SIMPOSIO / SYMPOSIA 2

Coordinador Dr. Claudio Coddou (UCN), "Purinergic Signaling on Pathophysiology: A latinoamerican view". Expositores: C. Coddou (UCN), F. Vazquez-Cuevas (UNAM), AH Ulrich (U. Sao Paulo), S. Buvinic (UCh)

REGULACIÓN DE RECEPTORES PURINÉRGICOS Y SU IMPLICANCIA EN LA SEÑALIZACIÓN DEL DOLOR. REGULATION OF THE PURINERGIC RECEPTORS AND ITS POTENTIAL ROLE ON PAIN SIGNALING.

The purinergic P2X2 receptor (P2X2R) is an ATP–gated ion channel widely expressed in the nervous system, and it is regulated by several allosteric modulators, including zinc, copper, calcium and by phosphorylation. P2X2R channel exhibits use-dependent desensitization (UDD), that consist in a progressive increase in receptor desensitization during repetitive agonist application. Interestingly, protein kinase Cyclin-dependent kinase 5 (Cdk5) regulated this phenomenon. We identified a phosphorylation site in the full-size variant P2X2aR (372TPKH375), which is absent in the splice variant P2X2bR. By co-immunofluorescence and co-immunoprecipitation, we observed an interaction between P2X2aR and Cdk5/p35 complex. Moreover, threonine phosphorylation was significantly increased in HEK293 cells co-expressing P2X2aR and p35. Electrophysiological recordings indicated a delay in development of UDD of P2X2aR/p35 but not of P2X2bR/p35 in HEK293 or Xenopus oocytes. A similar effect was found in P2X2a/3R heteromeric currents. The P2X2aR-T372A mutant was resistant to UDD. In endogenous cells, we observed P2X2R/Cdk5/p35 interactions by co-localization using immunofluorescence in primary culture of nociceptive neurons, and by co-immunoprecipitation in mouse trigeminal ganglia. Moreover, endogenous P2X2/3R mediated increases of $[Ca^{2+}]i$ in trigeminal neurons were Cdk5-dependent, since inhibition with roscovitine accelerated the desensitization kinetics of these responses. Behavioral tests in mice showed that α,β-meATP, an agonist of P2X2/3R and P2X3R, evokes facial pain responses as grooming and head flinching. Remarkably, these behaviors were significant suppressed in mice deficient of Cdk5 in nociceptive neurons (Cdk5 cKO) mice as compared with control littermates. These results indicate that the P2X2aR is a novel target for Cdk5-mediated phosphorylation, which might play important roles including in pain signaling.

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EL EJE ATP EXTRACELULAR/RECEPTOR P2X7 ES UN REGULADOR DE LA SOBREVIVENCIA CELULAR Y DE LA MIGRACIÓN EN CÉLULAS DERIVADAS DE CARCINOMA OVÁRICO. EXTRACELLULAR ATP/P2X7 RECEPTOR, A REGULATORY AXIS OF CELL SURVIVAL AND MIGRATION IN OVARIAN CARCINOMA-DERIVED CELLS.

It is well established that extracellular ATP (exATP) and its metabolites are important components of the tumor microenvironment (TME). These signaling molecules not only mediate cancer and stromal cells interactions but also regulate basic cellular processes by autocrine-paracrine mechanisms. Here, the actions mediated by exATP were studied in human ovarian carcinoma cell lines. SKOV-3 cells incubation with apyrase (APY, 10 U/mL), an ectonucleotidase, for 6 or 12 h reduced ERK and AKT phosphorylation levels, cell viability and migration and stress fibers. Furthermore, APY incubation increased transepithelial resistance. To determinate whether these observations rely on the activity of a purinergic receptor, p2ry2, p2ry4, p2ry6 and p2rx7 transcripts relative abundance was analyzed in SKOV-3 cells and then normalized against the level of expression in an untransformed cell line from ovarian surface epithelium (HOSE6-3). Noteworthy, it was found that P2RX7 is overexpressed three-fold times, consequently P2RX7 expression and function was studied in a non-metastatic (CAOV-3) and a metastatic (SKOV-3) cell line. P2RX7 transcript was amplified by reverse transcription and PCR. Moreover, the receptor was detected from immunoprecipitation of biotinylated plasma membrane proteins and immunoblot. Addition of BzATP (50 μM), an P2RX7 agonist, mediated ERK, p38, Jnk and AKT phosphorylation and also a dose-dependent increment in the intracellular Ca2+ concentration. Furthermore, P2RX7 inhibition decreased cell viability, migration and invasion, such as in APY experiments. Altogether, our results suggest that P2RX7 receptor signaling contributes with ovarian carcinoma derived cells viability and metastatic potential.

