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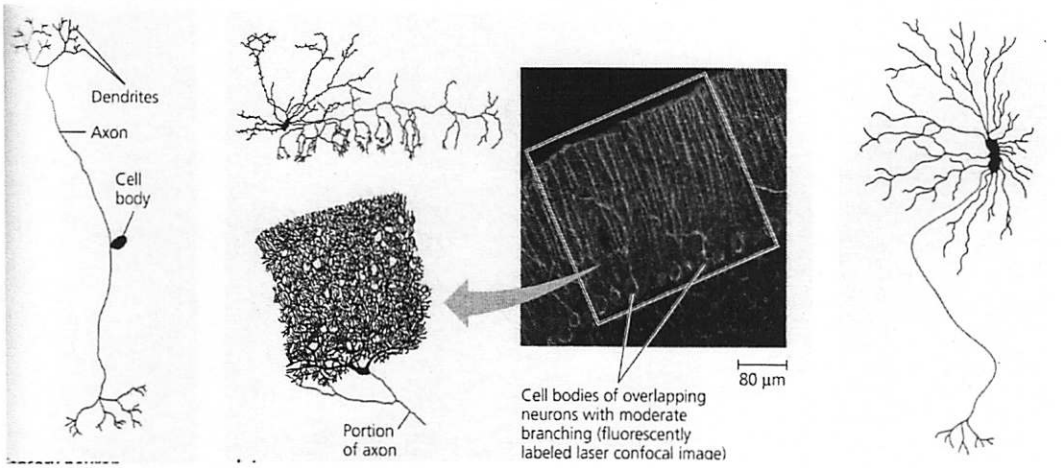
Understanding the Signaling Pathways Involved in the Process of Dendritic Growth in Sympathetic Neurons

Many neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Down's syndrome and the process of aging in humans are associated with changes in the neuronal morphology. Most of the studies on changes in neurons during these diseases have focused on axonal loss and cell death. However, it is clear that the process of aging and neurodegeneration is associated with retraction of dendrites (2). Therefore, understanding the process of dendritic growth and the establishment of the complexity of dendritic arbor is important for better comprehending the process of dendritic regression that we see during disease.

Dendrites are extremely important for normal neuronal function, for they receive and help convey nerve signals. Dendritic arbor (a measure of the length, complexity, and density of dendrites) (Figure 1) accounts for the great diversity among neurons. Neurons that integrate information from many neurons such as the Purkinje neurons in the cerebellum, which help maintain body position and motor coordination, have large dendritic arbor and many synapses compared to neurons that receive information from one or two neurons such as sensory neurons. Sympathetic neurons, which will be part of the focus of this research project, have a dendritic arbor intermediate between Purkinje cells and sensory neurons.



Sensory Neuron Purkinje Cell Sympathetic Neuron

Figure 1, modified from Campbell, Biology (1)

What naturally regulates dendritic growth? Research has shown that axons and dendrites respond to different growth factors and grow at different rates in response to these growth factors. A family of proteins called bone morphogenetic proteins (BMP) *is* known to specifically induce dendritic growth in different types of neurons in the central and peripheral nervous systems. First named for their ability to induce bone formation, BMPs have important effects in early embryonic development of many organs (4). When BMPs bind to receptors on sympathetic neurons, they activate a family of proteins known as SMADs. The SMADs then travel to the cell's nucleus, where they activate genes that promote dendritic growth (2).

Though BMPs are needed for induction of dendritic growth, the number of dendrites that the neurons put out and their length is not determined by BMPs. This suggests that there are other molecules that are important for specifying the complexity of the dendritic arbor in different neurons. These molecules most likely work in conjunction with BMPs and may be specific to particular neurons to specify the final shape and therefore function of these neurons.

The purpose of this proposed research project is to investigate two such pathways: Reactive oxygen species (ROS) and micro RNAs (miRNAs).

Dendritic growth due to ROS and miRNAs will be measured by culturing the neurons from embryonic rat superior cervical ganglia and treating them with BMPs alone, BMPs and antioxidants, ROS, miRNAs, and nothing (as a control). Tissue cultures can generate data relatively fast and are easy to work with, allowing many cultures to be run at once. As well, due to the ease of working with tissue cultures, the different projects described in this proposal dealing with ROS and miRNAs can be run in tandem. The cultures can then be analyzed for factors such as dendritic growth and cell death using resources available in the St. Mary's labs.

