Melatonin Exerts Direct Inhibitory Actions on ACTH Responses in the Human Adrenal Gland

Article *in* Hormone and Metabolic Research · February 2011

DOI: 10.1055/s-0031-1271693 · Source: PubMed

Melatonin Exerts Direct Inhibitory Actions on ACTH Responses in the Human Adrenal Gland

Authors
C. Campino[1,*], F. J. Valenzuela[2,*], C. Torres-Farfan[2], H. E. Reynolds[2], L. Abarzua-Catalan[2], E. Arteaga[1], C. Trucco[3], S. Guzmán[3], G. J. Valenzuela[4], M. Seron-Ferre[2,5]

Affiliations
Affiliation addresses are listed at the end of the article

Key words
- neurohormone
- steroidogenesis
- clock genes
- human adrenal function

Abstract

In nonhuman primates and rodents, melatonin acting directly on the adrenal gland, inhibits glucocorticoid response to ACTH. In these species, an intrinsic adrenal circadian clock is involved in ACTH-stimulated glucocorticoid production. We investigated whether these findings apply to the human adrenal gland by determining i) expression of clock genes in vivo and ii) direct effects of melatonin in ACTH-stimulated adrenal explants over a) expression of the clock genes *PER1* (Period 1) mRNA and BMAL1 [Brain-Muscle (ARNT)-like] protein, ACTH-induced steroidogenic acute regulatory protein (StAR), and 3β-hydroxysteroid dehydrogenase (3β-HSD) and b) over cortisol and progesterone production. Adrenal tissue was obtained from 6 renal cancer patients undergoing unilateral nephrectomy-adrenalectomy. Expression of the clock genes *PER1*, *PER2*, *CRY2* (Cryptochrome 2), *CLOCK* (Circadian Locomotor Output Cycles Kaput) and *BMAL1*, was investigated by RT-PCR in a normal adrenal and in an adenoma. In independent experiments, explants from 4 normal adrenals were preincubated in culture medium (6 h) followed by 12 h in: medium alone; ACTH (100 nM); ACTH plus melatonin (100 nM); and melatonin alone. The explants' content of *PER1* mRNA (real-time PCR) and StAR, 3β-HSD, BMAL1 (immuno slot-blot), and their cortisol and progesterone production (RIA) were measured. The human adrenal gland expresses the clock genes *PER1*, *PER2*, *CRY2*, *CLOCK*, and *BMAL1*. ACTH increased *PER1* mRNA, BMAL1, StAR, and 3β-HSD protein levels, and cortisol and progesterone production. Melatonin inhibited these ACTH effects. Our study demonstrates, for the first time, direct inhibitory effects of melatonin upon several ACTH responses in the human adrenal gland.

received 18.11.2010
accepted 12.01.2011

Bibliography
DOI http://dx.doi.org/10.1055/s-0031-1271693
Published online: February 17, 2011
Horm Metab Res 2011; 43: 337–342

ISSN 0018-5043

Correspondence
M. Seron-Ferre, PhD
Departamento de Fisiopatología
ICBM
Facultad de Medicina
Universidad de Chile
Salvador 486
Casilla 16038
Santiago
Chile
Tel.: +56/2/274 1560
Fax: +56/2/274 1628
mseron@med.uchile.cl

Introduction

Melatonin, the hormone of the night, has a wide range of physiological actions in such diverse tissues as the suprachiasmatic and other brain nuclei, pancreatic B cells, brown adipose tissue, diverse vascular territories, immune system, and also on steroid secreting glands [1–3]. Within the latter, we demonstrated that melatonin, acting directly on the adrenal gland, inhibits glucocorticoid response to ACTH in nonhuman primates, sheep, and rats [4–6]. Suggestive that this may also extend to humans, we found that the human adrenal gland expresses the MT_1 receptor and that melatonin acutely depresses cortisol response to ACTH in dexamethasone suppressed men [7].

We and others have demonstrated that in the nonhuman primate and rodent adrenal gland, ACTH stimulation of glucocorticoid production by the adrenal cortex involves an intrinsic adrenal circadian clock [8–10]. These clocks are sustained by the stimulatory and inhibitory transcriptional-translational feed-back loops of a group of genes, named clock genes, and their proteins. Of these, *PER1* is part of the inhibitory loop and *BMAL1* is part of the stimulatory loop. BMAL1 [Brain-Muscle (ARNT)-like] protein also drives a number of genes containing E-boxes in their promoters like the steroidogenic acute regulatory protein (StAR) [11], mediating cholesterol transport to the mitochondria; a limiting step in ACTH-stimulated adrenal cortisol production [12]. A possible relationship between melatonin, human adrenal cortisol response to ACTH and clock genes, is suggested by the direct inhibitory effect of melatonin on *BMAL1* and *PER2* mRNA expression demonstrated in the capuchin monkey adrenal gland [13].

