Nucleotide receptors in trigeminal satellite glial cells as new targets for the pharmacological control of migraine pain: in vitro and in vivo studies.

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The main aim of the present research project is to understand what occurs to pain transduction mechanisms of trigeminal sensory neurons in migraine, why they become hyperactive, and how we may stop them. We focused our studies on the role of the purinergic system in the neuron-to-glial cells communication within the trigeminal ganglion (TG), and its cross-talk with known pro-algogenic systems (such as bradykinin, BK, calcitonin gene-related peptide, CGRP, and prostaglandins). The final goal is the identification of new cellular and molecular players in the onset and maintenance of trigeminal-associated pain, for the development of new effective therapeutic strategies for migraine.
To this purpose, we set up an in vitro model of primary mixed neuron-glia or purified satellite glial cells (SGCs) cultures from TG from C57BL6 mice. G protein-coupled P2Y receptor function was evaluated by single cell calcium imaging, and the extracellular concentrations of CGRP were measured by an ELISA assay. Western blot experiments on $P2Y_1$ and $P2Y_2$ receptors subtypes were also performed. Concerning the role of metabotropic purinergic receptors, our data show that exposure of mixed-neuron glia cultures to BK induces neuronal release of CGRP, which in turn significantly potentiates the ADP-responsive $P2Y_1$ and the UTP-sensitive $P2Y_2$ receptor subtypes on surrounding SGCs. The increased activity of P2 receptors is not only due to increased receptor protein expression, but also, and especially for the $P2Y_1$ subtype, to modulation of the receptor localization to membrane lipid rafts. Interestingly, the anti-migraine drug sumatriptan fully inhibits both CGRP release and glial P2Y receptor potentiation. Moreover, exposure to BK leads to increased production of PGE_2, an effect completely abolished by the COX-1 inhibitor acetylsalicylic acid (ASA). The latter also blocks neuronal CGRP release. Taken together, these results suggest that a complex cross-talk between neuronal and glial cells takes place in the TG, involving pain mediators and extracellular nucleotides. Modulation of this network by known anti-migraine drugs, such as triptans and COX inhibitors, suggests that it might play an important role in the development of migraine pain.
The second part of the current project is aimed at evaluating the pro- or anti-algogenic role of P2Y receptors through their selective inhibition in vivo. To this purpose, we set up a sub-chronic inflammatory model characterized by inflammatory pain and trigeminal hypersensitivity, by injecting complete Freund adjuvant (CFA) into the temporomandibular joint (TMJ) of rats. CFA-injected animals showed ipsilateral mechanical allodynia and TMJ edema. Glial cell activation was then evaluated in the spinal-trigeminal system by immunohistochemistry, confirming that our model leads to trigeminal inflammation and sensitization. Western blot experiments on GFAP protein expression, a typical marker of glial cells, confirmed the presence of glial activation within the trigeminal ganglion starting from 24 hours up to 11 days after CFA injection. Moreover, $P2Y_1$ receptor subtype protein expression is upregulated in the ipsilateral trigeminal ganglia of CFA-injected rats starting from 1 week after injection, while for $P2Y_2$ receptor subtype a significant increase is observed starting from 72 h post-injection. Interestingly, the non-selective P2Y antagonist PPADS shows a strong analgesic effect on CFA-induced trigeminal inflammation, which is comparable to ASA-mediated analgesia.
These results suggest a possible the pro-algogenic role for P2Y receptors in the development of trigeminal sensitization and migraine pain, opening future perspectives of identifying innovative and more selective pharmacological approaches for the sake of those migraineurs who are insensitive to currently available drugs.