Domestic Animal Endocrinology 55 (2016) 11–24

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Interleukin 6 increases the in vitro expression of key proteins associated with steroidogenesis in the bovine adrenal zona fasciculata

S. McIlmoil, J. Strickland, A.M. Judd*

Department of Physiology and Developmental Biology and Neuroscience Center, Brigham Young University, Provo, UT, 84602, USA

ARTICLE INFO

Article history:
Received 17 June 2015
Received in revised form 20 September 2015
Accepted 11 October 2015

Keywords:
Interleukin 6
Zona fasciculata
Adrenal
Cytokine
Steroidogenesis

ABSTRACT

In this study, the in vitro effects of interleukin 6 (IL-6) on the messenger RNAs (mRNAs) and proteins for key steroidogenic factors in the bovine adrenal zona fasciculata (ZF) were determined. Bovine adrenal glands were obtained from an abattoir, and the ZF was isolated. Strips of ZF were then exposed to different concentration of murine IL-6 and/or adrenocorticotropic hormone (ACTH) for various intervals, the protein and mRNA extracted, and the mRNA and protein expression determined by real-time polymerase chain reaction and Western blots. Exposure (1 h) to IL-6 increased in a concentration-dependent manner (10-pg IL-6/mL, $P < 0.05$ vs control; 100-pg IL-6/mL, $P < 0.01$ vs control) the relative expression of the mRNAs and proteins for steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage enzyme (P450scc), 3β hydroxysteroid dehydrogenase type 2 (3β HSD), 17α-hydroxylase/17,20-lyase/17,20-desmolase (P450 17OH), steroid 21-hydroxylase (P450 21OH), steroid 11-β-hydroxylase type 1 (P450 11βOH), and steroidogenic factor 1 (SF-1), a nuclear factor that increases StAR and steroidogenic enzymes (SEs) expression. Similarly, IL-6 (10 pg/mL) increased the relative expression of proteins and mRNAs for StAR, P450scc, 3β HSD, P450 17OH, P450 21 OH, P450 11βOH, and SF-1 in a time-dependent manner (30 min, $P < 0.05$ vs control; 60, 120, and 240 min, $P < 0.01$ vs control). In contrast, IL-6 decreased in a concentration-dependent ($P < 0.01$ vs control for 1, 10, and 100 pg IL-6/mL) and time-dependent ($P < 0.05$ vs control for 30, 60,120, and 240 min of 10 pg IL-6/mL) manner the relative expression of the mRNA and protein for adrenal hypoplasia congenita-like protein (DAX-1), a nuclear factor that decreases expression of StAR and SEs. Incubation (1 h) of ZF with 100-nM ACTH increased ($P < 0.05$ vs control) the relative expression of StAR, P450scc, 3β HSD, P450 17OH, P450 21OH, P450 11βOH, and SF-1 and decreased ($P < 0.01$ vs control) the relative expression of DAX-1. Murine IL-6 (10 pg/mL) augmented ($P < 0.05$ vs ACTH) both the stimulatory and inhibitory effects of ACTH. Bovine IL-6 (100 pg/mL, 1-h incubation) also increased ($P < 0.01$ vs control) the relative expression of the proteins for StAR, P450scc, and SF-1 and decreased ($P < 0.01$ vs control) the relative expression of DAX-1. In summary, IL-6 increased ZF expression of StAR and 5 SEs, which may be mediated in part by decreasing DAX-1 expression and increasing SF-1 expression.

1. Introduction

The zona fasciculata (ZF) of the adrenal cortex releases cortisol on activation of the hypothalamic-pituitary-adrenal axis. Although a short-term increase in plasma cortisol has beneficial effects on the physiology of most

* Corresponding author. Tel.: 801 422 3179.
E-mail address: Allan_Judd@BYU.EDU (A.M. Judd).

0739-7240/$ – see front matter © 2016 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.domaniend.2015.10.003

animals, long-term increases in cortisol may have effects that are detrimental [1,2]. In cattle, sheep, and other domestic animals, these detrimental effects may include diminished vigor, decreased food consumption, poor digestive function, decreased weight gain, increased rates of infections, and diminished milk production [2–6]. In contrast, inadequate plasma cortisol may result in exaggerated immune and inflammatory responses that can result in extensive tissue damage and death of the animal [1,2,7].

During acute stress, the anterior pituitary releases adrenocorticotropic hormone (ACTH), and the plasma concentrations of ACTH and cortisol are generally positively correlated [2,8]. However, in various conditions including chronic inflammatory stress, there is a poor correlation between the plasma concentrations of ACTH and glucocorticoids in mice, rats, humans, cattle, dogs, sheep, and cats [2,8–15]. Therefore, it has been suggested that other factors in addition to ACTH may be regulating the release of cortisol and the rodent glucocorticoid corticosterone during some types of stress [2,8,16,17].

