Endothelin B receptor activation in postganglionic sympathetic neurons causes hyper-responsiveness to acetylcholine that is dependent on reactive oxygen species

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Postganglionic sympathetic neurons primarily receive cholinergic input from preganglionic neurons in the spinal cord and send noradrenergic output to innervated targets. Endothelin B receptors (ETBRs) are found in postganglionic sympathetic ganglia and activation of ETBRs via sarafotoxin 6c (S6c) leads to higher levels of reactive oxygen species (ROS). However, ETBR has not been localized in postganglionic sympathetic neurons and the effects of ETBR activation on neuronal activation have not been investigated. Using a specific antibody to ETBR, we found that it is localized primarily in the nuclei of postganglionic sympathetic neurons. We confirmed this result using subcellular fractionation followed by Western immunoblotting. To study sympathetic neuron activation, we measured calcium concentration in dissociated postganglionic sympathetic neurons from celiac ganglia of rats using the fluorescent calcium dye, fluo-4 AM. Neurons were incubated overnight with 100 nm S6c, or vehicle, and the change in calcium concentration was measured in response to 10 and 100 µM acetylcholine (ACh). In S6c incubated neurons, the increase in calcium concentration in response to both 10 and 100 µM of ACh was significantly higher than in vehicle incubated neurons. Incubation with the ROS scavenger apocynin (100 µM) eliminated the enhanced calcium response to ACh. These results indicate that activation of ETBRs confers hyper-responsiveness to ACh in sympathetic neurons via a ROS-dependent pathway.