[*] Both authors contributed equally to this work.

In the human adrenal gland, information about direct interactions of melatonin, clock genes and ACTH is not available. A limitation in studying such interactions is that only few clinical conditions require removal of a healthy human adrenal gland. In the present study, we used normal adrenal tissue from a small number of renal cancer patients undergoing laparoscopic unilateral nephrectomy-adrenalectomy for removal of an upper pole renal carcinoma. In these human adrenals, we explored the interactions of melatonin and clock genes under ACTH stimulation and its overall effect on cortisol production. We investigated: i) expression of clock genes in the human gland ex vivo and ii) direct effects of melatonin on ACTH-stimulated human adrenal gland explants over a) expression of the clock genes *PER1* mRNA and BMAL1 protein, ACTH-induced steroidogenic acute regulatory protein (StAR), and 3β-hydroxysteroid dehydrogenase (3β-HSD) and b) over cortisol and progesterone production.

Subjects and Methods

Subjects

Adrenal tissue was obtained from patients undergoing laparoscopic unilateral nephrectomy-adrenalectomy for removal of an upper pole renal carcinoma. Patients were recruited at the Hospital Clínico de la Pontificia Universidad Católica de Chile from cases not treated with medication that alters adrenal function. Adrenals from 6 renal cancer patients (48 to 77 years old) that met the criteria described below were collected at different dates over 2 years. 5 adrenal glands were removed between 10:00–12:00 h and one at 17:00 h. The experimental protocols were explained to the subjects and they provided written informed consent, according to the guidelines of the Declaration of Helsinki. The protocol was approved by the Ethical Committee of the Facultad de Medicina, Pontificia Universidad Católica de Chile.

Methods

Adrenal tissue preparation and experimental protocol

At each surgical intervention, samples of adrenal tissue (about 100–400 mg, judged to be mainly cortex by the surgeon's criteria) were sent to our laboratory and to pathology. As we could not know in advance whether the adrenals were normal, when the amount of tissue was adequate, adrenals were cut into explants as soon as received from the surgery room, and cultured following the experimental procedures described below. If tissue was scant, adrenals were collected only in TRIzol. Experiments were analyzed when the pathology report was normal. Adrenal samples from 2 patients were stored in TRIzol and processed later to identify expression of the clock genes *PER1*, *PER2*, *CRY2*, *CLOCK*, and *BMAL1* by RT-PCR. These adrenals were determined to be a normal gland and an adenoma. Adrenal specimens from the other 4 patients were reported by pathology as normal.

Fresh human adrenal gland explants were prepared, as previously described [4], immediately after receiving the surgical specimens. In brief, adrenal glands were cut in small explants (about 15–30 mg) which were mixed, suspended, and preincubated at 37 °C, 5% CO_2 for 6 h starting at 14:00 h. The adrenal tissue obtained at 17:00 h was maintained overnight at 4 °C, and treatment was initiated at 14:00 h the next day. At 20:00 h, explants were incubated in triplicate for 12 h in 2 ml medium alone (control) or containing 100 nM of 1–24 ACTH, 100 nM ACTH + 100 nM melatonin or 100 nM melatonin alone. Incubations ended at 08:00 h. After incubation, the medium was collected and stored at −20 °C to measure cortisol and progesterone production. The explants were weighed and processed in TRIzol for mRNA and protein extraction.

Our experiments explored the interaction between ACTH and melatonin at an approximate night time clock time interval (20:00 h to 08:00 h), hours at which the human adrenal is normally exposed to the nocturnal increase of melatonin [1]. The dose of ACTH was selected after corroborating in 2 independent experiments, that 100 nM elicits a maximal cortisol response in the human adrenal gland. The 100 nM dose of melatonin was chosen based on our previous studies using adrenal gland explants from other mammals [1–6, 14] in which 10–100 nM of melatonin inhibited ACTH-stimulated cortisol production.

Materials

DMEM-F12 and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St. Louis, MO, USA), 1–24 ACTH (Cortrosyn) from Organon Laboratories (Oss, Holland), TRIzol reagent from Invitrogen (Invitrogen Corp. Carlsbad, CA, USA). SV Total RNA Isolation System was purchased from Promega (Promega, Madison, WI, USA). Rabbit polyclonal antiserum anti-StAR and rabbit polyclonal antiserum anti-3β-HSD were generously donated by Prof. Ian Mason (University of Edinburgh, Scotland). Rabbit polyclonal antiserum anti-BMAL-1 (ab49421) was purchased from Abcam (Cambridge, UK). Goat anti-rabbit immunoglobulin antibody, polyvinylidene difluoride membranes (PVDF) and Amplified Opti-4CN kit were purchased from Bio-Rad (Bio-Rad Laboratories Inc., Hercules, CA, USA). Primers for *PER1*, *PER2*, *CRY2*, *CLOCK*, and *BMAL1* were purchased from Integrated DNA Technologies, Inc. (Coraville, IA, USA). SYBR Green Master Mix was purchased from Applied Biosystem (Foster City, CA, USA). Real-time PCR was performed in a Real-Time PCR system (StepOne, Applied Biosystem, Foster City, CA, USA).

