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Presidential Address

Neuropeptides as signal molecules in common with leukocytes and the hypothalamic–pituitary–adrenal axis

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Abstract

There exists a bidirectional regulatory circuit between the nervous and immune systems. This regulation has been shown to be mediated in part through neuroendocrine hormones and cytokines. Both systems have receptors for both types of signal molecules. The nervous system has receptors for cytokines and it also synthesizes cytokines. The immune system synthesizes and responds to cytokines. So, it is not too farfetched to believe that neuroendocrine peptide hormones could bind to leukocytes and modulate immune functions. However, it is not widely known that the immune system also synthesizes functional, neuropeptide hormones. This will be discussed in this paper citing a plethora of evidence. The aim of this paper is to summarize this evidence by using three neuropeptides that are synthesized by leukocytes and modulate immune functions as examples; corticotropin (ACTH), endorphin (END), and corticotropin releasing factor (CRF). The production and action of these three neuropeptides in the immune system will be explained. Finally, the potential physiological role of leukocyte-derived ACTH, END, and CRF in inflammation as a localized hypothalamic–pituitary-like axis is discussed.

Keywords: Psychoneuroimmunology; Neuroimmunology; HPA axis; ACTH; CRF; Endorphin; NF-κB; Neuroendocrine; Leukocytes

1. Introduction

Much progress has been made in psychoneuroimmunology (PNI) in the 28 years I have been involved in the field. Over the last year, I have had the honor of reading every abstract submitted for the annual meeting of the Psychoneuroimmunology Research Society. I also acted as guest editor for a series of reviews highlighting the developments of PNI to celebrate the 20th anniversary of *Brain Behavior and Immunity*. The molecular biology methodology combined with sophisticated behavioral approaches show PNI has come of age as a "mature science." To see how much the field in general has progressed during the past 20 years, I encourage you to read the 2007 series "20 Years of Brain Behavior and Immunity." In particular, PNI has made immense strides in understanding cytokines in the brain, sickness behavior, vagal afferent mediation of cytokine activity, sympathetic innervation and regulation of immune responses. Some of the early research topics have been slower to develop (or had fewer people interested in researching them). One such topic is the production of neuropeptides by leukocytes.

There are many reasons for the production of neuropeptides by the immune system being an under-developed topic. But, I believe the most prominent to be that the different specialties such as immunology and endocrinology do not know how to interpret or integrate such a finding in their systems. The difficulty immunologists have had in accepting PNI is well known and continues today. But the immunologists were also hesitant, at first to embrace cytokines since they were not antigen-specific factors. We still do not know what the essential role of IL-1 is, much less how a neuropeptide fits into the regulatory scheme of the immune system. This has been controversial from the very beginning. The first RO1 grant application Ed Blalock and I submitted on the topic prompted a site visit by the NIH reviewers to see if the findings were real. Even though

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there are over a hundred publications on the topic, it is rare to find leukocyte production of neuropeptides mentioned in immunology or neuroscience text books.

Many questions arose with the first evidence of leukocyte hormone production. Why does this production occur? What is the spectrum of neuropeptides produced? Does it occur through the same mechanisms as in the prototype tissues? And finally, what is the role of leukocyte production of neuropeptides? These are some of the same questions my laboratory has been trying to address, particularly in regard to corticotropin (ACTH) and corticotropin releasing factor (CRF). In this article I will discuss the production of three neuropeptides that are synthesized by leukocytes; ACTH, endorphin (END), and CRF and speculate on how they may interact in a localized circuit to affect immune responses.

