REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS, INTERNATIONAL
EDITION 20: 322 (2006)

A Nitric Oxide Mediated, cGMP-Independent Pathway for IL-6 Production in C2C12 Skeletal Myotubes

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Key words: Nitric oxide, cGMP, IL-6, skeletal muscle

We have previously observed that strenuous exercise leads to marked upregulation of interleukin-6 production. As nitric oxide (NO) is known to 1) increase after chronic exercise training and 2) modulate cytokine production, we sought to determine its effects on IL-6 release and study the mechanisms involved using an *in-vitro* model. C2C12 myoblasts were differentiated into myotubes after a 48hr exposure in low serum-containing medium and were then exposed to DETA-NONOate, an agent that releases NO with a half-life of 20hr at pH 7.4 and 37^0C. Such treatment resulted in a concentration-dependent stimulation of IL-6 that peaked at 1.5mM (control: 74.6±8.6, stimulated: 404.9±45.5 pg IL-6/mg total protein). Intracellular levels of cGMP were unaffected by DETA-NONOate suggesting that C2C12 cells lack soluble guanylyl cyclase (sGC); in addition, no immunoreactive sGC subunits could be detected in C2C12 lysates by western blotting. In line with these observations, the sGC inhibitor ODQ did not alter DETA-NONOate-induced IL-6 release. Moreover, unlike DETA-NONOate, the NO-independent sGC activator BAY 412272 and the cell-permeable cGMP analogue 8-bromo-cGMP failed to increase IL-6 production. DETA-NONOate-induced IL-6 release was attenuated by pre-treatment of myotubes with the MEK1/2 inhibitor PD-98059 (52.3±7.4 % inhibition) or the p38 inhibitor SB-203580 (74.46±2.6% inhibition), but not the JNK/SAPK inhibitor SP-600125. We conclude that NO-stimulated IL-6 in differentiated C2C12 myotubes is cGMP-independent and mediated by activation of MAPK members.