RT-PCR

Total RNA was extracted with TRIzol according to the manufacturer's instructions from one normal human adrenal, an adrenal adenoma, and from human adrenal explants after incubation. Total RNA concentrations were measured by absorbance at 260 nm. cDNAs were synthesized from 3 µg of total RNA using random hexamers. For semi-quantitative PCR reactions, samples were analyzed at 150–300 ng equivalents to total RNA per tube. Primers were: *PER2* (for: 5′-gaattcctcctgagaagat-3′, rev: 5′-ttgatgaagctggaccage-3′), *CRY2* (for: 5′-ggcctgggttgccaactatg-3′, rev: 5′-actgcaggacagccacaatcca-3′), *CLOCK* (for: 5′-cagggcaccaccaatagg-3′, rev: 5′-gcttccttgagaactcactgtg-3′) and *BMAL1 (*for: 5′-agcagatggatttttgtttgtcg-3′, rev: 5′-cgagaaacatactccatagat-3′) [15], *PER1* (for: 5′-agagcccatccccacccagcagtt-3′, rev: 5′-tcggcccgtcaggaaggaga-3′ [16]. All primers utilized pass through an intron, avoiding DNA amplification. PCR conditions for *PER2*, *CLOCK*, and *BMAL1* were: 4 min at 94 °C initial denaturation followed by 36 cycles of 1 min at 94 °C, 1 min at 54 °C, 2 min 72 °C, and 10 min of final extension at 72 °C. Conditions for *PER1* and *CRY2* were similar, except for using 60 °C and 61 °C, respectively, as annealing temperature. A control without template and a control without reverse transcription were included in each PCR run. PCR products were separated by electrophoresis on a 2% agarose-ethidium bromide gel. The gel image was captured with a digital camera (Olympus CamediaMaster 4.1, Tokio, Japan) using DocIT Software (UVO, Inc., Upland, CA). The expected PCR products

size for *PER1*, *PER2*, *CRY2*, *CLOCK*, and *BMAL1* were 404 bp, 282 bp, 525 bp, 386 bp, and 564 bp, respectively.

In adrenal explants, RNA was extracted as described above and then treated with DNAse using SV Total RNA Isolation System according to the manufacturer's instructions. *PER1* and *18S rRNA* gene expression was measured by real-time PCR system (StepOne, Applied Bioystem) using the primers for *PER1* described above and for *18S-rRNA* (for: 5′-gtaacccgttgaaccccatt-3′, rev: 5′-ccatccaatcggtagtagcg-3′ [17]). The final reaction volume was 12.5 µl (6.25 µl SYBR Green Master Mix, 0.375 µl sense and antisense primers 0.3 µM, 3.0 µl free nuclease water, and 2.5 µl cDNA). The melting temperature (melting point or Tm) of the PCR product for *PER1* was 84 °C and for *18S*-rRNA was 78 °C. Product size was confirmed by gel electrophoresis. The Per1 PCR product was purified using Wizard SV gel and PCR clean-up System (Promega), following the manufacturer's instructions. Sequencing was performed in a Genetic Analyzer ABI PRISM 310, Applied Biosystems, at the Unidad de Diversidad Molecular, Departamento de Ecología, Pontificia Universidad Católica de Chile. 92 % of the PCR product was completely sequenced showing 100 % identity with bases 582–929 of the reported sequence of human *PER1* (GenBank accession number NM_002616.21). Samples were measured using a standard curve constructed with serial dilutions of known quantities for *PER1* and *18S-rRNA* products. These products were prepared by conventional PCR, purified, amplified, and quantified by densitometry against a 300 bp DNA marker of known concentration, and finally stored in aliquots at −20 °C. Nontemplate controls were included in every PCR reaction and a cDNA pool was included to assess inter assay variability. The threshold cycle (Ct) of each sample and the internal control was interpolated in the respective standard curve. *PER1* and *18S*-rRNA concentration in each sample was measured in several cDNA dilutions (equivalent to 30 to 0.3 ng RNA for *PER1* and to 4 to 0.02 ng for *18S*-rRNA). The ratio *PER1*/*18S*-rRNA was calculated for each sample and normalized by calculating the percentage of each treatment with respect to control. Inter assay variation was 6 % for *18S-rRNA* and 10 % for *PER1*.

