Volume 121 · Supplement 2 · August 2017

BCPT

Basic & Clinical Pharmacology & Toxicology

37th SEF National Meeting with guest society: the British Pharmacology Society
18–21 June 2017
Barcelona, Spain

WILEY
Blackwell

Gal1R ligands. The results also indicate that MOR-Gal1R heteromers should be viewed as targets for the treatment of opioid use disorders.

Keywords: dopamine; galanin receptor; MAPK; opioid receptor; receptor heteromer; ventral tegmental area

P-75

CHARACTERIZATION OF THE ACTIONS OF THE NOVEL $P2Y_2$ RECEPTOR ANTAGONIST, AR-C118925XX

Muoboghare M.O., Drummond R., Kennedy C.

Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, Glasgow, United Kingdom.

Introduction: The endogenous nucleotides, adenosine 5′-triphosphate (ATP), uridine 5′-triphosphate (UTP), and uridine 5′-diphosphate (UDP) act via P2X and P2Y receptors. Due to the limited selectivity of most antagonists, the functions of many individual P2 subtypes are poorly characterised. A putative $P2Y_2$ antagonist, AR-C118925XX, has recently become available, so the aims were to quantify the action of AR-C118925XX at recombinant $P2Y_2$ receptors and then to determine the contribution of $P2Y_2$ receptors to nucleotide-evoked vasoconstriction.

Methods: Recombinant human $P2Y_1$, $P2Y_2$, $P2Y_4$ and $P2Y_{11}$ receptors, stably expressed in 1321N1 cells, were grown on glass coverslips, which were placed in a fluorimeter, and intracellular Ca^{2+} measured using the Ca^{2+}-sensitive dye, Cal-520AM. Concentration-response curves (CRC) were constructed by superfusing cells with agonists ($P2Y_1$-ADP, $P2Y_2$-UTP, $P2Y_4$ and $P2Y_{11}$-ATP), in the absence and presence of AR-C118925XX. The Hill equation was fitted to the data and a Schild plot generated using the EC_{50} values. 5 mm rings of rat intrapulmonary artery (rIPA) were mounted under isometric conditions *in vitro* at 37°C. Contractions were elicited by addition of P2Y agonists to the bath.

Results: In 1321N1 cells expressing $hP2Y_2$ receptors, UTP (10 nM-3 μM) evoked a concentration-dependent rise in intracellular Ca^{2+} (EC_{50}=54 nM, 95% cl. 43–67 nM, $n = 5$). Increasing concentrations of AR-C118925XX (10 nM-1 μM), produced a progressive rightward shift in the UTP CRC, with no decrease in maximum response ($n = 6$ each). These inhibitory effects were fully reversible. Schild analysis produced a pA_2=8.30 and slope=0.985. In contrast, AR-C118925XX (1 μM) had no effect at human $P2Y_1$, $P2Y_4$ and $P2Y_{11}$ receptors ($n = 5$ each). In rIPA UTP, ATP and UDP (300 μM) evoked reproducible contractions that were unaffected by AR-C118925XX (1 μM) ($n = 4$ each).

Conclusion: AR-C118925XX is a potent, competitive and reversible $P2Y_2$ antagonist, making the identification of the physiological functions of $P2Y_2$ receptors possible. $P2Y_2$ receptors do not appear to play a role in nucleotide-evoked contractions of rIPA.

Keywords: P2Y receptors AR-C117825XX pulmonary artery

P-76

SIGNALING OF THE FRACTALKINE RECEPTOR CX_3CR1 AND ITS NATURAL GENETIC VARIANTS: IMPACT OF RECEPTOR NON-SYNONYMOUS SINGLE NUCLEOTIDE POLYMORPHISMS

García-Silva A.[1], de la Fuente R.A.[1], Izquierdo A.G.[1], Loza M.I.[1], Costas J.[2], Castro M.[1]

[1]Universidade de Santiago de Compostela, Center for Research in Molecular Medicine and Chronic Diseases (CIMUS), 15782 Santiago de Compostela, Spain; [2]Instituto de Investigación Sanitaria (IDIS) de Santiago de Compostela, Complexo Hospitalario Universitario de Santiago de Compostela (CHUS), Servizo Galego de Saúde (SERGAS), Santiago de Compostela, Spain

