TACSM Abstract

Quantification Method of P2X3 Receptors in Rat DRG Neurons: Western Blotting

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ABSTRACT

Skeletal muscle contractions are known to evoke pressor and cardioaccelerator responses in part by stimulating P2X3 receptors found on the peripheral endings of afferents. In diabetic patients, this pressor response is exaggerated. What is currently not known is whether P2X3 receptors play a role in evoking this exaggerated response. **PURPOSE**: The purpose of this project was to quantify P2X3 receptors in the L4 and L5 dorsal root ganglia (DRG) neurons in both healthy and type 1 diabetic rats using western blot analysis. **METHODS:** We injected 50 mg/kg streptozotocin (STZ) or the vehicle (CTL) i.p in fasted female and male Sprague Dawley rats and then waited at least 7 days for the rats to become diabetic. We then performed a laminectomy in the anesthetized rats to expose the spinal cord and roots. Using a dissecting microscope, we removed the L4 and L5 DRG from the spinal column. The DRG are the cell bodies of the peripheral afferents found in the hindlimb musculature. The DRG were placed in HBSS (is this buffer?) and stored at -80°C until analysis. For quantification, samples were lysed and proteins were isolated using the NucleoSpin RNA/Protein Kit (Macherey-Nagel, Bethlehem, PA, USA). A Qubit 3.0 Fluorometer was used to quantify the protein concentration of each sample so that equal protein concentrations could then be loaded onto a Bolt Bis-Tris (4-12%) gel. Following electrophoresis, the proteins were transferred to a membrane before being probed with a rabbit polyclonal P2X3 antibody (Alomone Labs), followed by an anti-rabbit secondary antibody conjugated to alkaline phosphatase (Life Technologies). The membrane was then exposed using a ChemiDoc XRS and the results analyzed using BioRad's Quantity One imaging software. **RESULTS:** We were able to detect P2X3 receptor proteins. When compared with a molecular weight ladder, P2X3 receptor proteins were 54kDa, which is similar to the molecular weight of P2X3 receptors quantified in other studies. **CONCLUSION:** This method of quantifying P2X3 receptors in DRG neurons allows for a comparison between non-diabetic and diabetic rats. Further analyses are required to determine whether the quantity of P2X3 receptors in L4 and L5 DRG neurons is different in diabetic rats compared to non-diabetic rats.