Semi-quantification of BMAL1, StAR, and 3β-HSD protein levels in human adrenal gland explants

The protein levels of BMAL1, StAR, and 3β-HSD in adrenal explants from the adrenal glands of 3 patients were measured by an amplified slot-blot technique using specific antibodies, which recognize single bands by Western blot of 68 kDa, 30 kDa, and 43 kDa, respectively [8]. The yield of protein from the adrenal gland explants of the fourth patient was insufficient to perform the measurements. Total adrenal proteins were extracted from adrenal explants by TRIzol following the manufacturer's instructions. The protein concentration was measured by spectrophotometry at 280 nm using 16.66 µM albumin, as standard. 10 micrograms of protein in 50 µl of SDS 1 % w/v were applied to wet PVDF membranes (0.45 µm) followed by the procedure previously described [8]. Each blot image was scanned using a digital Scanner (AGFA SNAPSCAN 310, Mortsel Belgium), then optical density (pixels/mm^2) of a square of fixed size comprising the spot, was measured with the software Scion Image (Scion-Corporation, http://www.scioncorp.com). Membranes stained with Coomasie blue were used as additional loading controls. Each sample was measured in 3 independent assays. BMAL1, StAR, and 3β-HSD protein levels were expressed per µg of protein and normalized by calculating the percentage of each treatment with respect to control treatment.

Cortisol and progesterone production by human adrenal gland explants

Concentrations of cortisol and progesterone in culture medium from incubated adrenal glands from 4 patients were measured by RIA, as described previously [18]. Inter- and intra-assay coefficients were less than 15 %. Cortisol and progesterone production were expressed per mg of tissue. Control cortisol production per mg of adrenal tissue ranged from 21.2 to 157.8 pmol (7.7–57.2 ng). Control progesterone production ranged from 0.20 to 1.31 pmol (63–413 pg). For both steroids, the data were normalized by calculating the percentage of each treatment with respect to control in each experimental protocol.

Data analysis

Data were expressed as mean ± S.E.M. Differences between means were analyzed by repeated measures ANOVA and Newman-Keuls as post hoc test. Data expressed as percentage was transformed to arcsine before analysis. Statistical analysis was performed using GraphPad Prism 4 Software. Differences were considered significant at $p < 0.05$.

Results

Expression of clock genes by the human adrenal gland ex vivo

We detected expression of *PER1*, *PER2*, *CRY2*, *CLOCK*, and *BMAL1* genes in the samples assayed immediately after surgery, reflecting the in vivo situation as close as possible. As shown in **Fig. 1**, PCR products of the expected size for these genes were present in a normal adrenal gland as well as in the adrenal adenoma. Using cDNA equivalent to 150 ng of RNA, weak bands for *CRY2*, *PER2*, *CLOCK*, and *BMAL1* were obtained. In contrast, a heavily saturated band was obtained for *PER1* suggesting a higher abundance than that of the other genes. Given this observation, and the limitations in the amount of RNA obtained in adrenal explants after DNAse treatment, we decided to quantify just *PER1* expression in adrenal explants by real-time PCR.

Melatonin inhibits *PER1* mRNA and BMAL1 protein expression induced by ACTH in human adrenal gland explants

Quantification of *PER1* content in human adrenal explants revealed that ACTH (100 nM) treatment increased expression of *PER1* mRNA. At 12 h of incubation, *PER1* expression was about 3 times higher than in control explants and explants treated only with melatonin (**Fig. 2a**). The increase of *PER1* mRNA induced by ACTH was suppressed by addition of 100 nM melatonin to the culture medium. Treatment with melatonin alone decreased

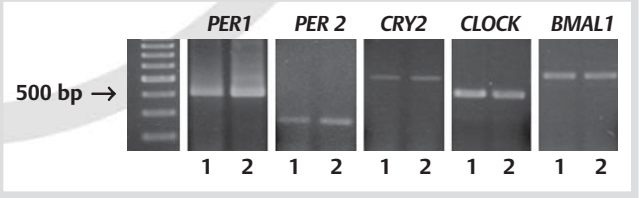

Fig. 1 Expression of the clock genes *PER1*, *PER2*, *CRY2*, *CLOCK*, and *BMAL1* by RT-PCR in 2 human adrenal glands. PCR products for *PER1* (404 bp), *PER2* (282 bp), *CRY2* (525 bp), *CLOCK* (386 bp), and *BMAL1* (564 bp) were obtained from cDNA dilutions equivalent to 150 ng of RNA. Lane 1: normal adrenal and Lane 2: adrenal adenoma.

