Steroids xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Involvement of adenosine monophosphate activated kinase in interleukin-6 regulation of steroidogenic acute regulatory protein and cholesterol side chain cleavage enzyme in the bovine zona fasciculata and zona reticularis

Matharage S.I. De Silva, Adam W. Dayton, Lance R. Rhoten, John W. Mallett, Jared C. Reese, Mathieu D. Squires, Andrew P. Dalley, James P. Porter, Allan M Judd*

Department of Physiology and Developmental Biology and Neuroscience Center, Brigham Young University, 4005 LSB, Provo, Utah 84602, United States

ARTICLE INFO

Keywords:
Interleukin-6
Adenosine monophosphate activated kinase
Adrenal zona fasciculata
Adrenal zona reticularis
Steroidogenesis
StAR

ABSTRACT

In bovine adrenal zona fasciculata (ZF) and NCI-H295R cells, interleukin-6 (IL-6) increases cortisol release, increases expression of steroidogenic acute regulatory protein (StAR), cholesterol side chain cleavage enzyme (P450scc), and steroidogenic factor 1 (SF-1) (increases steroidogenic proteins), and decreases the expression of adrenal hypoplasia congenita-like protein (DAX-1) (inhibits steroidogenic proteins). In contrast, IL-6 decreases bovine adrenal zona reticularis (ZR) androgen release, StAR, P450scc, and SF-1 expression, and increases DAX-1 expression. Adenosine monophosphate (AMP) activated kinase (AMPK) regulates steroidogenesis, but its role in IL-6 regulation of adrenal steroidogenesis is unknown. In the present study, an AMPK activator (AICAR) increased ($P < 0.01$) NCI-H295R StAR promoter activity, StAR and P450scc expression, and the phosphorylation of AMPK (PAMPK) and acetyl-CoA carboxylase (PACC) (indexes of AMPK activity). In ZR (decreased StAR, P450scc, SF-1, increased DAX-1) ($P < 0.01$) and ZF tissues (increased StAR, P450scc, SF-1, decreased DAX-1) ($P < 0.01$), AICAR modified StAR, P450scc, SF-1 and DAX-1 mRNAs/proteins similar to the effects of IL-6. The activity (increased PAMPK and PACC) ($P < 0.01$) of AMPK in the ZF and ZR was increased by AICAR and IL-6. In support of an AMPK role in IL-6 ZF and ZR effects, the AMPK inhibitor compound C blocked ($P < 0.01$) the effects of IL-6 on the expression of StAR, P450scc, SF-1, and DAX-1. Therefore, IL-6 modification of the expression of StAR and P450scc in the ZF and ZR may involve activation of AMPK and these changes may be related to changes in the expression of SF-1 and DAX-1.

1. Introduction

The mammalian stress response involves the activation of the hypothalamic-pituitary-adrenal axis resulting in glucocorticoids release from the adrenal zona fasciculata (ZF) [1,2]. During acute stress, adrenocorticotrophic hormone (ACTH) is the primary regulator of glucocorticoid release [1,2]. However, during chronic stress involving inflammation and/or infection, there is a poor correlation between ACTH and glucocorticoid concentrations [3–9]. Thus, other factors regulate glucocorticoid concentrations in chronic inflammatory stress [3–9]. One of these factors may be interleukin-6 (IL-6) in that IL-6 increases glucocorticoid release through ZF IL-6 receptors [3,6,9–15]. Furthermore, adrenocortical cells of rats, humans, cattle, pigs, and baboons express IL-6 protein or mRNA [3,7–11,15–18] and various secretagogues increase IL-6 release from adrenocortical cells [15–17,19]. The adrenal cortex also contains immune cells that release IL-6 [7,10]. Various studies support a physiological/pathophysiological role for IL-6 in regulating glucocorticoid release by directly stimulating the ZF [8,9,14,20–28].