2. Pro-opiomelanocortin (POMC) production in the immune system

The finding that leukocytes produce neuropeptides came early in the development of the PNI field. When I joined Ed Blalock's laboratory he was studying interferon's (IFN) novel, endocrine-like effects (another fundamental family of cytokines that was slow to be accepted as an immunological regulatory factor). Blalock found that IFN-α exhibited adrenergic activity on mouse myocardial cells and would increase their beat frequency in addition to inducing an antiviral response in the cells (Blalock and Stanton, 1980). This was a specific, IFN-α receptor-mediated effect because IFN is a species-specific cytokine and neither human IFN-α or murine IFN-γ had an effect on the murine myocardial cells. We started looking at other hormonal activities associated with IFNs (Smith and Blalock, 1981b). Mouse adrenal tumor cells (Y-1) and melanoma cells both responded to IFN-α. The Y-1 cells changed morphologically to a more rounded shape and the melanoma cells increased their pigmentation. ACTH causes similar changes in both cell types. One possible explanation other than an IFN receptor-mediated effect, was a structural homology (primary or tertiary) such that IFN could bind the ACTH receptor (melanocortin 2 receptor; MC2R). This was before the sequence of IFN-α was known, but was what our preliminary evidence suggested, a structural homology or common sequence between ACTH and IFN-α (Blalock and Smith, 1980). With further investigation we determined that the ACTH was co-expressed with the IFN in our system and present in the partially purified IFN preparation (Smith and Blalock, 1981a). Thus it was discovered that leukocytes could synthesize a neuropeptide.

Other hormones and neuropeptides have also been shown to be produced by, and have activity on, lymphocytes. Examples include CRF, END, enkephalins (ENK), growth hormone, prolactin, thyroid stimulating hormone (TSH), arginine vasopressin/oxytocin, vasoactive intestinal peptide, luteinizing hormone, substance P, and others (Kelley et al., 1986; Davila et al., 1987; Blalock et al., 1989; Hughes and Chin, 1994). Since leukocyte neuropeptide production occurs in many organisms, including less advanced ones such as invertebrates, this feature has been conserved through evolution. To be maintained it must serve a fundamental role (Stefano and Smith, 1995). These other neuropeptides and hormones likely have important roles in the systems. However, this paper will focus on ACTH, END, and CRF interactions in the immune system.

ACTH was the first and probably the best, characterized neuropeptide produced by leukocytes (Smith, 1994). ACTH was first observed in cell culture supernatant fluid from lymphocytes stimulated with Newcastle disease virus (NDV) to produce IFN (Smith and Blalock 1981a). The IFN could be removed from the preparation by proteolytic digestion and acid treatment to which ACTH is stable. Purification and biochemical characterization proved the leukocyte product to be actual ACTH. The leukocyte-derived ACTH had the same electrophoretic mobility on polyacrylamide gels, same migration on reverse phase high performance liquid chromatography (HPLC), was recognized by anti-ACTH antiserum, and was co-produced with END (Smith et al., 1990; Smith and Blalock 1981a). Leukocyte ACTH would induce mouse Y-1 adrenal cells to round up morphologically and to secret corticosteroids. The amino acid sequence of the purified, ACTH peptide and that of a cDNA from RT-PCR of the mRNA was identical with that from the pituitary (Smith et al., 1990; Smith and Blalock 1981a). Since ACTH and endorphin are cleavage products of POMC, we believe that the same POMC gene expressed in the pituitary gland is also expressed in leukocytes.

ACTH is produced by lymphocytes in response to various inducers in addition to NDV (Smith and Blalock 1981a; Westly et al., 1986). Other inducers include bacterial lipopolysaccharide (LPS) (Harbour-McMenamin et al., 1985; Smith and Blalock 1981a), CRF (Smith et al., 1986), and other viruses such as human immunodeficiency (HIV) (Smith et al., 1992; Hashemi et al., 1998), and Epstein–Barr (EBV) viruses (Oates et al., 1988).

The first studies to determine if leukocytes produced ACTH in vivo used a surgical approach to delete the primary source of the neuroendocrine hormones. Hypophysectomized mice were injected with NDV, and at multiple time points sacrificed, to measure serum corticosterone and then the splenocytes were stained by immunofluorescence for ACTH (Smith et al., 1982). There was a time-dependent rise in serum corticosterone that correlated with ACTH production by the splenocytes. Interestingly, pretreatment of the mice with dexamethasone prevented the splenocytes' ACTH production and serum corticosterone elevation. Thus leukocytes produce a biologically relevant amount of ACTH and corticosteroids are negative regulators of leukocyte ACTH similar to pituitary ACTH. Using an avian model of combined hypophysectomized and bursectomized animals, Bayle et al. showed B-lymphocytes produced ACTH in vivo in response to a bacterial antigen