Per1 mRNA expression to values lower than in control explants in 3 of the 4 adrenal glands studied, but the difference between means was not statistically significant.

ACTH treatment increased the expression of BMAL1 protein at 12 h of incubation in the adrenal explants from the 3 patients studied (◦ **Fig. 2b**). This increase was abrogated by addition of 100 nM melatonin to the culture medium. Expression of BMAL1 protein in explants treated with melatonin alone was not different from that in control or ACTH treated explants.

Melatonin inhibits cortisol and progesterone production induced by ACTH and suppresses StAR and 3β-HSD protein levels in human adrenal gland explants

ACTH (100 nM) treatment stimulated cortisol and progesterone production in human adrenal explants (◦ **Fig. 3a, b**). ACTH treated explants produced nearly 3 times the amount of cortisol and about 2 times the amount of progesterone than control explants treated with medium alone (range 2.2- to 3.1-fold and 1.3- to 3.2-fold, respectively) and contained about twice the amount of StAR and 3β-HSD protein than control explants (◦ **Fig. 3c, d**). Incubation with 100 nM melatonin blocked the ACTH stimulation of cortisol and progesterone production and of StAR and 3β-HSD protein levels near to control values. Treatment with 100 nM melatonin alone had no effect on cortisol or progesterone production and on protein expression of StAR and 3β-HSD respect to control treatment (◦ **Fig. 3a–d**).

Discussion

Our study provides evidence of expression of circadian clock genes in the human adrenal and report a novel stimulatory effect of ACTH upon *PER1 mRNA* and BMAL1 protein expression. Melatonin inhibited the former responses as well as the known stimulation by ACTH of StAR and 3β-HSD protein levels and of cortisol and progesterone production. Thus, notwithstanding the limitations imposed by the number of cases and amount of tissue available, our study demonstrates, for the first time, direct inhibitory effects of melatonin in several aspects of the human adrenal gland response to ACTH.

The expression of *PER1*, *PER2*, *CRY2*, *CLOCK*, and *BMAL1* mRNA in the human adrenal cortex samples, measured immediately after surgery, suggests that in vivo the human adrenal gland contains a circadian clock. Clock gene expression represents the contribution of diverse cell types present in the adrenal cortex: steroidogenic cells, endothelial cells, fibroblasts and possibly of a few chromaffin cells. Clock gene expression has been reported in the nonhuman primate adrenal gland and rodent adrenal gland [13, 19, 20]. In the former species, adrenal gland explants sustain oscillatory expression of clock genes in culture, situation not tested in the present experiments. However, measured at a single time point, 12 h of incubation, clock gene expression persisted in cultured human adrenal explants. Furthermore, at this time interval, we found that *PER1* mRNA levels were higher in ACTH treated explants than in control explants incubated with medium alone. Additionally, with the increase in *PER1* mRNA levels, the human adrenal explants incubated with ACTH for 12 h, contained higher levels of BMAL1 protein than the control explants. Given the reciprocal interaction between BMAL1 and *PER1*, the increase in BMAL1 protein in response to ACTH may be responsible for the *PER1* mRNA increase [21, 22]. On the other hand, it is known that increases in *PER1-2*, most likely accompa-

