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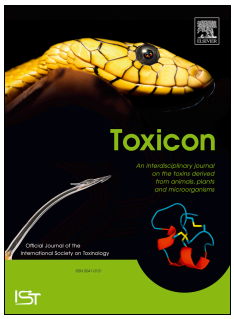

Inhibition of the proteasome partially attenuates atrophy in botulinum neurotoxin treated skeletal muscle

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Title

Inhibition of the proteasome partially attenuates atrophy in Botulinum neurotoxin treated skeletal muscle.

Running heading

Proteasome inhibition during neurotoxin-induced atrophy.

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Abstract

Botulinum neurotoxin type A (BoNT/A) is used as a therapeutic tool to induce chemical denervation of spastically contracted muscles, yet the neurotoxin can also cause skeletal muscle atrophy. The underlying proteolytic mechanisms that induce this atrophy remain unclear. Our previous work has highlighted increased ubiquitin proteasome system (UPS) activity in soleus muscle of male Sprague Dawley rats following hind limb injection of BoNT/A, with the chymotrypsin-like activity of the 20s proteasome the most active. Thus, we chose to inhibit 20s proteasome activity in BoNT/A injected hind limb to determine the effect on soleus muscle atrophy. Epoxomicin is commonly used to inhibit the proteasome *in vivo*, binding specifically and irreversibly to the 20s proteasome catalytic subunits. Daily subcutaneous injections of epoxomicin abolished BoNT/A-induced elevations in 20s chymotrypsin-like activity both 3 days and 10 days post BoNT/A injection. Furthermore, BoNT/A-induced elevations in polyubiquitination remained elevated in BoNT/A + epoxomicin treated muscle, presumably due to epoxomicin's inhibition of the proteasome causing a back-up of polyubiquitinated proteins. Despite inhibition of the proteasome, epoxomicin was insufficient to significantly attenuate soleus muscle fiber atrophy 3 days following BoNT/A injection however, 10 days of daily epoxomicin injection was sufficient to spare ~20% of muscle wasting. The mechanism of the remaining 80% of BoNT/A-induced atrophy presumably occurs via mechanisms outside of the 20s proteasome.

Key words