TACSM Abstract

Detection of $P2X_3$ in DRG Using an Automated Approach to Immunoblotting, Jess

JUNGHOON LEE[1], AUDREY J. STONE[1], & MICHELLE L. HARRISON[2]

Autonomic Control of Circulation Laboratory; Department of Kinesiology & Health Education; The University of Texas; Austin, TX. [1] Health & Integrative Physiology Laboratory; Department of Kinesiology & Health Education; The University of Texas; Austin, TX. [2]

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Advisor / Mentor: Stone, Audrey J. (Audrey.stone@austin.utexas.edu)

ABSTRACT

Exaggerated cardiovascular (CV) responses to exercise can lead to adverse CV events. Previous studies have reported that $P2X_3$ receptors, found on the peripheral endings of afferents, contribute to an exaggerated exercise pressor reflex in individuals with CV-related diseases. One way to investigate the role played by these receptors in CV pathophysiology is through immunoblotting. The Jess (Protein Simple) provides an automated option for the protein separation and immunoblotting of the traditional Western Blot and allows for total protein staining, an improvement over the use of loading controls to normalize for sample loading variability. **PURPOSE:** The purpose of this study was to develop a protocol, using the Jess, for quantifying $P2X_3$ receptor protein expression in the L4 and L5 dorsal root ganglia (DRG) of healthy and type 1 diabetic rats. **METHODS:** Streptozotocin (STZ), 50 mg/kg, or a vehicle (CTL) was injected i.p into fasted Sprague Dawley rats (n=7 each group). After a minimum of 3 weeks, L4 and L5 DRG were excised, immediately placed in HBSS, and then stored at -80°C until subsequent analyses. For quantification, samples were lysed and protein was isolated (Macherey-Nagel) and then quantified (Qubit protein assay kit). For an initial *Optimization Run* a single test sample lysate was used; different protein concentrations (0.1 - 1.4 mg/ml) were tested against multiple Anti-$P2X_3$ (Novus Biologicals) dilutions (1:25 – 1:250). Sample lysates (3 µl) and required reagents were loaded into a microplate as per manufacturer's instructions and then the microplate and capillaries were loaded into the Jess. Over 3 hours; protein separation, antibody incubations, washes, and detection were all performed automatically within the Jess. The data from that run provided the optimal protein concentration and antibody dilution that were then used for the *Sample Run*, which involved running the CTL and STZ sample lysates. For the *Sample Run*, protein normalization reagent was added to the microplate in order to normalize for sample loading variability using total protein staining. **RESULTS:** *Optimization Run* - A protein concentration of 1.4 mg/ml and a dilution of 1:250 for the $P2X_3$ antibody were found to be optimal. This determination was based on the combination of a low background signal from the antibody and a detectable target protein signal. *Sample Run* - We found that $P2X_3$ receptor protein expression decreased in STZ rats compared to CTL rats (0.82 ± 0.09 vs 1.00 ± 0.19; n=7 both groups, p=0.03). **CONCLUSION:** Using the Jess, an automated protocol was developed that detected differences in $P2X_3$ receptor protein expression in rats with and without diabetes. Advantages over the traditional Western Blot include: a run time of 3 hours, reduced user-associated variability thru automation, and the capability of using total protein staining to normalize for sample loading variability. Technological advances such as the Jess are a step towards addressing current rigor and reproducibility concerns.