The zona reticularis (ZR) of the adrenal cortex releases adrenal androgens [29]. Although ACTH stimulates adrenal androgen release, other factors are involved in the regulation of this release because plasma concentrations of adrenal androgens and ACTH are often not correlated [29]. Acute stress increases plasma adrenal androgens [30–32] whereas prolonged stress decreases plasma adrenal androgens [31–34]. Plasma adrenal androgens concentration and/or adrenal androgen release also decrease during aging [29,35–37]. Similar to the ZF, ZR adrenal androgen release may be regulated by IL-6 in that IL-6 inhibits androgen release from bovine ZR cells [15,18] and there is evidence that the direct inhibitory effects of IL-6 on the ZR may have physiological/pathophysiological relevance in the decrease of adrenal androgens during the latter stages of inflammatory stress and aging

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

https://doi.org/10.1016/j.steroids.2018.02.009
Received 24 October 2017; Received in revised form 1 February 2018; Accepted 22 February 2018

[31–38].

The biochemical mechanisms through which IL-6 modifies adrenal function have been partially determined in the ZF and ZR and in NCI-H295R cells [18,39,40]. In the ZF [39] and NCI-H295R cells [40], IL-6 increases the expression of the protein/mRNA of steroidogenic acute regulatory protein (StAR) and cholesterol side chain cleavage enzyme (P450scc protein; CYP11A1 gene) that are key proteins involved in steroidogenesis [2,41]. This cytokine also increases the expression of the nuclear factor steroidogenic factor 1 (SF-1), that enhances StAR and P450scc expression [42,43]. In contrast, IL-6 inhibits the expression of adrenal hypoplasia critical region, on chromosome X, gene 1 (alternate name adrenal hypoplasia congenita-like protein) (DAX-1), a nuclear factor that decreases StAR and P450scc expression [44,45]. In the ZR, IL-6 has the opposite effect as in the ZF in that StAR, P450scc, and SF-1 expression is decreased and DAX-1 expression is increased [18].

In skeletal muscle, IL-6 stimulates the uptake of glucose through a mechanism dependent on adenosine monophosphate activated kinase (AMPK) [46]. Furthermore, AMPK regulates steroidogenesis [47–52]. Thus, we hypothesized that AMPK activation may alter the expression of steroidogenic proteins in ZF and ZR tissues and NCI-H295R cells. Furthermore, it was postulated that IL-6 effects on steroidogenesis in the ZF and ZR may involve AMPK-dependent mechanisms. Therefore, this study was designed to answer three questions: 1) What are the effects of AMPK activation on the expression of StAR, P450scc, SF-1 and DAX-1 in NCI-H295R cells and ZF and ZR tissues and does AMPK activation mimic the effects of IL-6? 2) Does IL-6 active AMPK in the ZF and ZR? 3) Does AMPK activation mediate the changes in StAR, P450scc, SF-1, and DAX-1 induced by IL-6 in the ZF and ZR?

2. Experimental

2.1. Cell culture media, solutions, AMPK activator and inhibitor, and IL-6 reagent

Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12) (ThermoFisher Scientific, Carlsbad, CA, USA) was utilized in all experiments involving NCI-H295R cells. Complete DMEM/F12 containing penicillin G and streptomycin (ThermoFisher Scientific), ITS supplement (Sigma-Aldrich, St. Louis, MO, USA) and Corning Nu Serum (ThermoFisher Scientific), serum-free DMEM/F12 medium containing only penicillin and streptomycin, DMEM/F12 medium supplemented with only ITS, and high serum DMEM/F12 medium were formulated as previously outlined [40]. Serum-free Roswell Park Memorial Institute medium 1640 (RPMI 1640) utilized for the ZF and ZR experiments, phosphate-buffered saline (PBS), protein extraction buffer for western blots, and luciferase extraction buffer were prepared as previously described [18,39,40].

Compound C (Cayman Chemical Company, Ann Arbor, MI, USA), was dissolved in DMSO at a concentration of 10 mM and the stock solution was stored in 5 μL aliquots at −80 °C. Compound C was then added to serum-free RPMI medium immediately before the start of each experiment. The AMPK activator 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) [47,48] (Sigma-Aldrich) was directly added to either serum-free RPMI medium (ZF and ZR experiments) or serum-free DMEM/F12 medium (NCI-H295R experiments) immediately before experiments. Recombinant bovine IL-6 (Genway Biotech San Diego, CA, USA) was dissolved in sterile serum-free RPMI-1640 medium (50 μg/mL), stored as 10 μL aliquots at −80 °C, and diluted with incubation media immediately before each experiment.

