Kalyanaraman et al. BMC Pharmacology and Toxicology 2013, 14(Suppl 1):O26
http://www.biomedcentral.com/2050-6511/14/S1/O26

BMC Pharmacology & Toxicology

ORAL PRESENTATION Open Access

Non-genomic thyroid hormone signaling through NO/cGMP/PKGII

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From 6th International Conference on cGMP: Generators, Effectors and Therapeutic Implications
Erfurt, Germany. 28-30 June 2013

Background

Skeletal integrity requires continuous bone remodelling by osteoblasts and osteoclasts, and thyroid hormone (TH) is a key regulator of bone remodelling. Excess TH (hyperthyroidism) causes net bone loss, resulting in osteoporosis and increased fracture risk; lack of TH (hypothyroidism) also increases fracture risk because bones become brittle from decreased bone turnover [1]. TH stimulates bone formation and resorption through processes that are only partly defined; it enhances osteoblast proliferation and differentiation, and induces osteoblast production of the osteoclast differentiation factor RANKL (receptor activator of nuclear factor-κB ligand). Nuclear TH receptors (THR-α and THR-β) act as transcriptional regulators and generate the hormone's classic "genomic" effects [1]. In different cell types, TH also has transcription-independent ("non-genomic") effects, including stimulation of the MEK/Erk and PI3K/Akt/mTOR kinase cascades, but the molecular mechanisms mediating these non-genomic effects are largely unknown.

Results

We found that physiological concentrations of 3,5,3'-triiodo-L-thyronine (T3, 10^{-9} to 10^{-11}M), but not reverse-T3, rapidly increase NO production, and activate Src, Erk, and Akt in osteoblasts. These TH effects required THR-α, but were independent of THR–β. We identified a novel, membrane-bound THR-α isoform that mediates T3-induced Erk/Akt activation, but does not affect transcription from TH response element-containing promoters. Signalling via the newly-discovered THR-α isoform was blocked by inhibitors of NO synthase, guanylate cyclase (sGC), or protein kinase G (PKG), and was defective in endothelial NO synthase (eNOS)- or PKG II-deficient osteoblasts. We showed previously that NO/cGMP induce osteoblast proliferation through PKG II activation of Src and Erk, and established a mechanism for Src activation by PKG II [2]. We now show that TH enhances osteoblast proliferation and survival, and induces *osteocalcin* and *fos* family gene expression in a NO/cGMP/PKG-dependent fashion via "non-genomic" activation of Erk. In contrast, TH-induced expression of the osteoclast regulator RANKL occurs independently of NO, suggesting a classic "genomic" effect via nuclear THR-α. Consistent with these results, treatment with the sGC activator cinaciguat increased bone formation in hypothyroid mice, without affecting osteoclast numbers.

Conclusion

We conclude that anabolic effects of TH in osteoblasts are mediated predominantly by non-genomic TH signalling, via activation of a novel, membrane-bound THR-α isoform, with subsequent activation of eNOS, sGC, PKGII, Src, Erk, and Akt. Our results are consistent with the phenotype of THR-α knockout mice and the role of NO in bone biology.

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Published: 29 August 2013

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doi:10.1186/2050-6511-14-S1-O26
Cite this article as: Kalyanaraman *et al.*: Non-genomic thyroid hormone signaling through NO/cGMP/PKGII. *BMC Pharmacology and Toxicology* 2013 **14**(Suppl 1):O26.