



PRÊMIO JOSÉ RIBEIRO DO VALLE 2008

O prêmio José Ribeiro do Valle, oferecido a cada ano pela SBFTE, visa identificar a cada ano os melhores trabalhos científicos desenvolvidos por jovens investigadores na área da Farmacologia. Entre os trabalhos inscritos para esta décima-primeira edição do prêmio, foram selecionados cinco finalistas, que fizeram apresentações de seus respectivos trabalhos perante comissão julgadora, em sessão pública durante o 40º Congresso Brasileiro de Farmacologia e Terapêutica Experimental, em Águas de Lindóia, SP. O resultado foi o seguinte:

Primeiro lugar

Identification of inverse agonism of atropine at denervated skeletal muscle is linked to constitutively active muscarinic receptors. Andrade-Lopes, A. L.; Chiavegatti, T.; Pires-Oliveira, M.; Godinho, R. O. UNIFESP – Farmacologia.

Introduction: Studies from our group showed that G-protein coupled muscarinic acetylcholine receptors (mAChR) are expressed at noninnervated skeletal muscle, being downregulated by neuromuscular synapse formation and upregulated following adult muscle denervation, which suggests that mAChR could contribute to early development of the neuromuscular synapse and to reinnervation of adult skeletal muscle. Intriguingly, these receptors are abundant in situations where the endogenous agonist acetylcholine is absent. Considering these facts, we studied mAChR functionality at denervated skeletal muscle evaluating receptor coupling to specific G α isoforms, in the presence or absence of muscarinic agonists. **Methods:** Adult male Wistar rats were phrenicectomized for 7 to 28 days, the hemi-diaphragms were removed and membrane fractions (50 μ g/well, n=5) were incubated with 0.1 nM [³⁵S]GTP γ S (non-hydrolysable GTP analogue) and 50 μ M GDP, in the absence or presence of oxotremorine-M (Oxo M, 0-100 μ M) and/or 100 nM atropine. Nonspecific binding was determined with 50 μ M unlabeled GTP γ S. To discriminate the activated G α isoforms, [³⁵S]GTP γ S functional binding assay using membrane from 14-day-denervated muscles was followed by immunoprecipitation using antibodies against G α s, G α q and G α i. cAMP production was also measured in these membranes after Oxo-M and/or atropine incubation. **Results:** Oxo-M stimulated [³⁵S]GTP γ S binding (basal = 32.1 \pm 2.4 fmol/mg protein) increasing by 54% and 46% the activation of G α s and G α i, respectively, with no effect on G α q. Oxo-M (1-100 μ M) also reduced by 20% cAMP production, indicating the preferential coupling of mAChR to G α i protein. Interestingly, atropine alone (100 nM) reduced by 50% and 20% the [³⁵S]GTP γ S basal binding and cAMP production. The negative efficacy of atropine alone (inverse agonism) indicates that at least part of mAChR expressed at denervated diaphragm is constitutively active and coupled to G α s protein. **Discussion:** Our results show that mAChR expressed at denervated skeletal muscle could promiscuously activate G α i and G α s proteins when occupied by an agonist. Even more relevant is the fact that a fraction of mAChR could activate G α s protein in an agonist-independent manner, which is consistent with the negative efficacy of atropine. This phenomenon may contribute to physiological adaptation of muscle fiber to neurotrophic factor deprivation, including the main neurotransmitter acetylcholine. The ability of receptors to activate G-protein in the absence of an agonist has changed the classical concepts of pharmacology, increasing the complexity of GPCR signaling mechanisms. Apoio Financeiro: FAPESP and CNPq

Segundo lugar

The molecular mechanism of peripheral antinociceptive action of opioids: activation of PI3K/AKT/Nitric Oxide/K⁺_(ATP) channel signaling pathway. Cunha, T. M.¹; Duarte, H. L. L.²; Lotufo, C. M. da C.¹; Funez, M. I.¹; Verri Jr., W. A.¹; Sachs, D.¹; Souza, G. R.¹; Teixeira, M. M.²; Cruz, J. S.²; Cunha, F. de Q.¹; Ferreira, S. H.¹ ¹FMRP-USP - Farmacologia; ²UFMG- Bioquímica e Imunologia;