2.2. Cell culture

NCI-H295R cells (American Type Culture Collection, Manassas, VA, USA) were perpetuated [40] and the cells inoculated into cell culture vessels for two types of experiments. For StAR promoter assays, 1.25×10^5 NCI-H295R cells were inoculated into each well of two 12-well Corning Costar cell culture plates (Sigma-Aldrich) containing 2 mL complete DMEM/F12 medium. For experiments involving measurements of protein and mRNA expression, 1×10^6 cells were inoculated into Nunclon 100 mm cell culture dishes (Sigma-Aldrich) containing 12 mL complete DMEM/F12 medium. During all experiments, the cells were incubated at 37 °C in a 5% CO_2:95% air atmosphere and the cellular morphology observed with an inverted microscope (Talaval 31, Zeiss, Jana, Germany) to monitor cellular viability. There were no observable changes in cellular morphology in any treatment group.

2.3. Effects of AICAR on the expression of StAR and P450scc proteins and AMPK and acetyl-CoA carboxylase phosphorylation in NCI-H295R cells

The NCI-H295R cells were grown in 100 mm dishes until they were 70–80% confluent. On the day of the experiment, the complete DMEM/F12 medium was removed from the cells and replaced with 5 mL serum-free DMEM/F12 medium (control) or AICAR in serum-free DMEM/F12 medium. In time-course experiments, the cells were exposed to the control or AICAR (1 mM) medium for 30, 60, or 180 min. To determine the concentration-related effects of AICAR, the NCI-H295R cells were exposed to the control medium or serum-free medium containing 0.5, 1.0, or 2 mM AICAR for 60 min. Following the completion of the experiment, the protein was extracted from the cells and stored at −90 °C [43].

2.4. Effects of AICAR on StAR promoter activity in NCI-H295R cells

The NCI-H295R cells in 12-well plates were incubated for 18–24 h before the start of experiments. These cells were then transfected with 1 μg/well of the basic plasmid pGL2 (plasmid control) (Promega, Madison, WI, USA) (wells 1 and 2 on each 12-well plate) or with 0.5 μg/well of a 6456 base pair pGL2 plasmid containing the StAR promoter (858 bp) region linked to the luciferase gene (provided by Dr. Jerome F. Strauss III, Virginia Commonwealth University, Richmond, VA, USA) and 0.5 μg/well of pSV-β-galactosidase control plasmid (Promega) as an internal control for transfection efficiency (wells 3–12 on each 12-well plate). These transfections were accomplished with Lipofectamine LTX with PLUS Reagent (ThermoFisher Scientific) [40]. Generally, two 12-well plates containing NCI-H295R cells were utilized for each experiment. Following completion of the transfection, the transfection mixture was removed from the wells and replaced with 1 mL/well of high serum (2×) DMEM/F-12 medium without ITS or antibiotics and the cells were incubated for 16–24 h.

On the day of the experiment, the high serum DMEM/F12 medium was removed from the cells, replaced with 1 mL of DMEM/F-12 containing only ITS, and the cells incubated at 37 °C for 30 min. The time-dependent and concentration-dependent effects of AICAR on StAR promoter activity were then determined. For the concentration-dependent experiments, the DMEM/F12 medium containing only ITS was removed from the cells. Wells 1 and 2 and 13 and 14 (on second plate) (basic pGL2 control) received 1 mL of serum-free DMEM/F12 medium as a control for non-specific luciferase and β-galactosidase activity. The remaining 20 wells were incubated for 60 min in 1 mL of serum-free DMEM/F12 medium containing the following treatments: wells 3–6 (no AICAR, control), wells 7–10 0.5 mM AICAR, wells 11 and 12 and 15 and 16 (second plate 1.0 mM AICAR), wells 17–20 1.5 mM AICAR, and wells 21–24 2.0 mM AICAR. In some experiments, the control and order of the various AICAR concentrations were reversed to eliminate any positional bias.

The time-course experiments were executed by staggering the starting incubation time of the various treatment groups and incubating the cells in either 1 mL control medium or 1 mL medium containing 1 mM AICAR. At 180 min before the termination of the experiment, the DMEM/F12 medium containing only ITS was removed from the cells in wells 1, 2, 13 and 14 (basic pGL2 control) and these wells received 1 mL of serum-free DMEM/F12 medium as a control for non-specific