Journal of Steroid Biochemistry & Molecular Biology 109 (2008) 224–229

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry and Molecular Biology

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

From integrative signalling to metabolic disorders☆

Mélissa Otis[a,1], Marie-Claude Battista[a,1], Mylène Provencher[b], Shirley Campbell[c], Claude Roberge[a], Marcel Daniel Payet[d], Nicole Gallo-Payet[a,*]

[a] Service of Endocrinology, Department of Medicine, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, 3001, 12th Avenue North, Sherbrooke, Quebec, CanadaJ1H 5N4
[b] Centre de recherché, Hôpital Ste-Justine, 3175 Côte Ste-Catherine, Montréal, Quebec, CanadaH3T 1C5
[c] Department of Pharmacology, University of Vermont, Sherbrooke, Quebec, CanadaJ1H 5N4
[d] Department of Physiology and Biophysics, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Quebec, CanadaJ1H 5N4

ARTICLE INFO

Keywords:
Adrenocortical cells
Angiotensin II
ACTH
Seladin-1
Collagen
Fibronectin

ABSTRACT

The adrenal cortex undergoes constant dynamic structural changes, a key element in ensuring integrative functionality of the gland. Studies have shown that the cellular environment can modulate cell functions such as proliferation and steroid secretion. For example, 3-day treatment with angiotensin II promotes protein synthesis with a concomitant decrease in proliferation of glomerulosa cells, when cultured on fibronectin, but not on collagen IV or laminin. These effects involve close interaction between cytoskeleton-associated proteins and activation of p42/p44^{mapk} and p38 MAPK pathways. On the other hand, adrenocorticotropin hormone (ACTH), which is clearly the most potent stimulus of fasciculata cells, induces specific modulation of targeted proteins, when cells are cultured on collagen IV, but not on fibronectin or laminin. In particular, ACTH treatment leads to increased expression of Seladin-1 and induces the relocalization of Seladin-1 from the cytoplasm to the nucleus, both *in vivo* and in culture conditions, in adult rats and in human fetal adrenal glands. As a whole, these results indicate that Seladin-1, together with collagen IV, is able to modulate ACTH responsiveness. Hence, Seladin-1 may participate in the regulation of steroidogenesis when localized in the cytoplasm, while conversely protecting cells against oxidative stress generated by intense ACTH stimulation when massively localized in the nucleus.

1. Introduction

The adult adrenal cortex is composed of three concentric layers, the zona glomerulosa, the zona fasciculata and the zona reticularis, all of which present different morphological and functional properties. The zona glomerulosa secretes the mineralocorticoid aldosterone while the zona fasciculata secretes glucocorticoids and, in primates, the zona reticularis synthesizes deshydroepiandrosterone (for a review, see [1,2]). The adrenal cortex has the property of adapting its morphology to environmental conditions. For instance, *in vivo*, a sodium-deficient diet increases cell proliferation and width of the zona glomerulosa. On the other hand, hypophysectomy decreases [3], while ACTH treatment increases, adrenal gland volume [4] and vascularization.

Angiotensin II (Ang II) is considered to be the main hormonal stimulus of the zona glomerulosa [5,6], enhancing both aldosterone secretion and proliferation *in vivo* [7,8] and protein synthesis *in vitro* [9]. Adrenocorticotropin hormone (ACTH) is the main stimulus of the zona fasciculata, stimulating cortisol/corticosterone secretion. ACTH acts not only on the immediate stimulation of adrenal steroid synthesis and release, but also increases the expression of a number of genes including those involved in steroidogenesis [10]. Despite numerous studies using both animal and human models, the precise molecular mechanisms by which Ang II and ACTH stimulate growth and secretory activities are complex and still poorly understood.

In addition to Ang II and ACTH, the cytoskeleton and extracellular matrices (ECM) also participate in adrenocortical cell growth and secretion [11,12]. To our knowledge, there is no information as to whether ECM alone are involved in basal maintenance of adrenocortical functions and whether the effects of Ang II and ACTH could be modulated by the main ECM. This review highlights two significant examples of interactions between ECM and hormones, in both glomerulosa and fasciculata cells. The first instance describes how Ang II and fibronectin matrix interact to modulate prolifera-

☆ Presented at the 12th International Congress on Hormonal Steroids and Hormones & Cancer' (Athens, Greece, 13–16 September 2006).

* Corresponding author. Tel.: +1 819 564 5243; fax: +1 819 564 5292.
E-mail address: Nicole.Gallo-Payet@USherbrooke.ca (N. Gallo-Payet).

[1] Both first authors.

0960-0760/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2008.03.010

tion and protein synthesis in glomerulosa cells. The second example illustrates how collagen IV is able to modulate ACTH-induced intracellular localization of a protein postulated to play a key role in adrenocortical function.

2. Materials and methods

2.1. Preparation of cell cultures and steroid measurements

Glomerulosa and fasciculata cells were obtained from adrenal glands of female Long Evans rats weighing 200–250 g, and isolated according to the method previously described in detail [9,13]. All protocols were approved by the Animal Care and Ethics Committee of our institution. Fetal adrenal glands were obtained from fetuses aged 17–18 weeks (post-fertilization) at the time of therapeutic abortion and processed as previously described [14]. The project was approved by the human subject review committee of our institution. Cells were cultured at 37 °C in a humidified atmosphere composed of 95% air–5% CO_2. Cells were incubated in the absence or presence of Ang II or ACTH for 3 days or for 2 h incubations at 37 °C in 95% air–5% CO_2. Steroids were assayed by RIA, using specific antisera and tritiated steroid as tracer.

