Módszerek A HD kultúrák sejtjeiből előállított mintákban RT-PCR, Western blotok, NR1 siRNS, NMDA receptorok agonistáinak és anatgonistáinak alkalmazása a tenyésztés során, intracelluláris Ca^{2+}-koncentráció mérések, ionáramok detektálása egész-sejt patch clamp technikával.
Eredmények A HD kultúrák sejtjei glicin-receptorként működő NR3 típusú alegységeket is tartalmazó, így csekély glutamát és NMDA-érzékenységű NMDA receptorokat expresszálnak. A szokásos NMDA-agonisták a porcképződést nem befolyásolták, az NR2B alegységhez kötődő gátlószer ifenprodil, és a glicin fokozták a porcképződést. Az NMDA-receptorok agonistái gyenge Ca^{2+}-áramot és kifejezett K^+-áramot indukáltak a sejteken, a glicin hatásait jelenleg vizsgáljuk. Az NR1 alegység siRNS transzfekcióval elért tranziens géncsendesítése meggátolta a porcképződést, erősen csökkentette a sejtproliferációt és megszüntette a HD-kultúrák sejtjeinek nagy frekvenciájú Ca^{2+}-oszcillációit.
Következtetés Eredményeink arra utalnak, hogy az NMDA receptorműködés kismértékű, átmeneti farmakológia gátlása a porcképződést serkenti, míg a tranziens géncsendesítéssel elért kifejezett működéskiesés blokkolja a kondrogenezist.
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E08-04 IMAGING ATP-INDUCED CALCIUM TRANSIENTS IN THE HEMICOCHLEA OF HEARING MICE

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Aims The process of converting the mechanical sound waves into electrical signals is developed by the co-work of functionally different subunits of the inner ear. The organ of Corti is the effective transducer, where the functions of the different cell types are modulated by ATP. Beyond its role in sensing, ATP-evoked processes are thought to be the part of a defending system in the inner ear against noise and other damages. ATP generates $[Ca^{2+}]i$ changes in these cells. Our aim was to characterize the ATP-evoked Ca^{2+} response in different supporting cells of the organ of Corti of hearing mice.
Methods We have developed a method of fluorescence Ca^{2+} imaging in acute mouse hemicochlea preparation. The advantage of the preparation is that it is prepared from hearing mice (>P15) and keeps the original tissue structure. After bulk loading of the preparation with the calcium reporter dye Fura-2/AM, the fluorescence changes were followed with a cooled CCD camera based ratio imaging system. Drugs were applied to the perfusion buffer.
Results Average basal $[Ca^{2+}]i$ was 59, 47 and 87 nM in pillar-, Deiters- and Hensen cells, respectively. Short perfusion of ATP induced a dose-dependent increase in $[Ca^{2+}]i$. The ATP response was repeatable with no desensitization using at least 10 min stimulation interval. Applying ATP (50μM) two times with 20 min intervals was used in further experiments to elucidate the sources of Ca^{2+}. The non-selective P2 blocker PPADS caused a significant reduction in the Ca^{2+} signals in all three types of cells. Both withdrawal of Ca^{2+} and application of the endoplasmic reticulum Ca^{2+}-ATPase inhibitor CPA between the two ATP stimulus resulted in a significant inhibition of the second response. RT-PCR analysis of P2X and P2Y receptor mRNAs in dissected organ of Corti supported the involvement of both ionotropic (extracellular Ca^{2+} dependent) and metabotropic (intracellular Ca^{2+} store-dependent) purinergic receptors.
Conclusion ATP evokes Ca^{2+} response in supporting cells of the organ of Corti through stimulation of both ionotropic (P2X) and metabotropic (P2Y) purinergic receptors. These signal transduction processes are supposed to play a role in the pathogenetic pathways of certain sensorineural hearing losses ATP is involved in.

E28-03 ANALYSIS OF LIGAND-DEPENDENT CHANGES IN ESTROGEN RECEPTOR- AND THYROID HORMONE RECEPTOR MRNA AND PROTEIN EXPRESSION IN THE DEVELOPING RAT CEREBELLUM

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Aims Estrogen (E2) and thyroid hormones (THs) are important regulators of CNS development and function. These hormone-regulated events involve the binding of hormone ligands to their receptors that function as transcription factors in the orchestration of developmental processes. Recent reports implicate a complex mechanism through which E2 and THs interact to regulate the expression levels of E2 receptors (ERs) and TH receptors (TRs) to precisely mediate developmental signals. We examined the effects of the presence or absence of E2 and THs on the expression of their receptor mRNAs and proteins.
Methods Cerebellar granule cell cultures were treated with either E2, T3, T4 or a combination of these hormones, and resulting receptor mRNA and protein expression levels were determined. We also determined the effects that glial cells might have on the regulation of ER-TR expression levels.
Results 1. ER and TR expression levels depend on the individual or combined presence of E2 and THs; 2. Glial cells mediate the hormonal regulation of neuronal ER-TR expression; 3. Loss of tissue integrity results in characteristic changes in ER-TR expression levels.
Conclusion Observations suggest that a fine equilibrium of E2 and THs is required for the precise orchestration of cerebellar development. Comparison of results from in vitro and in situ samples revealed a shift in receptor expression levels after loss of tissue integrity, likely indicating possible adjusting/regenerative mechanisms after cerebellar tissue injury.
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