PAR-1 Alters Morphology of Schwann Cells As Assessed by Cytoskeletal Staining

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ABSTRACT

The purpose of this study was to investigate the morphological changes associated with activation of PAR-1 in Schwann cells. Previous studies have shown that PAR-1 is activated by SEILRN, a synthetic thrombin-like peptide chain that mimics the serine protein Thrombin [1,3]. Differences in morphologies were studied using fluorescent imaging of the actin filaments in the cytoskeleton of the Schwann cells. The number of cell processes or extensions was counted for each cell as a quantitative indicator of cell morphology (Figure 1).

PAR-1 is a large G-class protein that sits in the membrane of many cells in the body and can be activated by thrombin. Thrombin activation leads to a PAR-1 response that can induce multiple cellular activities, including cell shape changes, cell death, inhibited repair/regeneration, and reduction in cell proliferation and growth [2,3]. Nervous cells and their response to PAR-1 is of significant interest due to the fact that vascular damage in areas of the nervous system has been shown to impair the nervous system’s ability to repair itself. Previous work has shown that oligodendrocytes in the CNS show a morphological response to thrombin/SEILRN and PAR-1 activation; however, less is understood about the response of Schwann cells in the PNS [1].

Schwann cells are neuroglial cells found in the peripheral nervous system that myelinate the axons of motor neurons insulate nerve fibers outside the central nervous system. Schwann cells function by localizing an axon by an extension of their plasma membrane and then wrapping themselves around the numerous times, resulting in an insulating cover around the entire length of the membrane.

INTRODUCTION

The purpose of this study was to investigate the morphological changes associated with activation of PAR-1 in Schwann cells. Previous studies have shown that PAR-1 is activated by SEILRN, a synthetic thrombin-like peptide chain that mimics the serine protein Thrombin [1,3]. Differences in morphologies were studied using fluorescent imaging of the actin filaments in the cytoskeleton of the Schwann cells. The number of cell processes or extensions was counted for each cell as a quantitative indicator of cell morphology (Figure 1).

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METHODS

Cells were cultured from a previously cryo-frozen line of Schwann cells using the standard Dulbecco’s modified (DME) + FBS) media. Schwann cells were placed in a 24 well plate at a 500 cell/ml concentration with 1 ml in each well and given 24 hours for attachment. At 24 hours, cells were treated with either 1 uM, 2 uM, or 3 uM or 0 uM (control) SEILRN. Figure 2 shows the setup for each plate treatment. Immunocytochemistry was used to stain cells, actin filaments, nuclei, and, ideally, focal adhesion points, at each plate’s respective time point: 12 hr, 24 hr, 48 hr, and 96 hr. Specific antibodies were used against actin and vimentin. Cells were imaged and imaged in 5 locations of each well, approximating the center, and four quadrants of the well for each picture. The images were then analyzed by tallying the number of cells in each treatment and time point with 0, 1, 2, 3, or 4+ cell processes.

RESULTS

Figure 4 shows the percentage of cells with 0, 1, 2, 3 or 4+ processes for each treatment group and time point. A Pearson’s Chi Square Test was run for each frequency distribution, using the proportion of cells in each treatment or control group. Relative percentages of cells with 0, 1, 2, 3, and 4+ processes were significantly different between the variable PAR-1 responses that can occur. It has been shown that various body cells respond differently to thrombin and its inhibitors especially in reference to vascular damage in the nervous system (3). Therefore, the PAR-1 treatment may have a compensatory effect on Schwann cells, while increasing the amount of thrombin (or damage) would be detrimental.

Figure 5: Bi polar Schwann cell Behavior

In order to assess the point at which thrombin or SEILRN treatments become significant, further study should be completed using smaller concentrations. As the PAR-1 treatments were significant it would be informative to test concentrations in between 0 uM and 2 uM in the future. In addition, using alternative cell classifications (other than cell processes) to assess cell morphology may be of benefit. For example, staining focal adhesion points and excluding mitotically active cells would provide clarity into cell morphology and classification during study.

DISCUSSION

As a general trend treated cells are more likely to have fewer extensions than untreated, especially at 2 and 3 uM concentrations. The distribution of the 1 uM treatment was slightly more erratic and this may be attributed to the variety of PAR-1 responses that can occur. It has been shown that various body cells respond differently to thrombin and its inhibitors especially in reference to vascular damage in the nervous system (3). Therefore, the PAR-1 treatment may have a compensatory effect on Schwann cells, while increasing the amount of thrombin (or damage) would be detrimental.

Of note to Schwann cell behavior are the frequency of bipolar or 2 process category cells. Although higher in the control, they were seen frequently at all treatments and are assumed to denote an “searching” behavior. These cells were most often found together with other cells of the same category as shown in Figure 5 below.

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RECOGNITIONS


Furman University; Howard Hughes Medical Institution: RET Grant Program

Figure 4: Percentage of cells with 0, 1, 2, 3 or 4+ processes for each treatment group and time point.

Figure 3: Example of stained cell image

Figure 2: Well Plate Treatment Map

Figure 1: Counting Cell Processes

Figure 4: Percentage of cells with 0, 1, 2, 3 or 4+ processes for each treatment group and time point.