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γ-Aminobutyric Acid Inhibits Synergistic Interleukin-6 Release and Increases Intracellular Cytokine Content in C6 Astrocytoma Cells In Vitro

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Abstract

Alzheimer’s disease (AD) is a neurodegenerative disorder that is characterized by memory loss and is the most common cause of dementia. It has been hypothesized that pro-inflammatory cytokines induce the inflammation that is believed to be the cause of the neuronal death that is associated with AD. γ-aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the Central Nervous System, possessing membrane hyperpolarization or depolarization activities. A decline in GABA may enhance cytokine release in Alzheimer’s disease resulting in neuroinflammation. Therefore, we investigated the GABA-mediated suppression of the synergistic release of interleukin-6 (IL-6) induced by interleukin-1β (IL-1β) and tumor necrosis factor α (TNF-α). In this study, our aim was to determine the sub-cellular location of the accumulated IL-6 within Rat C6 astrocytoma cells, and to determine the receptor through which GABA is acting to cause the intracellular accumulation of IL-6. We hypothesize that the accumulation occurs within the Golgi apparatus and that the GABA receptor is acted upon to inhibit the release of IL-6.

Methods

- Rat C6 astrocytoma cell line was used as model system
- Immunofluorescence microcopy
  - Used to determine subcellular location of accumulated IL-6
- Enzyme-Linked Immunosorbent Assay (ELISA)
  - Used to determine concentration of IL-6 in C6 Rat Astrocytoma supernatant and lysate
  
- Immunofluorescence microcopy
  - Accumulation of IL-6 was disordered throughout the cell with little or no accumulation within the Golgi apparatus.
  - Cells treated with GABA and TI showed the greatest intracellular accumulation of IL-6

- Enzyme-Linked Immunosorbent Assay
  - GABA dose-dependently suppressed the TI-mediated release of IL-6
  - GABA causes an intracellular accumulation of IL-6
  - Neither phaclofen nor bicuculline completely reversed the GABA suppression of the TI-mediated IL-6 release.
  - Basolin and muscimol yielded similar levels of suppression of IL-6 release.

Results

- Effect of GABA and TI on extracellular levels of IL-6 in C6 astrocytoma cells in vitro: Cells were pre-treated with 0.1-10 mM GABA for 1 h. Cells were subsequently treated for 24 h with either GABA or TI. Supernatants were collected and the data are presented as mean SEM of triplicate observations from a single representative experiment repeated four times.

Conclusion

We conclude that the intracellular accumulation of IL-6 is not located solely in the Golgi apparatus and in fact the diffuse distribution leads us to believe that GABA is acting at the cellular membrane to inhibit the release of IL-6. The immunofluorescence microscopy confirms an overall increase in the intracellular content of IL-6 in GTI treated cells as also shown by ELISA. When treated with GABA receptor antagonists or agonists the levels of associated IL-6 release were very similar, leading us to further conclude that GABA is not selectively acting on the GABA receptor or the GABAγ receptor alone, but instead acts on both receptors to suppress the TI mediated synergistic release of IL-6.

References


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