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MEETING ABSTRACT

A1.3

Regulatory interaction of cytoskeleton-associated proteins with soluble guanylyl cyclase in vascular smooth muscle

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Background: Nitric oxide (NO) and its target soluble guanylyl cyclase (sGC) play a central role in vessel homeostasis by initiating vasodilation via the NO/cGMP signaling pathway. In that regard sGC has become an important target in the therapy of cardiovascular diseases. Thus, compounds have been developed to pharmacologically modulate the enzyme. However, little is known about endogenous modulators of sGC. In a previous study we found an increase in maximal sGC activity *in vitro* caused by the presence of cytosolic preparations from porcine coronary arteries. The active principle, further referred to as sGC-activating factor (sGC-AF), could be enriched by ammonium sulfate fractionation of cytosols, indicating that it might be a protein. In the present study we isolated and identified the constituents of sGC-AF.

Methods: Column chromatography with an Äkta FPLC system utilizing hydrophobic interaction, ion exchange, and size exclusion (gel filtration), respectively, was used to isolate sGC-AF. SDS-PAGE and LC-MS/MS were conducted to identify the obtained proteins. sGC-AF activity was monitored by co-incubation of purified bovine lung sGC with the respective fractions and measurement of conversion of [α-^{32}P]GTP to [^{32}P]cGMP under stimulation with NO.

Results: The developed purification strategy yielded a protein mixture consisting of three major bands in SDS-PAGE. The respective 100, 70 and 40 kDa bands were identified as gelsolin, annexin A6, and actin, respectively. These proteins are all related to or part of the cytoskeleton/contractile elements of smooth muscle. Investigations addressing the interaction of sGC-AF with sGC revealed a heme-dependent mode of action. It was independent from the applied NO donor and was not linked to a redox process. In addition, the effect was not mimicked by bovine serum albumin or superoxide dismutase.

Discussion: The proteins obtained in the present study are all closely linked to the contractile apparatus of smooth muscle. While actin represents the basic framework of contractile elements, gelsolin and annexin A6 are involved in severing and membrane anchoring of actin, respectively. As the individual proteins were inactive it is presumed that sGC-AF is a protein complex rather than a single protein. The increase in activity caused by sGC-AF was similar to that observed with saturating concentrations of the sGC stimulator BAY 41-2272. This indicates that sGC-AF converts the NO-bound enzyme into a more active conformation. These results point towards an interaction of proteins of the contractile apparatus with sGC in a regulatory manner.

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