BMC Pharmacology



Poster presentation

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cGMP signaling pathway is involved in Leyding cell stress response Silvana Andric*1, Natasa Stojkov¹, Marija Janjic¹, Stanko Stojilkovic² and Tatjana Kostic¹

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from 3rd International Conference on cGMP Generators, Effectors and Therapeutic Implications Dresden, Germany. 15–17 June 2007

Published: 25 July 2007

BMC Pharmacology 2007, 7(Suppl 1):P1 doi:10.1186/1471-2210-7-S1-P1

This abstract is available from: http://www.biomedcentral.com/1471-2210/7/S1/P1

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The ability of stress to interfere with the Leydig cell steroidogenesis has been published previously by others and our laboratory, but the intracellular signaling pathways involved in this process have not been identified and described precisely. In this study, we examined the effects of immobilization stress (1 × 120 min, 2 × 120 min within 2 days, and 10 × 120 min within 10 days) on nitric oxide (NO)-cGMP signaling pathway in rat Leydig cells from adult rats. Purified Leydig cells were isolated form freely moving (controls) and immobilized rats, a procedure that required about 8 hrs, and were then subjected to RT-PCR and functional analyses. Results showed that immobilization stress was accompanied with a decrease in mRNA transcripts for phosphodiesterase 5 and multidrug resistance protein 5 in all three experimental groups,

and with an increase in transcripts for inducible NO synthase in 1×120 - and 2×120 -min treatments, whereas the mRNA levels for endothelial NO synthase, soluble guanylyl cyclase, protein kinase G, and cyclic nucleotide-gated channels were not affected by single and repetitive immobilization. In parallel, *in vivo* androgen levels and *in vitro* Leydig cell steroidogenesis were also affected by 1×120 -and 2×120 -min immobilization treatments, but recovered after 10×120 -min treatment. Experiments with inhibitors of soluble guanylyl cyclase revealed the dual effect of stress-induced NO production on Leydig cells steroidogenesis: inhibitory effect is mediated directly by this messenger, and stimulatory effect is mediated through NO-dependent cGMP signaling pathway.