The cytokine interleukin 6 (IL-6) may be one of the factors involved in the regulation of cortisol release during inflammatory stress [2,8,16–18]. The injection of IL-6 into rodents and humans increases the release of ACTH and cortisol or the rodent glucocorticoid corticosterone [2,8,16,17]. Although some effects of IL-6 on glucocorticoid release are dependent on ACTH release from the anterior pituitary, it has been hypothesized that IL-6 in the plasma and/or locally produced IL-6 in the adrenal gland may directly stimulate the adrenal cortex to release glucocorticoids [2,8,16,17]. In support of this hypothesis, IL-6 protein and messenger RNA (mRNA) are expressed in the adrenal cortex of rats, humans, cattle, pigs, and baboons [2,8,16–21], and IL-6 is released from cultured adrenocortical cells from cattle and rats [16,18,20]. Furthermore, IL-6 increases the in vitro release of cortisol from human and bovine adrenocortical cells and corticosterone from rodent adrenocortical cells [16,17,19,22–26] probably through binding to the IL-6 receptors expressed in these tissues [16,19,26]. In support of a role of IL-6 receptors in the stimulation of cortisol release from adrenal cells, radioactive IL-6 binds to isolated ZF cells with a binding affinity similar to that of IL-6 receptor characterized in other tissues [16,26]. Further evidence supporting a physiological role of IL-6 directly on cortisol release from the ZF is that the concentrations of IL-6 that stimulate cortisol release from the bovine ZF cells in vitro (10–1,000 pg/mL) [26] are found in the plasma of normal humans (0.5–1,300 pg/mL) and cattle (12–3,000 pg/mL) [21,27]. In addition, infections and inflammation in domestic animals increase IL-6 synthesis and IL-6 plasma concentrations, and these conditions are accompanied by an increase in plasma cortisol concentrations [2,7]. Furthermore, experiments in rodents have provided support for a physiological role of IL-6 directly on the adrenal gland to increase glucocorticoid release [28–32]. Moreover, under some conditions in sheep, humans, and dogs, there is a positive correlation between plasma IL-6 and plasma cortisol [27,33,34].

Although IL-6 may have a physiological role in stimulating cortisol release from the adrenal ZF, the biochemical mechanisms through which IL-6 augments glucocorticoid release from adrenocortical cells have not been determined. In this study, we determined the effects of in vitro IL-6 on the ZF expression of the mRNAs and proteins of 6 key proteins involved in cortisol synthesis: 5 steroidogenic enzymes (SEs) and steroidogenic acute regulatory protein (StAR). In addition, we determined the effects of in vitro IL-6 on the expression of the mRNAs and proteins for the nuclear factors steroidogenic factor 1 (SF-1) that enhances the expression of SEs and StAR [35,36] and adrenal hypoplasia congenita-like protein (dosage-sensitive sex reversal, adrenal hypoplasia congenital, critical region on the X chromosome, gene 1 or DAX-1) that decreases the expression of SEs and StAR [35,37,38].

2. Materials and methods

2.1. Materials

Serum-free Roswell Park Memorial Institute 1640 medium (RPMI) (Life Technologies, Carlsbad, CA, USA) and complete RPMI were prepared as previously described [18,20,21,26]. Adrenocorticotropic hormone fragment 1-24 (Sigma-Aldrich, St. Louis, MO, USA, catalog number A0298), which sequence is conserved across most species including cattle and humans, was dissolved in sufficient sterile water to reach a concentration of 100-μM ACTH and stored in aliquots at −20°C. Three different preparations of IL-6 were used: recombinant murine IL-6 from J. Van Snick (Ludwig Institute, Brussels, Belgium), recombinant murine IL-6 (PeproTech, Rocky Hill, NJ, USA), and recombinant bovine IL-6 (Genway Biotech, San Diego, CA, USA). The IL-6 preparations were diluted with complete RPMI medium until a concentration of 1-μg IL-6/mL was achieved and stored in aliquots at −80°C. Serum-free RPMI medium was used to dilute the ACTH and IL-6 to the desired concentration immediately before each experiment. The recombinant IL-6 from Dr Van Snick was used for most of the experiments in this study with the purchased murine and bovine IL-6 used only in the last study to demonstrate that IL-6 from different sources had similar effects.

2.2. Cattle used in experiment

The adrenal glands used in these experiments were isolated from adult female cattle of mixed breeds (beef and dairy) of various ages that were sold to a local abattoir (Deseret Meat, Spanish Fork, UT, USA) because of various conditions including age, poor milk production, failure to get pregnant, or thinning of herds. Adrenal glands were collected at approximately 8:00 AM, and the animals had been removed from the pens for approximately 1 h before they were sacrificed. Adrenal glands were collected from the carcasses of cattle that had been sacrificed about 5 min previously.

2.3. Isolation of bovine adrenal tissue

The ZF from bovine adrenal glands (2 to 3 glands from different cows for each experiment) was isolated as explained previously [20,21,26]. The single gland harvested