REVIEW OF CLINICAL PHARMACOLOGY AND
PHARMACOKINETICS INTERNATIONAL
EDITION 24: 120 (2010)

Inhibition of the Inflammatory Response of Activated Microglia by a Novel 17 Spiro Analog of Neurosteroid Dehydroepiandrosterone

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Key words: Microglia, BV2 cells, inflammation, DHEA, BNN27

INTRODUCTION

Activated microglia plays an important role in the pathogenesis of the majority of neurodegenerative disorders, such as Alzheimer's, Parkinson's disease and Multiple Sclerosis. Upon activation, microglia, which possesses the role of the CNS macrophages, produces and secretes most of the pro-inflammatory cytokines including Tumor Necrosis Factor α (TNFα), Interleukin 6 (IL-6), and Interleukin 1α and β (IL-1α and IL-β). Recent studies have shown that microglia also expresses the necessary biosynthetic enzymes for the production of neurosteroids, such as Dehy-droepiandrosterone (DHEA), Pregnanolone and Allopregnanolone (Allo). DHEA is a multi-functional neurohormone exerting both neuroprotective effects as well as possible modulatory actions on the immune system. Although there are several studies on the role of DHEA on peripheral macrophages, the impact of neurosteroids on the production of inflammatory mediators from microglia cells has not been yet elucidated. Based on the above, the aim of our study was to characterize in details the role of DHEA and its synthetic analog BNN27 (a 17 spiro-neurosteroid which is recently developed in our laboratory, deprived of endocrine properties), on the inflammatory response of activated microglia cells.

METHODS

For our studies we used the murine microglia BV2 cell line. BV2 cells have been shown to maintain many microglia responses. The experimental procedure was as follows: BV2 cells were stimulated with lipopolysaccharide (LPS) in the absence or presence of DHEA or BNN27 (100 nM) for several time points (3, 6, 12, 24, and 48 hr following stimulation with LPS). The levels of TNF-α and IL-6 were determined by ELISA and proliferation rate was measured using the MTT assay. Moreover, the possible effect of DHEA and BNN27 on apoptotic cell death of BV2 cells was examined by Flow Cytometry Analysis (FACS).

RESULTS AND DISCUSSION

Our results showed that BNN27 significantly reduced the secretion of TNF-α following activation with LPS in every time point tested. However, DHEA did not have a similar effect, due to its possible metabolic conversion, by specific steroid converting enzymes that BV2 cells produce. Neither DHEA nor BNN27 affected the levels of IL-6 in the time points tested. In addition, the synthetic analog also reduced the proliferation rate of BV2 cells after LPS stimulation, thus further indicating its anti-inflammatory role, since increased rates of proliferation characterize activated microglia. Studies in progress aim to elucidate the role of DHEA and BNN93 on apoptosis. In summary, our findings provide evidence for the possible pharmacological use of the synthetic analog BNN27 in the inhibition of inflammatory responses of activated microglia and thus ultimately, in the combined treatment of various neurodegenerative disorders.