**THE SMALL MOLECULE SMOOTH MUSCLE MYOSIN INHIBITOR, CK-2018571, SELECTIVELY INHIBITS ATP HYDROLYSIS AND RELAXES SMOOTH MUSCLE IN VITRO**

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**INTRODUCTION**

Smooth muscle myosin is a mechanical enzyme that hydrolyzes ATP to generate mechanical force; ultimately all signaling pathways that regulate smooth muscle tone converge on the regulation of the motor protein. We used a high throughput screen to identify compounds that inhibit the ATPase activity of smooth muscle myosin; optimization of the initial hit compounds has resulted in compounds with nanomolar potency. A potent representative of this chemical series, CK-2018571, inhibits the steady-state ATPase activity of human smooth muscle myosin at approximately 10-fold lower concentrations than are required to inhibit non-muscle myosin. The most closely related myosin II and has greater specificity versus striated muscle myosin III. Transient kinetic studies demonstrate that CK-2018571 inhibits the myosin-catalyzed hydrolysis of the y-phosphate group of ATP, with no effect on nucleotide binding or release from the enzyme. Actin co-sedimentation assays indicate that CK-2018571 stabilizes a weak actin-binding conformation of myosin in the presence of ATP. Consistent with this mechanism, CK-2018571 relaxes skinned rat tail smooth muscle tissue. Importantly, this relaxation occurs regardless of whether the skinned muscle has been activated by calcium or by phosphorylation of the myosin regulatory light chain, supporting evidence that CK-2018571 relaxes smooth muscle tissue by direct inhibition of activated smooth muscle myosin. The ability of CK-2018571 to relax intact tracheal smooth muscle and aortic ring preparations suggests this mechanism may prove useful in diseases of smooth muscle hypercontractility, such as hypertension and asthma.

**RESULTS**

**CK-2018571 Selectively Inhibits The ATPase Activity of Smooth Muscle Myosin**

**CK-2018571 Serves ATP Hydrolysis ...

**CK-2018571 Promotes Weak Actin-Binding**

**Materials and Methods**

**Bioassays:** Assays were performed in low salt PM12 buffer (12 mM K-PIPES, 2 mM MgAc, pH 6.8).

**RESULTS**

1. CK-2018571 selectively inhibits the ATPase activity of smooth muscle myosin as compared to other myosin isoforms (non-muscle myosin, cardiac and skeletal myosin).
2. CK-2018571 inhibits smooth muscle myosin in a weak actin-binding state, consistent with its ability to relax smooth muscle tissue in vitro.
3. CK-2018571 inhibits the chemical chauactivity of ATP by smooth muscle myosin, a mechanism distinct from previously identified myosin inhibitors such as blebbistatin and BFA.
4. CK-2018571 inhibits calcium-induced force development in skinned calcium artery and relaxes skinned rings activated by thapsigargin, consistent with relaxation occurring as a consequence of the direct inhibition of smooth muscle myosin.

**CONCLUSIONS**

**REFERENCES**


Blebbistatin: an actin-based smooth muscle contraction, evidence for the involvement of actin that is bound to the triton insoluble fraction even in the absence of Ca**2+**. J Biol Chem. 2019;294:20386-20396.