The chemokine receptor CX_3CR1 is a $G_{i/o}$ G protein-coupled receptor (GPCR) expressed in monocytes, NK cells, T lymphocytes, astrocytes and microglia, among other cells, and it plays an important role in inflammation and immunity as well as in neuron-microglia communication in the Central Nervous System [1]. Its known ligand is fractalkine (CX_3CL1), the sole member of the CX_3C chemokine subfamily. Genomic studies have identified non-synonymous single nucleotide polymorphisms (nsSNPs) in the *CX_3CR1* gene. Specifically, the common receptor genetic variant CX_3CR1-V249I/T280M has been associated with faster progression to disease in HIV-infected patients, cardiovascular atheroprotection, increased risk of age-related macular degeneration, and obesity [2]. We aimed to investigate the possible functional impact of the currently identified nsSNPs of CX_3CR1 on the pharmacology of this receptor.

Receptor interaction with G protein-coupled receptor kinase 2 (GRK2) and beta-arrestins were investigated in transfected HEK293 cells by bioluminescence resonance energy transfer (BRET)-based assays. Our results indicate that the CX_3CR1-V249I/T280M receptor variant interacts with more efficacy than the wild type receptor with beta-arrestins 1 and 2 (Emax 146% and 204% of wild type, respectively) and GRK2 (Emax 310% of wild type) in response to fractalkine. The functional impact of this observation on the dynamics and compartmentalization of the receptor signaling is being further investigated. Being GRKs and beta-arrestins crucial regulators of G protein-dependent and -independent signaling of GPCRs [3], our findings expand our current knowledge on the signaling pathways modulated by CX_3CR1 and their possible implications in physiological and pathological condition.

Acknowledgements: This work is funded by the Spanish Ministry of Economy and Competitiveness (MINECO) (SAF2014-57138-C2-1-R).

References:

[1] Smit M.J. Lira S.A. and Leurs R. (eds) (2011) Chemokine Receptors as Drug Targets, WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim; Paolicelli R.C. and Gross C.T. (2011) Neuron Glia Biol 7: 77–83; Bachelerie F. et al. (2013) Pharmacol Rev 66: 1–-79.

[2] Faure S. et al. (2000) Science 287: 2274–2277; McDermott D.H. et al. (2003) J Clin Invest 111: 1241–1250; Combadière C. et al. (2007) J Clin Invest 117: 2920–2928; Sirois-Gagnon D. et al. (2011) Obesity (Silver Spring) 19: 222–7; Wu J. et al. (2014) Dis Markers 913678.

[3] Schmid C.L. and Bohn L.M. (2009) Pharmacol Ther 121: 285–93; Shukla A.K. et al. (2011) Trends Biochem Sci 36: 457–469.

P-77

GLIBENCLAMIDE INHIBITS ATP-SENSITIVE K CURRENTS IN THE CANTU SYNDROME

Nieto-Marín P., Utrilla R.G., Alfayate S., Tinaquero D., Pérez-Hernández M., Matamoros M., Tamargo J., van der Heyden M., Delpón E., Caballero R.

Dpto. de Farmacología. Fac. de Medicina. Universidad Complutense de Madrid. CIBERCV. Madrid. España; Dpt. of Molecular Cell Biology. Utrecht University. Utrecht. Holanda

(*KCNJ11*) and β-subunits SUR2A (*ABCC9*). Cantu syndrome (CS) is a rare genetic disorder, which leads to multiple alterations including hypotension and cardiac abnormalities. CS is caused by mutations in *KCNJ8* and *ABCC9* that reduce channel sensitivity to ATP-blockade, increasing K_{ATP} channel activity. Glibenclamide, a K_{ATP} blocker, has been proposed as a potential treatment for CS. However, it is unknown whether CS-associated mutations are sensitive to K_{ATP} modulating drugs. We analyzed the effects of glibenclamide on pinacidil-activated K_{ATP} currents (I_{KATP}) generated by channels with Kir6.1 (V65M and G343D) and SUR2A (H60Y, S1020P, and S1054Y) CS-associated mutations. I_{KATP} were recorded in Chinese Hamster Ovary (CHO) cells transiently transfected with the cDNA encoding WT or mutant Kir6.x and SUR2A subunits by whole-cell patch-clamping. Pinacidil increased I_{KATP} generated by Kir6.1WT+SUR2AWT and Kir6.2WT+-SUR2AWT channels in a concentration-dependent manner, the effective concentration 50 (EC_{50}) being 1.9 ± 0.03 and 4.9 ± 1.1 μM, respectively. The EC_{50} values for pinacidil were significantly increased