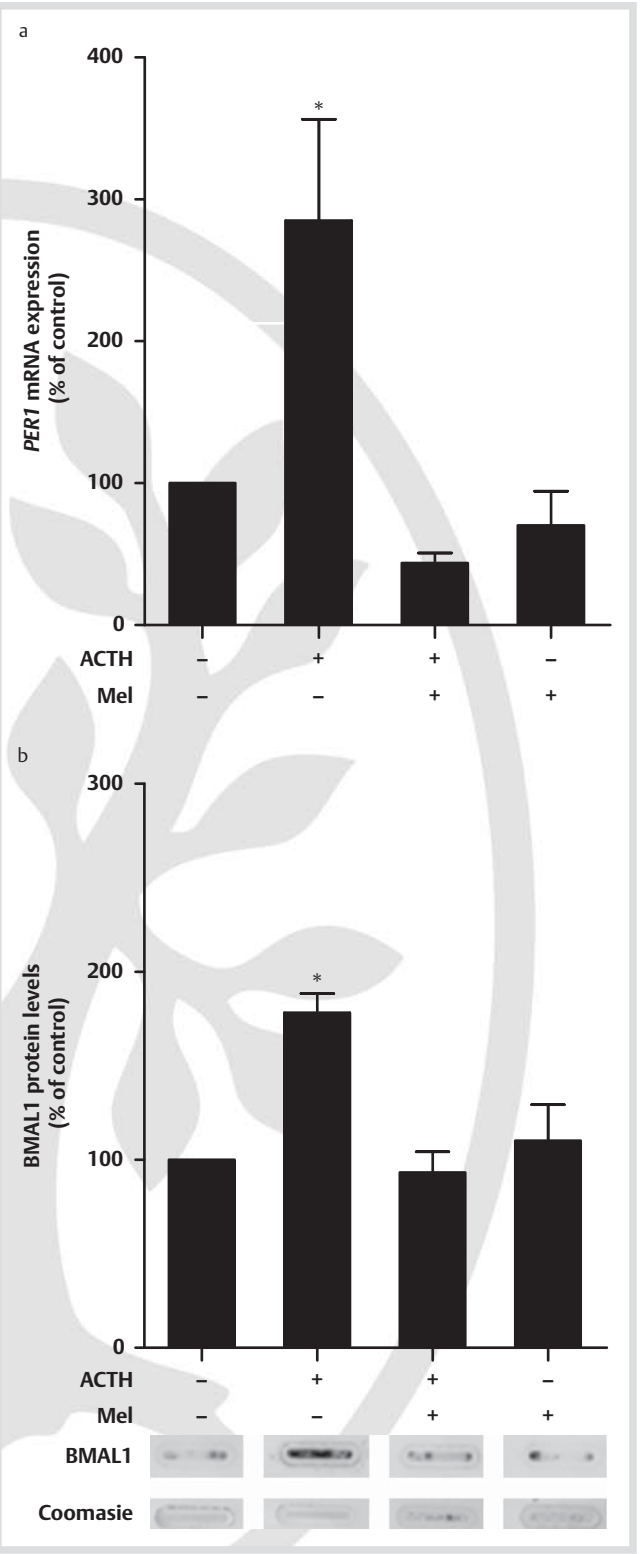

Fig. 2 Mean ± S.E.M inhibitory effect of melatonin (Mel) on *PER1* mRNA expression (Panel **a**, n = 4) and on BMAL1 protein levels (Panel **b**, n = 3) induced by ACTH in human adrenal gland explants. A representative immuno slot-blot for BMAL1 protein levels is shown in graph **b**. The data were normalized by calculating in each experiment the percentage of the treatment against the respective control. * Different from control, ACTH + Mel and Mel, $p < 0.05$ (ANOVA for repeated measures and Newman-Keuls' post hoc test).