Introduction: The increased intensity of nociception during inflammation (hypernociception) is primary due to the sensitization of specific classes of nociceptive neurons. Opioids directly block

hypernociception via the activation of nitric oxide/cGMP/PKG/K⁺_(ATP) channels signaling pathway. In the present study we tested whether the initiation of this signaling cascade depends on the stimulation of the PI3K γ /AKT. **Methods:** Mechanical hypernociception was evaluated in rats and mice using a modification of Randall-Sellito test and an electronic version of the von Frey test, respectively. The expression of μ and K opioid receptors, PI3K γ , substance P (SP) and TRPV1 in dorsal root ganglion (DRG) neurons was detected by confocal microscopy. Phosphorylated AKT expression was analyzed by western blot of DRG culture neurons. Nitric oxide production by DRG neurons was evaluated using fluorescent DAF indicator. K⁺ currents in DRG neurons were determined by patch-clamp in the whole cell model. **Results:** Confocal analysis showed that PI3K γ is expressed in DRG neurons, mainly in neurons of small size, which also express TRPV1, SP, μ -, k-opioid receptors and also in IB₄ positive neurons. Non-selective (wortmannin, 1-10 μ g/paw) or selective (AS 605240, 10-90 μ g/paw) pharmacological inhibition of PI3K γ prevented in rats the peripheral anti-hypernociceptive action of morphine (6 μ g/paw), DAMGO (μ -agonist, 1 μ g/paw) and U50488 (k-agonist, 10 μ g/paw). In PI3K γ deficient mice (-/-) mice morphine, DAMGO as well U50488 did not show peripheral anti-hypernociceptive effect. Further investigating the PI3K γ downstream signaling pathway, the treatment with an AKT selective inhibitor (3-30 μ g/paw) also prevents morphine, DAMGO and U50488 anti-hypernociceptive effects. Corroborating with this *in vivo* observation, incubation of DRG culture neurons with morphine (10 μ M), DAMGO (1 μ M) or U50488 (100 nM) produce an increase in AKT phosphorylation. This activation was prevented by naloxone (10 μ M), PI3K γ inhibitor (AS 605240; 100 nM) and found reduced in cultured neurons of PI3K γ (-/-). Incubation of DRG neurons with morphine induced an increase of nitric oxide production which was inhibited by PI3K γ and AKT inhibitors. Further supporting these findings, electrophysiological recordings showed that morphine up-regulates K⁺_(ATP) currents in DRG neurons through activation of PI3K γ /AKT/NO pathway. **Discussion:** The present results suggest that the peripheral blockade of hypernociception by opioids seems to be dependent on an initial activation of PI3K γ /AKT signaling pathway, which is ultimately responsible for the activation of nitric oxide/cGMP/PKG/K⁺_(ATP) channels pathway. Apoio Financeiro: Fapesp and CNPq

Menção Honrosa

Heme modulates SMC proliferation via NADPH oxidase activation: counter-regulatory role for HO-1 system. Moraes, J. A. de¹; Assreuy, J.²; Arruda, M. A.³; Barja-Fidalgo, T. C.³ ¹UERJ - Farmacologia Bioquímica e Celular; ²UFSC - Farmacologia; ³UERJ - Farmacologia

PI3-K delta isoform up-regulates L-type Ca currents and increases contractility in 2.1 streptozotocin-induced diabetic mice aorta. Pinho, J. F.¹; Medeiros, M. A. A.²; Rezende, B.A.³; Côrtes S. F.³; Andrade, S. P.¹; Campos, P. P.¹; Cruz, J. S.⁴; Lemos, V. S.¹ ¹UFMG - Fisiologia e Biofísica; ²UFPB - LTF; ³UFMG - Farmacologia; ⁴UFMG - Bioquímica e Imunologia

Mast cell involvement in silica-induced pulmonary fibrosis in mice. Farias-Filho, F. A.; Lucena, S. L.; Martins, M. A.; Cordeiro, R. S. B.; Silva, P. M. R. e FIOCRUZ - Fisiologia e Farmacodinâmica

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