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Y-Aminobutyric acid inhibits synergistic interleukin-6 release and increases intracellular cytokine content in C6 astrocytoma cells in vitro

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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

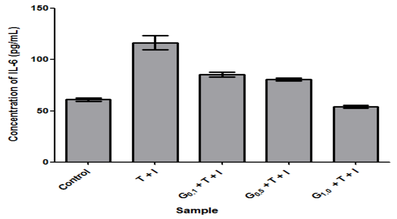
Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by memory loss and is the most common cause of dementia. It is has been hypothesized that pro-inflammatory cytokines induce the inflammation that is believed to be the cause of the neuronal death that is associated with AD. 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 1-β (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 $GABA_B$ receptor is acted upon to inhibit the release of IL-6.

Methods

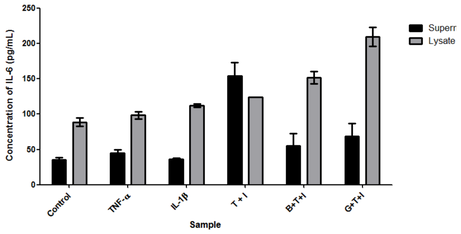
- Rat C6 astrocytoma cell line was used as model system
- Immonofluorescence microscopy
  - Used to determine subcellular location of accumulated IL-6
- Enzyme-Linked Immunosorbent Assay (ELISA)
  - Used to determine concentration of IL-6 in C6 Rat Astrocytoma supernatant and lysate

Results

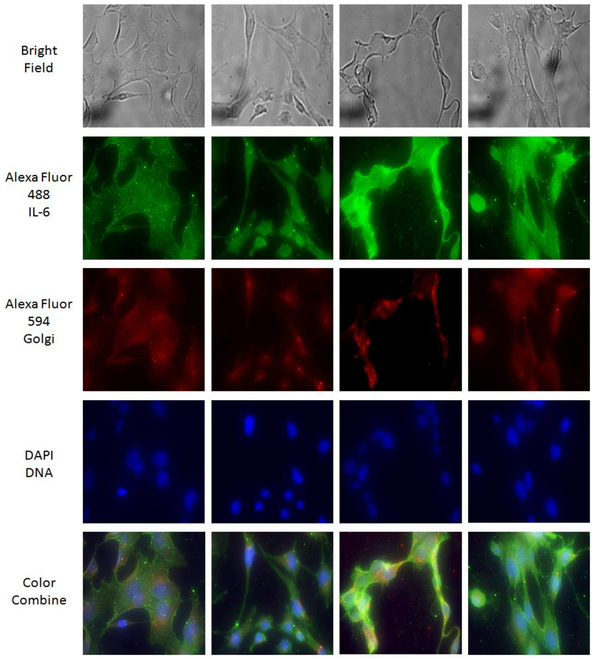
- Immunofluorescence microscopy
  - Accumulation of IL-6 was disbursed throughout the cell with little or no accumulation within the Golgi apparatus.
  - Cells treated with GABA and TI showed the greatest intracellular accumulation of IL-6
- Enzyme-Linked Immunosorbent Assay
  - GABA dose-dependently 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.
  - Baclofen and muscimol yielded similar levels of suppression of IL-6 release



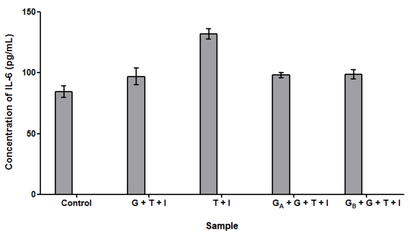
Effect of GABA and TNF-α plus IL-1β (TI) on extracellular levels of IL-6 in rat C6 astrocytoma cells *in vitro*. Cells were pre-treated with 0.1-1.0 mM GABA for 1 h. Cells were subsequently treated for 24 hours simultaneously with GABA and TI for 24 h ELISA was used to determine extracellular IL-6 concentrations (Pierce). The data are presented as a ± SEM of triplicate observations from a single representative experiment repeated four times.



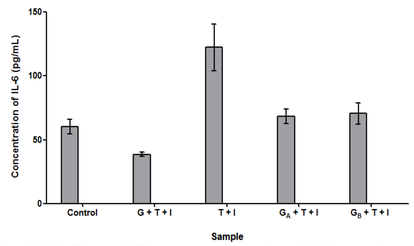
Effect of GABA and brefeldin-A (BFA) on cytokine-driven alterations of intracellular and extracellular IL-6 accumulations in rat C6 astrocytoma cells *in vitro*. Cells were pre-treated with either GABA (1 mM) or BFA (0.5. mg/ml) for 1 h. Cells were subsequently treated for 24 h with TNF- α (100 ng/mL), IL-1β (50 ng/mL), or both cytokines (*i.e.*, TI). GABA or BFA were also included with TI for 24 h as indicated. Supernatant was removed and cellular lysates generated using M-PER (Pierce). ELISA was used to determine medium and lysate IL-6 concentrations (Pierce). The data are presented as mean ± SEM of triplicate observations from a single representative experiment repeated four times.



Effect of GABA or brefeldin A (BFA) on IL-1β and TNF- α(TI) mediated synergistic expression of IL-6. Cells were pre-treated with GABA (1 mM) or BFA (0.5 mg/mL) for 1 h followed by co-treatment with IL-1β (50 ng/mL) and TNF- α (100 ng/mL) for 24 h. Cells were visualized by fluorescent microscopy. IL-6 was visualized using a monoclonal mouse anti-rat IL-6 antibody (Santa Cruz). Golgi was visualized using a polyclonal rabbit ant-rat TGN-38 antibody. Control = untreated; TI = TNF- α /IL-1β co-treatment; GTI = GABA/ TNF- α /IL-1β co-treatment; BTI = BFA/ TNF- α /IL-1β co-treatment. Data are representative of 3 independent trials.



Effect of GABA, TI, and $GABA_A$ receptor antagonist ,or $GABA_B$ receptor antagonist on extracellular levels of IL-6 (pg/mL) in rat C6 astrocytoma cells *in vitro*. Cells were pre-treated with the $GABA_A$ antagonist phaclofen (100 μM) or the $GABA_B$ antagonist bicuculline (20 μM) for 1 h followed by co-exposure to either antagonist plus GABA (1mM) for 1h. Supernatants were collected following a 24 h treatment of cells with antagonist plus GABA in the presence of TI. ELISA was used to determine supernatant IL-6 concentrations (Pierce). The data are presented as mean ± SEM of quadruplicate observations from a single representative experiment repeated three times.



Effect of GABA, TI, and $GABA_A$ receptor agonist, or $GABA_B$ receptor agonist on extracellular levels of IL-6 (pg/mL) in rat C6 astrocytoma cells *in vitro*. Cells were pre-treated with GABA (1mM), $GABA_A$ receptor agonist muscimol (100 μM) or the $GABA_B$ receptor agonist baclofen (100 μM) for 1 h. Supernatants were collected following a 24 hour treatment with GABA or agonist in the presence of TI. ELISA was used to determine supernatant IL-6 concentrations (Pierce). The data are presented as mean ± SEM of quadruplicate observations from a single representative experiment repeated two 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 $GABA_A$ receptor or the $GABA_B$ receptor alone, but instead acts on both receptors to suppress the TI mediated synergistic release of IL-6.

References

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Acknowledgements

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