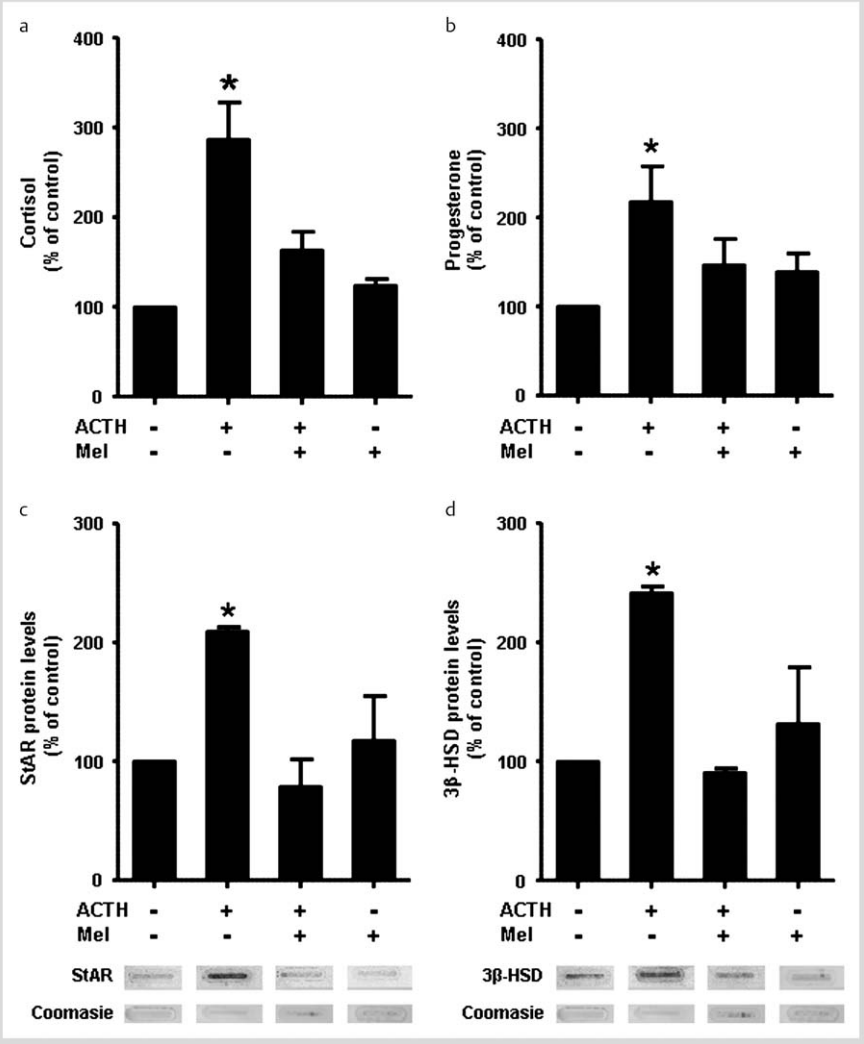

Fig. 3 Mean ± S.E.M inhibitory effect of melatonin (Mel) on cortisol and progesterone production, and on StAR and 3β-HSD protein levels induced by ACTH in human adrenal gland explants.
Panel **a**: Cortisol production (n = 4).
Panel **b**: Progesterone production (n = 4).
Panel **c**: StAR protein content (n = 3);
Panel **d**: 3β-HSD protein content (n = 3). A representative immuno slot-blot for StAR and 3β-HSD protein levels is shown below the respectively graph. The data were normalized by calculating in each experiment the percentage of the treatment against the respective control. * Different from control, ACTH + Mel, and Mel, $p < 0.05$ (ANOVA for repeated measures and Newman-Keuls' post hoc test).

nied by increases in *CRY1-2* expression, could result in PER/CRY protein mediated upregulation of *BMAL1* transcription [23].

The ACTH induction of *PER1* mRNA expression and of BMAL1 protein levels in the human adrenal gland explants was inhibited by simultaneous treatment with melatonin. In addition, melatonin inhibited the 4 other responses to ACTH studied: increases in StAR and 3β-HSD protein expression and in cortisol and progesterone production. Similar direct inhibitory effects of melatonin over ACTH-stimulated cortisol production have been shown in the NCIH295R human adrenocortical carcinoma cell line [24] and in the adrenals of several species over ACTH-stimulated glucocorticoid and progesterone production and 3β-HSD mRNA expression [4–6, 8, 14]. In the conditions of the present experiments, ACTH induction of BMAL1 protein coincided with an increased expression of StAR. This protein is inducible by BMAL1 [11] whereas its expression is decreased in corpora lutea, testis, and adrenal gland of *BMAL1* (–/–) female and male mice that show low progesterone and testosterone levels, respectively [25, 26]. Clock genes may participate in the regulation of other steroidogenic enzymes as *CRY* null mice that have excessive aldosterone production and hypertension, overexpress 3β-HSD6 in the adrenal zona glomerulosa [27]. 2 lines of evidence, using knockout rats and direct clock gene knock-in, showed that adrenal glucocorticoid response to ACTH in rats and nonhuman primates requires an intact adrenal clockwork [8–10]. On the other hand, melatonin has been shown to directly inhibit clock gene expression in the capuchin monkey adrenal [13] and in striatal neurons of mice [28]. In the present experiments, we cannot identify the precise human adrenal cell type upon which melatonin acted. In adrenal explants, the anatomic architecture remains, thus steroidogenic cells are in close contact with endothelial cells and fibroblasts, and possibly, a few chromaffin cells dispersed in the cortex, are potential providers of paracrine signals that modulate steroid response to ACTH [29]. Albeit the former, the similitude of the pattern of change of cortisol and progesterone production and of StAR and 3β-HSD protein content of human adrenal gland explants in response to ACTH and ACTH plus melatonin, was strikingly similar to that of *PER1* mRNA and BMAL1 protein content, suggesting that melatonin acted on the link between ACTH, clock genes, and adrenal steroidogenesis. In vivo, adrenal cortex regulation of cortisol production is complex, involving the coordinated action of ACTH, adrenal innervation, and local adrenal factors [29–31], and possibly melatonin [4]. In the human adrenal gland, the present experiments, notwithstanding their limitations, suggest the involvement of adrenal clock gene expression in the mechanisms by which ACTH stimulates cortisol production and by which melatonin directly inhibited this production. Elucidating further the interaction between ACTH, clock genes, and melatonin in the human adrenal gland can provide new insights into the regulation of cortisol production, a key hormone for physiological homeostasis.

Declaration of Interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Acknowledgements

This work was supported by grants from Department of Women's Health, Arrowhead Regional Medical Center, Colton, CA, USA; Fondo Nacional de Ciencia y Tecnología (Fondecyt 1050833 and 1090381); SOCHED 2009-07; and Departamento de Endocrinología, Facultad de Medicina, Pontificia Universidad Católica de Chile. F.J.V and L.A-C were supported by Ph.D. fellowships from Comision Nacional de Ciencia y Tecnología (Conicyt). We thank Prof. Ian Mason (Reproductive & Developmental Sciences Division, University of Edinburgh School of Clinical Sciences and Community Health) for StAR and 3β-HSD antibodies and Ms. Auristela Rojas for assistance in RIAs.