Reactive oxygen species (ROS) are types of free radicals found in cells and formed primarily in the mitochondrial electron transport chain of cellular respiration. They include hydrogen peroxide, hydroxyl, and superoxide radicals. In large quantities, ROS can cause damage to DNA and kill cells, but cells counteract the harmful effects of ROS by using enzymes derived from antioxidants. Previous research by Dr. Chandrasekaran has shown that antioxidants of different classes such as diphenylene iodinium, nordihydroguaiaretic acid, and vitamins C and E all have the ability to inhibit BMP induced dendritic growth (2). These data lead to the idea that reactive oxygen species in low levels are necessary for dendritic growth in sympathetic neurons. This is not the first time that beneficial effects of ROS have been detected. Low levels of ROS have been shown to be important for signaling pathways downstream from growth factors (6). This research project will try to understand how ROS fits into the BMP pathway by addressing the following questions:

1. Are ROS generated in sympathetic neurons during dendritic growth?
2. What are the specific effects of ROS on dendritic growth with or without BMP?

It is important to determine whether or not ROS are generated in sympathetic neurons during dendritic growth. This part of the research project will be carried out with the collaboration of Dr. Pam Lein at UC Davis, who has given prior permission to use a machine—Seahorse—to measure minute amounts of ROS at the cellular level. If ROS are confirmed to be in sympathetic neurons during dendritic growth, then the question becomes whether ROS can induce dendritic growth by themselves or if they need BMPs to be present to induce growth. To accomplish this, the specific effects of ROS on dendritic growth with or without the presence of BMPs will be measured. This can be done by artificially generating ROS using xanthine oxidase in a test tube, exposing the sympathetic ganglia to ROS in the presence or absence of BMPs, and then measuring the length of dendrites as a result. If ROS are able to induce dendritic growth without BMPs, it suggests that ROS could work via SMADs or by a novel pathway. On the other hand, if they need BMPs to induce growth, ROS could potentiate BMPs and have synergistic effects on dendrite extension.

Another way to examine the same question is to look at antioxidants and their interaction with the BMP pathway. It is not known whether antioxidants inhibit dendritic growth by directly or indirectly blocking BMP signaling. Whether the inhibition is direct or indirect can be determined by focusing on SMAD activation: If the inhibition is direct, SMADs will not travel to the nucleus, and if inhibition is indirect it could involve novel pathways. Using antibodies that detect activated SMADs, the location of SMADs in the presence and absence of antioxidants and BMP will be examined. In addition, other BMP target genes will be examined to see if they are present when treated with antioxidants. In summation, the data from this part of project will enable us to determine the role of reactive oxygen species and antioxidants in the process of dendritic growth and retraction in sympathetic neurons.

For the second part of the project, we will be considering the possibility that specific miRNAs, present within certain neurons, may acts as modulators of BMP signaling. MiRNAs are non-coding RNA sequences 24-30 nucleotides long that help regulate messenger RNAs. They are highly conserved sequences that recent research has shown to have key roles in the nervous system, specifically in neuron development and function (3). Recent research has further found that specific miRNAs—miR-297, miR-206, and miR-124a—are present in the cell bodies of sympathetic neurons, suggesting that they have an important role in dendrite formation and neurological diseases (5). Also, recent data indicate that BMP signaling pathways can be regulated by micro RNAs in cardiac precursors during embryonic development (7). These two pieces of data lead us to ask the question of whether these micro RNAs in sympathetic neurons can act as cell specific regulators of dendritic growth. To address this question, it will first be determined if the levels of miR-297, miR-206, and miR-124a change in sympathetic neurons in the presence of BMPs and during the process of dendritic growth. This can be accomplished by in situ hybridization and Northern Blotting. Next, the sympathetic ganglia will be treated with miR-297, miR-206, and miR-124a each individually and in combination in the presence and absence of BMPs to look at the effects on dendrites. This part of the project will lead to a better understanding of the role of micro RNA during the development of sympathetic neurons and maybe a novel branch in the BMP signaling pathway within these neurons.

This proposed research project will generate data that gives more information on the effects of ROS and miRNAs on the BMP pathway and dendritic growth. Ultimately, this research would give more understanding into the mechanisms and chemicals that affect dendrite morphology—which can have applications in neuroregeneration and many diseases where

deficiencies in dendritic growth are known to occur, such as in Alzheimer's disease, Parkinson's disease, and Down's syndrome.

References

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