 

# NITRIC OXIDE MODULATION AND CALCIUM DYSREGULATION IN BRAIN ENDOTHELIAL AND ASTROCYTE CELL CO-CULTURES DURING INFLAMMATION

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## ABSTRACT:

Nitric Oxide (NO) is an important signaling molecule in brain, and is expressed in both normal and disease states such as inflammation. Calcium ($Ca^{++}$) is a second messenger of many signaling pathways and plays a central role in neuronal physiology. Impairment in its homeostasis can cause extreme functional alterations. The objective of this work was to investigate potential effects of inflammatory stimuli on $Ca^{++}$ dynamics in astrocyte and brain microvascular endothelial cell (BMVEC) cultures both separately and as co-cultures, to test if astrocytes have a negative feedback effect on cell networks. Both BMVECs and astrocytes make up the blood brain barrier (BBB), which protects the brain, but also can serve as a barrier to delivery of potentially therapeutic drugs as well. NO and $Ca^{++}$ changes have been shown to affect BBB permeability. This research quantifies NO synthesized in response to an inflammatory stimulus to BMVECs and astrocytes individually, and in a coculture model, *in vitro*. We observed that for the same inflammatory stimulus, 100ng/ml of Tumor Necrosis Factor (TNF) + 5ug/ml of lipopolysaccharide (LPS), BMVECs produce a significantly higher concentration of NO compared to astrocytes. In the co-culture model, the astrocytes provided negative feedback to countercheck the high NO production from BMVECs. Stimulated BMVECs showed higher cell proliferation as indicated by changes in culture pH, and formed quantifiable oval lumen structures as patterns seen as part of the cell networks. Intracellular $Ca^{++}$ concentration ($[Ca^{++}]_i$) dynamics was recorded for BMVECs and astrocytes treated with the inflammatory agents. When these cells were then stimulated with ATP and glutamate (Glu), cells showed $Ca^{++}$ peaks with significantly higher amplitude and notable $Ca^{++}$ oscillations. In contrast, astrocytes showed a smaller response to ATP and Glu. These findings lay out more details on inflammation and BBB permeability and suggest a cue on how BMVECs and astrocytes may interact in regulating BBB permeability. It also provides a base for investigating modulation of these cell signaling molecules using nanomaterials towards moderating BBB permeability.
**Keywords:** Blood-Brain Barrier, Nitric Oxide, Inflammation, Calcium activity, Co-culture, Negative feedback

## INTRODUCTION

The BBB is a barrier between the brain's blood vessels and the cells and components that make up brain tissue. Tight junctions in endothelial cells of brain micro vessels (BMVECs) that makes BBB prevents entry of most potential drugs for neurological and mental disorders to readily cross the barrier and reach into the brain, posing an immense challenge in the treatment of brain disorders [1]. While BMVECs line the luminal surface of the brain blood vessels the abluminal side is wrapped by a basement membrane, about 80% to 99% of which is covered by astrocytes [2]. The close proximity of BMVECs and astrocytes suggests a crucial role of astrocyte-derived factors in the formation and maintenance of BBB[3]. Inflammatory reactions are an important contributor to neuronal damage in a wide variety of neurodegenerative disorders, and both TNF and LPS can be used to induce inflammation in vitro including the production of NO[4]. Communication (paracrine actions) between BMVECs and astrocytes have been suggested involving TNF that may affect BBB permeability [5], and LPS and TNF effects for astrocytes and BMVECs can be receptor-mediated [6],[7]. One study has suggested negative feedback of astrocytes on NO-mediated effects on $[Ca^{++}]_i$ dynamics in microglia (possibly via