coordination and platelet aggregation. Genetic deletion of the P2Y12R in mice reproduced the effect of P2Y12R antagonists on mechanical hyperalgesia in inflammatory and neuropathic pain models, on acute thermal nociception and on the induction of spinal IL-1β. Here we report the robust involvement of the P2Y12R in inflammatory pain. The anti-hyperalgesic effect of P2Y12R antagonism could be mediated by the inhibition of both central and peripheral cytokine production and involves α7-receptor mediated efferent pathways.
The results are published (Horváth G, Gölöncsér F, Csölle C, Király K, Andó RD, Baranyi M, Koványi B, Máté Z, Hoffmann K, Algaier I, Baqi Y, Müller CE, Von Kügelgen I, **Sperlágh B.** Neurobiol Dis. 2014 70:162-78).

10.) ATP-Evoked Intracellular Ca^{2+} Signaling of Different Supporting Cells in the Hearing Mouse Hemicochlea

Hearing and its protection is regulated by ATP-evoked Ca^{2+} signaling in the supporting cells of the organ of Corti, however, the unique anatomy of the cochlea hampers observing these mechanisms. For the first time, we have performed functional ratiometric Ca^{2+} imaging (fura-2) in three different supporting cell types in the hemicochlea preparation of hearing mice to measure purinergic receptor-mediated Ca^{2+} signaling in pillar, Deiters' and Hensen's cells. Their resting $[Ca^{2+}]_i$ was determined and compared in the same type of preparation. ATP evoked reversible, repeatable and dose-dependent Ca^{2+} transients in all three cell types, showing desensitization. Inhibiting the

Ca^{2+} signaling of the ionotropic P2X (omission of extracellular Ca^{2+}) and metabotropic P2Y purinergic receptors (depletion of intracellular Ca^{2+} stores) revealed the involvement of both receptor types. Detection of P2X2,3,4,6,7 and P2Y1,2,6,12,14 receptor mRNAs by RT-PCR supported this finding and antagonism by PPADS suggested different functional purinergic receptor population in pillar versus Deiters' and Hensen's cells. The sum of the extra- and intracellular Ca^{2+}-dependent components of the response was about equal with the control ATP response (linear additivity) in pillar cells, and showed supralinearity in Deiters' and Hensen's cells. Calcium-induced calcium release might explain this synergistic interaction. The more pronounced Ca^{2+} leak from the endoplasmic reticulum in Deiters' and Hensen's cells, unmasked by cyclopiazonic acid, may also suggests the higher activity of the internal stores in Ca^{2+} signaling in these cells. Differences in Ca^{2+} homeostasis and ATP-induced Ca^{2+} signaling might reflect the distinct roles these cells play in cochlear function and pathophysiology.

The study was the part of a collaboration with the lab of T. Zelles and the results are published (Horváth T, Polony G, Fekete Á, Aller M, Halmos G, Lendvai B, Heinrich A, **Sperlágh** B, Vizi ES, Zelles T., Neurochem Res. 2016 Jan 22. [Epub ahead of print]

In addition to original research articles, we have also published review articles on the P2X7 receptors as a potential therapeutic target in CNS (**Sperlágh B**, Illes P., P2X7 receptor: an emerging target in central nervous system diseases Trends Pharmacol Sci. 2014 35(10):537-47.); the role of purinergic receptor in neuroinflammation (Beamer E, Gölöncsér F, Horváth G, Bekő K, Otrokocsi L, Koványi B, **Sperlágh B**, Neuropharmacology. 2015 Sep 16. pii: S0028-3908(15)30111-8. doi: 10.1016/j.neuropharm.2015.09.019. [Epub ahead of print]), and an Editorial of the Special Issue of Neuropharmacology entitled "Purines in neurodegeneration and neuroregeneration" (Illes P, Verkhratsky A, Burnstock G, **Sperlagh B**. Neuropharmacology. 2016 Jan 15. pii: S0028-3908(16)00002-2. doi: 10.1016/j.neuropharm.2016.01.020. [Epub ahead of print]).

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