2.2. Proliferation assays and protein synthesis measurements

Cell proliferation was measured using fluorescence BrdU incorporation. After appropriate stimulation, cells were fixed, permeabilized and incubated with anti-BrdU Alexa Fluor-594 (1:500). The relative amount of protein synthesis was determined by assessing tritiated phenylalanine incorporation, as described by Otis et al. [9].

2.3. Immunofluorescence studies

For immunofluorescence detection of fibronectin, frozen sections of rat adrenal glands were fixed, incubated with anti-FN (1:50), followed by incubation with a goat secondary antibody conjugated to Alexa-Fluor 488 nm (Molecular Probes, Eugene, OR). Nuclei were stained with DAPI and the sections were mounted with Vectashield mounting medium and examined under a Nikon Eclipse 300 microscope (Mississauga, Ont., Canada) equipped with a CoolSnap fx digital camera (Roper Scientific, Tucson, AZ). Images were acquired using a 40× objective.

The Seladin-1 antiserum was produced by Affinity Bioreagents (CO, USA). Rabbits were immunized with the same peptide as that used by Greeve et al. [15], consisting of amino acid residues 203–218 of Seladin-1 H_3N–TPSENSDLFYAVPWSC–COOH, maleimide conjugated to keyhole-limpet hemocyanin (KLH). This sequence is identical in humans, rats and mice. Immunofluorescence *in situ* was performed as described previously [16,17]. Tissue sections and cells were incubated with the primary anti-Seladin-1 antiserum or with the pre-immune sera (both 1:1000) followed by incubation with a secondary conjugated anti-mouse antibody coupled with Alexa-Fluor 488 nm or 594 nm (1:500; green or red) for 1 h at room temperature. Sections and cells were stained with DAPI (1:1000; blue) for visualization of nuclei. In all cases, no specific staining was observed when primary antiserum was replaced by pre-immune serum at the same dilution and exposure time. Images were acquired using identical camera settings for contrast and brightness.

2.4. PCR and DNA microarray studies

Total RNA was isolated using RNAaqueous-4PCR according to the manufacturer's recommendations. DNA microarrays were performed as described by Battista et al. [18], also had last reference (Battista, 2007).

2.5. Subcellular fractionation and Western blotting

Subcellular protein fractioning was performed on freshly isolated zona fasciculata cells by using the ProteoExtract® subcellular proteome extraction kit according to the manufacturer's instructions (Calbiochem). Total protein extraction and Western blotting was performed as previously described [9]. Membranes were incubated with anti-Seladin-1 (dilution 1:1000).

3. Results and discussion

3.1. Proliferation and protein synthesis of adrenocortical cells cultured on various extracellular matrices

Glomerulosa cells easily proliferate in culture (doubling in 2 days) comparatively to fasciculata cells, which grow much slower [13,19]. Proliferation of glomerulosa and fasciculata cells was stimulated on all ECM tested (fibronectin, laminin and collagen IV) compared to plastic conditions, with the highest rate observed on collagen IV (Fig. 1A and B). Protein synthesis of glomerulosa cells was decreased on laminin and fibronectin, whereas basal protein content of fasciculata cells was not affected by the various matrices (Fig. 1C and D). Interestingly, addition of Ang II did not modify proliferation induced by laminin or collagen IV, but did inhibit proliferation observed on plastic and on fibronectin, with a concomitant increase in protein synthesis (Fig. 1A and C). On the other hand, ACTH treatment increased proliferation of glomerulosa (not shown) and fasciculata cells only on collagen IV (Fig. 1B). ACTH treatment also increased protein synthesis on plastic, fibronectin and collagen IV, but not on laminin (Fig. 1D). Based on these observations, the effects of Ang II and FN in zona glomerulosa cells and of ACTH and collagen IV in zona fasciculata cells were examined in more detail.

3.2. Proliferation and protein synthesis in glomerulosa cells: interaction between Ang II and fibronectin

We previously investigated which signalling pathways were possibly involved in basal proliferation as well as which intracellular proteins were targeted in the shift from proliferation to hypertrophy elicited by Ang II. Ang II induces a time-dependent increase in p42/p44^{mapk} phosphorylation and a rapid and transient increase in p38 MAPK phosphorylation, both of which are involved in the observed increase in protein synthesis and in the expression of p27^{Kip1}, which, in turn, is responsible for inhibition of the cell cycle [9]. Ang II also increases the expression levels of StAR and 3β-HSD which are dependent on p42/p44^{mapk} and p38 MAPK activation. This suggests that p42/p44^{mapk} and p38 MAPK are involved in Ang II-stimulated aldosterone production by enhancing expression of StAR and 3β-HSD proteins [20]. These results complement the observations that, in bovine adrenal glomerulosa cells, p42/p44^{mapk} is involved in Ang II-induced phosphorylation of cholesterol ester hydrolase [21] and p38 MAPK in the Ang II-induced release of calcium from intracellular pools [22].

On the other hand, the cytoskeleton modulates the action of Ang II in the adrenal gland [11,23]. A recent study from our group [20] has provided evidences that basal proliferation requires a well-structured organization of actin filaments into stress fibers and a specific Rho/ROCK-dependent activation of p44^{mapk} (ERK1). The increase in cell protein content by Ang II