in the channels composed by V65M (5.5 ± 0.03 μM) and G343D (8.4 ± 0.04 μM) Kir6.1 + SUR2AWT subunits. Pinacidil-induced increase of Kir6.2WT+SUR2A H60Y and Kir6.2WT+SUR2A S1020P was similar to that of Kir6.2WT+SUR2AWT channels. Unexpectedly, pinacidil failed to increase I_{KATP} generated by Kir6.2WT+SUR2A S1054Y channels. Glibenclamide inhibited currents generated by Kir6.1WT+SUR2AWT and Kir6.2WT+SUR2AWT in a similar extent, yielding inhibitory concentration 50 (IC_{50}) values (0.3 ± 0.1 and 0.22 ± 0.06 μM, respectively) within the range of therapeutic concentrations of glibenclamide. The presence of the V65M mutation in Kir6.1 significantly reduced the glibenclamide potency (IC_{50}=1.6 ± 0.04 μM), while the rest of the mutations in Kir6.1 (G343D) or SUR2A (H60Y and S1020P) did not affect the glibenclamide-induced block. These results demonstrate that most of the CS-associated mutations reduced channel response to pinacidil, whereas only the V65M mutation in Kir6.1 affected channel sensitivity to glibenclamide.

Keywords: Kir6.x, SUR2A, glibenclamide, Cantu Syndrome, cardiac

P-78

THE PARKINSON'S DISEASE-ASSOCIATED GPR37 RECEPTOR IS AN ADENOSINE A_{2A} RECEPTOR REPRESSOR IN MICE

Morató X.[1,2], *Luján R.*[3], *López-Cano M.*[1,2], *Gandia J.*[1,2], *Stagljar I.*[4], *Watanabe M.*[5], *Cunha R.*[6], *Fernande-Dueñas V.*[1,2], *Ciruela F.*[1,2]

[1]Unitat de Farmacologia, Departament Patologia i Terapéutica Experimental, Facultat de Medicina, IDIBELL, Universitat de Barcelona, L'Hospitalet de Llobregat, Spain; [2]Institut de Neurociències, Universitat de Barcelona, Spain; [3]IDINE, Departamento de Ciencias Médicas, Facultad de Medicina, Universidad Castilla-La Mancha, Albacete, Spain; [4]Donnelly Centre, University of Toronto, M5S 3E1, Canada; [5]Department of Anatomy, Hokkaido University School of Medicine, Sapporo 060-0818, Japan; [6]CNC-Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

GPR37, also known as parkin-associated endothelin-like receptor (Pael-R), is an orphan G protein-coupled receptor that has been related with Parkinson's disease (PD) neuropathology. Interestingly, the genetic blockade of GPR37 enhances cell surface expression of striatal dopamine transporter (DAT), resulting in reduced dopamine content in the striatum. In addition, it has been shown that deletion of GPR37 triggers anxiolytic-like effects and sensitizes mice to adenosine A_{2A} receptors (A_{2A}R)-mediated signaling. Here we report that GPR37 and A_{2A}R physically and functionally interact both in living cells and in native tissue. Thus, by using biochemical techniques (i.e. co-immunoprecipitation and proximity ligation assay) we demonstrated a physical interaction between these two receptors in the striatum. Also, by means of post-*embedding immunogold-electron microscopy* (EM) *techniques* detection it was demonstrated a close proximity at the postsynaptic level of striatal synapses. On the other hand, GPR37 deletion promoted striatal A_{2A}R cell surface expression, which correlated well with an increased A_{2A}R agonist-mediate cAMP accumulation both in primary striatal neurons and synaptosomes from striatum. Furthermore, GPR37-KO mice showed enhanced catalepsy induced by an A_{2A}R agonist and an increased response to A_{2A}R antagonist-mediated locomotor activity. Overall, these results demonstrate for the first time an important role for GPR37 controlling A_{2A}R expression and function in the striatum, which may be of interest for the treatment of PD.

Acknowledgements: This work was supported by MINECO/ISCIII (SAF2014-55700-P and PIE14/00034), the Catalan government (2014 SGR 1054), ICREA (ICREA Academia-2010), Fundació la Marató de TV3 (Grant 20152031) and IWT (SBO-140028). Also supported by the PhD fellowship FPI (BES-2012-052722).

