Channel catfish, *Ictalurus punctatus*, leukocytes secrete immunoreactive adrenal corticotropin hormone (ACTH)

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Abstract

The interrelationships between the neuroendocrine and immune systems are becoming more understood, at least in mammalian systems. The most characterized of these relationships is that of hormonal signaling within the hypothalamo-pituitary-adrenal (HPA) axis. CNS-perceived signals stimulate the release of corticotropin releasing hormone (CRH) which in turn stimulates the release of pituitary corticotropin (ACTH) and ultimately the release of adrenal-cortex-derived corticosteroids. We demonstrate that channel catfish peripheral blood mononuclear cells, a channel catfish B-cell line (1G8) and a T-cell line (28S.1), constitutively and in response to CRF, secrete a molecule that is reactive with a mammalian RIA for ACTH (irACTH). The T-cell line was the most responsive to CRH and may provide a valuable model for understanding the interrelationships between the neuroendocrine and immune systems in lower vertebrates. Lymphoid derived ACTH, or ACTH-like products, in fish, as well as higher vertebrates, may represent a paracrine or autocrine control on lymphocyte function and immune regulation.

Introduction

Research in immunoendocrinology continues to elucidate the interrelationships between the immune and endocrine systems. Historically, the effects of physiological stress on immune function was assumed to be a direct result of adrenal corticosteroid production in response to pituitary corticotropin (ACTH) which is produced centrally in response to corticotropin releasing hormone (CRH). Thus, the effects of ACTH on immune cells were assumed to be indirect through the production of corticosteroids. Recent work has demonstrated that communication between the neuroendocrine and immune systems is bi-directional, involving shared messengers, receptors and effectors (Weigent and Blalock 1987). Corticotropin, a neuropeptide of pituitary origin was the first endocrine mediator to be found endogenously produced by cells of the immune system (Blalock and Smith 1980). This protein produced by human leukocytes and in murine splenic macrophages (Lolait et al. 1984) was similar in molecular weight and amino acid sequence to pituitary ACTH (Smith et al. 1990). Macrophages produce ACTH constitutively, however production has been documented in mammalian lymphocytes only after stimulation (Lyons and Blalock 1995; Olsen et al. 1992). ACTH production can be induced in lymphocytes by a variety of stimulants including viral infection, bacterial products (e.g., lipopolysaccharide, LPS), tumor cells, as well as by corticotropin releasing hormone (CRH), arginine vasopressin and oxytocin (Weigent and Blalock 1995).

While the primary function of ACTH is to stimulate the adrenal cortex secretion of corticosteroids, its direct function within the immune system is still in question. Various *in vitro* studies have found ACTH to both potentiate and inhibit the cellular immune response, depending upon the culture conditions. In C57BL/6 mouse spleen cell preparations the plaque forming cell response to both SRBC and DNP-Ficoll
was suppressed by ACTH (Johnson et al. 1982). However, in studies with human tonsillar B-cells, ACTH, when administered at physiological concentrations, was found to enhance B-cell proliferation and enhance, but not induce, Ig production (Alvarez-Mon 1985). ACTH has also been shown to inhibit LPS-induced TNF production in mice (Fantuzzi et al. 1995).

To date, most of the research in immunoen-docrinology has utilized mammalian models. Although the immunomodulation of glucocorticoids in the teleost is fairly well characterized, reports of the production by, and direct actions of ACTH, on fish immune cells are unclear. Two recent studies report the isolation of ACTH-like material from the neutrophils, monocytes and basophils of two species of teleost, *Crassius auratus* and *Coris julis* (Ottaviani et al. 1992). However, it has not been found in fish lymphocytes (Ottaviani et al. 1995).

This study was designed to investigate the production of ACTH by teleost lymphocytes using channel catfish (*Ictalurus punctatus*) as a model. The research utilized both peripheral blood lymphocyte (PBL) cultures and immortal cell lines. The effect of various concentrations of CRH on ACTH production was also examined. An important component of this work was the validation of a radioimmunoassay developed for detecting ACTH in a mammalian system for use with channel catfish.

**Materials and methods**

**Radioimmunoassay (RIA)**

All samples were assayed for ACTH[1–24] using a radioimmunoassay (RIA) kit (Diagnostics Products Corp., Los Angeles, CA). The kit utilizes a double antibody competitive binding assay for human ACTH [1–24] and includes primary anti-serum (rabbit anti-human ACTH [1–24]), an iodinated ACTH tracer ([125I]ACTH), lyophilized human ACTH [1–24] calibrators and a precipitating solution consisting of a secondary antibody (goat anti-rabbit gamma globulin) and dilute polyethylene glycol (PEG) in saline. According to the manufacturer the antisera is highly specific for ACTH with very low crossreactivity and accurate over a broad range of values. The kit is designed to measure human ACTH in a clinical setting which necessitated validation of the assay’s ability to quantify irACTH in channel catfish lymphocyte culture supernatants.

Channel catfish cell lines 1G8 (B-lymphocytes), F13L, and 28S.1 (both T-lymphocytes) (Clem et al. 1996) were generously provided by Dr. Norman Miller, University of Mississippi Medical School, Jackson, MS. Cell lines were placed in T-75 tissue culture flasks at an initial density of 5 × 10⁸ cells per flask. All reagents were obtained from Sigma, St. Louis, MO unless otherwise stated. The cells were cultured in channel catfish A/L5 medium which contains a 1:1 mixture of Aim V/Leibowitz’s-15 media (both from Gibco BRL, Gaithersburg, MD USA) supplemented with 100 U ml⁻¹ penicillin, 100 μg ml⁻¹ streptomycin, 2 mmol L-glutamine, 1% of a 7.5% sodium bicarbonate solution, 10% H₂O, 0.2% Fungizone, 50 μmol β-mercaptoethanol and 5% pooled heat-inactivated channel catfish serum, hereafter referred to as complete media (CM). The cells were incubated at 27°C under 5% CO₂ for 5 days, then removed from the flasks, centrifuged at 250 × gravity (g) for 10 min and supernatants collected and frozen at −70°C. Samples were later assayed using the ACTH [1–24] RIA.

Parallelism was tested for by assaying diluted samples of the supernatants. Samples from both cell lines were diluted 50% and assayed. A dilution curve was prepared with the T-cell supernatant diluted to 50%, 33% and 25%. Additionally, samples of 1G8 supernatants were spiked with known amounts of ACTH [1–24] using calibrators included in the RIA kit and assayed. In this and subsequent experiments irACTH concentrations were calculated from a logit-log calibration curve with a separate standard curve for each run of the assay. SAS® (1996, SAS Institute, Cary, NC) was used to calculate irACTH concentration and the sensitivity of each assay.

**irACTH production by peripheral blood leukocytes**

Four channel catfish were bled by caudal vein puncture into vacutainer tubes containing lithium heparin. Blood from each fish was diluted 1 part in 6 (1:5) with Hank’s balanced salt solution (HBSS) adjusted to an isomolarity of channel catfish plasma (270–280 mOsmol). A 1.077 g cm⁻³ histopaque cushion was layered under the cell suspension and centrifuged for 30 min at 300 × g. Cells at the interface were removed, washed 3 times in HBSS and resuspended in 1 ml of CM. Trypan blue exclusion was used to determine viability and cells were counted using a hemocytometer.