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P2Y2 RECEPTOR SIGNALING IN GABAERGIC DIFFERENTIATION AND HUNTINGTON DISEASE. P2Y2 RECEPTOR SIGNALING IN GABAERGIC DIFFERENTIATION AND HUNTINGTON DISEASE.

Huntington's disease (HD) is an autosomal dominant inherited disease caused by at least 35 repetitions of the N-terminal CAG trinucleotide (glutamine) in the Huntington's gene (Htt). We used as in vitro disease models induced pluripotent iPS cells obtained from HD patients and Htt-gene edited embryonic stem cells, which were induced to neuronal differentiation into GABAergic neurons. Calcium oscillations were tracked by real-time fluorescence and luminescence microscopy to analyse the correlative relationship between calcium transient activity and rhythmic proneuronal transcription factor expression in embryonic stem cells after stable transfection with ASCL-1 or neurogenin-2 promoter-protein fused to the luciferase reporter gene. We show that pharmacological activity manipulation of P2Y2 and P2X7 purinergic receptors induced a two-step process of neuronal differentiation. In vitro models of Huntington's disease (HD) showed increased basal intracellular calcium concentration together with augmented apoptosis rates and lacked spike-like calcium oscillations and P2Y2 receptor activity, agreeing with deficiency of ASCL-1 expression activation and GABAergic differentiation. Our results suggest that HD may have developmental origins based on inefficient GABAergic differentiation, shedding new light on the mechanisms underlying neurogenesis of inhibitory neurons.

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NUCLEÓTIDOS EXTRACELULARES COMO REGULADORES DE LA PLASTICIDAD MUSCULAR Y DE LA CONVERSACIÓN CRUZADA MÚSCULO-HUESO. EXTRACELLULAR NUCLEOTIDES IN MUSCLE PLASTICITY AND MUSCLE-BONE CROSSTALK.

The musculoskeletal system is a highly coordinated machinery, where muscles and bones are remodeled together under physiological or pathological conditions. I have focused my research on the role of extracellular nucleotides in skeletal muscle plasticity (demand-dependent muscle remodeling). My research group has shown that after membrane depolarization of skeletal fibers, ATP is released through a pannexin 1 channel, and then P2Y/P2X receptors are activated to increase intracellular Ca2+ and modify gene expression. Thus, extracellular ATP (eATP) is a relevant mediator in Excitation-Transcription Coupling in skeletal muscle. We have shown that all the molecular interactors of this pathway, such as CaV1.1 (voltage sensor), Pannexin1 (ATP channel) and P2Y receptors, bind in a multiprotein complex in the T tubule of skeletal fibers. In addition to the beneficial role of eATP in muscle plasticity and metabolism, we have observed that resting eATP is greatly increased in conditions such as muscular dystrophy or aging muscles. It appears that unregulated levels of eATP are detrimental to skeletal muscle. We are currently studying the role of eATP in muscle atrophy and bone loss. We have shown that the eATP pathway is overexpressed in muscles atrophied by disuse or by injection of botulinum toxin. The latter leads to overexpression of interleukin-1 and interleukin-6 from skeletal fibers, the chronic increase of which mediates both muscle atrophy and bone loss. Thus, we propose that dysregulation of eATP signaling could be a mediator of musculoskeletal deterioration after muscle disuse or paralysis.

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