P-79

CELLULAR BIOLOGY OF HUMAN CARDIAC NAV1.5-KIR2.1 CHANNEL COMPLEXES

Matamoros M., Pérez-Hernández M., Utrilla R.G., Tinaquero D., Nieto-Marín P., Alfayate S., Tamargo J., Delpón E., Caballero R.

Department of Pharmacology, School of Medicine, Universidad Complutense de Madrid, CIBER*CV*, Ramón y Cajal square, 28040-MADRID

Cardiac Kir2.1 and Nav1.5 channels generate the inward rectifier K^+ (I_{K1}) and the Na^+ (I_{Na}) currents, respectively. Nav1.5 and Kir2.1 channels exhibit positive reciprocal modulation and the augmented presence of Nav1.5 and Kir2.1 channels at the membrane could be the result of an increase in the protein synthesis and forward trafficking and/or to a decrease in channel internalization. Here we compared the differential characteristics of these processes when Nav1.5 and Kir2.1 channels are expressed together or separately. The proximity ligation assays demonstrated that Nav1.5 and Kir2.1 proteins are in close proximity to each other (<40 nm apart) in the membrane of ventricular myocytes, suggesting that they form complexes. Patch-clamp experiments in heterologous transfection systems demonstrated that the inhibition of endoplasmic reticulum (ER) to Golgi transport with Brefeldin A did not abolish the positive reciprocal modulation between Kir2.1 and Nav1.5 channels and that the internalization time constants of Kir2.1 (5.1 ± 0.5 h) and Nav1.5 (4.9 ± 0.4 h) channels were not modify when they were coexpressed with Nav1.5 and Kir2.1 channels, respectively. Either when they were coexpressed or not, the inhibition of dynamin-dependent endocytosis similarly reduced the internalization of Nav1.5 and Kir2.1 channels. Inhibition of the dynein/dynactin motor suggested that it is involved in the backward and forward traffic of Kir2.1 and Nav1.5, respectively. Conversely, Nav1.5-Kir2.1 complexes were forwarded by the dynein/dynactin motor. Nav1.5 but not Kir2.1 were ubiquitinated by Nedd4-2 ubiquitin-protein ligase and degraded by the proteasome. Nav1.5-Kir2.1 complexes were also degraded following this route as demonstrated by the overexpression of Nedd4-2 and the inhibition of the proteasome with MG132. We concluded that there is a pool of Kir2.1 and Nav1.5 channels that form complexes whose biology is similar to that of the Nav1.5 channels.

Keywords: Nav1.5 channel, Kir2.1 channel, cardiac, intracellular traffic

P-80

A METHOD FOR QUANTIFYING BIASED SIGNALLING OF INVERSE AGONISTS

David Hall.[1], *Jesús Giraldo.*[2,3]

[1]Fibrosis and Lung Injury DPU, GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts, SG1 2NY, UK; [2]Institut de Neurociències and Unitat de Bioestadística, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain; [3]Network Biomedical Research Center on Mental Health (CIBERSAM)

Biased agonism, the ability of an agonist to selectively trigger specific signalling pathways when they act at a given receptor, is an increasingly frequently described pharmacological phenomenon. Several methods to quantify the bias of agonists have been proposed, for example the $\Delta\Delta\log(\tau/Ka)$ parameter of Kenakin and co-workers (Kenakin et al, 2011, ACS Chem. Neurosci. 3, 193–203). However, all of the methods used for the analysis of biased signalling focus on the analysis of agonists as they are based on the use of the Operational model. However, the classical Operational model does not account for constitutive receptor signalling and cannot be used to analyse the properties of inverse agonists. Here we report a method for the analysis of ligand bias based on the Operational model of Slack & Hall (Slack & Hall, 2012, Br. J. Pharmacol. 166, 1774–1792) which accounts for receptor constitutive activity and hence can be used to analyse biased signalling in response to inverse agonists as well as agonists. This method also has the advantage of providing a measure of absolute rather relative bias since it is derived from estimates of ligand intrinsic

© 2017 The Authors
Basic & Clinical Pharmacology & Toxicology © 2017 Nordic Association for the Publication of BCPT (former Nordic Pharmacological Society).