Affiliations

[1] Departamento de Endocrinología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[2] Programa de Fisiopatología, Instituto de Ciencias Biomédicas (ICBM), Facultad de Medicina, Universidad de Chile, Santiago, Chile
[3] Departamento de Urología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile
[4] Department of Women's Health, Arrowhead Regional Medical Center, Colton, CA, USA
[5] Universidad de Tarapaca, Arica, Chile

References

1 *Reiter RJ, Tan DX, Fuentes-Broto L.* Melatonin: a multitasking molecule. Prog Brain Res 2010; 181: 127–151
2 *Dubocovich ML, Markowska M.* Functional MT1 and MT2 melatonin receptors in mammals. Endocrine 2005; 27: 101–110
3 *Ekmekcioglu C.* Melatonin receptors in humans: biological role and clinical relevance. Biomed Pharmacother 2006; 60: 97–108
4 *Torres-Farfan C, Richter HG, Rojas-Garcia P, Vergara M, Forcelledo ML, Valladares LE, Torrealba F, Valenzuela GJ, Seron-Ferre M.* mt1 Melatonin receptor in the primate adrenal gland: inhibition of adrenocorticotropin-stimulated cortisol production by melatonin. J Clin Endocrinol Metab 2003; 88: 450–458
5 *Torres-Farfan C, Valenzuela FJ, Mondaca M, Valenzuela GJ, Krause B, Herrera EA, Riquelme R, Llanos AJ, Seron-Ferre M.* Evidence of a role for melatonin in fetal sheep physiology: direct actions of melatonin on fetal cerebral artery, brown adipose tissue and adrenal gland. J Physiol 2008; 586: 4017–4027
6 *Richter HG, Torres-Farfan C, Garcia-Sesnich J, Abarzua-Catalan L, Henriquez MG, Alvarez-Felmer M, Gaete F, Rehren GE, Seron-Ferre M.* Rhythmic expression of functional MT1 melatonin receptors in the rat adrenal gland. Endocrinology 2008; 149: 995–1003
7 *Campino C, Valenzuela FJ, Arteaga E, Torres-Farfan C, Trucco C, Velasco A, Guzmán S, Seron-Ferre M.* Melatonin reduces cortisol response to ACTH in humans. Rev Med Chile 2008; 136: 1390–1397
8 *Torres-Farfan C, Abarzua-Catalan L, Valenzuela FJ, Mendez N, Richter HG, Valenzuela GJ, Seron-Ferre M.* Cryptochrome 2 expression level is critical for adrenocorticotropin stimulation of cortisol production in the capuchin monkey adrenal. Endocrinology 2009; 150: 2717–2722
9 *Son GH, Chung S, Choe HK, Kim HD, Baik SM, Lee H, Lee HW, Choi S, Sun W, Kim H, Cho S, Lee KH, Kim K.* Adrenal peripheral clock controls the autonomous circadian rhythm of glucocorticoid by causing rhythmic steroid production. Proc Nat Acad Sci USA 2008; 105: 20970–20975
10 *Oster H, Damerow S, Kiessling S, Jakubcakova V, Abraham D, Tian J, Hoffmann MW, Eichele G.* The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. Cell Metabolism 2006; 4: 163–173
11 *Nakao N, Yasuo S, Nishimura A, Yamamura T, Watanabe T, Anraku T, Okano T, Fukada Y, Sharp PJ, Ebihara S, Yoshimura T.* Circadian clock gene regulation of steroidogenic acute regulatory protein gene expression in preovulatory ovarian follicles. Endocrinology 2007; 148: 3031–3038
12 *Sewer MB, Waterman MR.* ACTH modulation of transcription factors responsible for steroid hydroxylase gene expression in the adrenal cortex. Microsc Res Tech 2003; 61: 300–307
13 *Valenzuela FJ, Torres-Farfan C, Richter HG, Mendez N, Campino C, Torrealba F, Valenzuela GJ, Seron-Ferre M.* Clock gene expression in adult primate suprachiasmatic nuclei and adrenal: is the adrenal a peripheral clock responsive to melatonin? Endocrinology 2008; 149: 1454–1461
14 *Torres-Farfan C, Richter HG, Germain AM, Valenzuela GJ, Campino C, Rojas-García P, Forcelledo ML, Torrealba F, Seron-Ferre M.* Maternal melatonin selectively inhibits cortisol production in the primate fetal adrenal gland. J Physiol 2004; 554: 841–856
15 *Torres-Farfan C, Rocco V, Monsó C, Valenzuela FJ, Campino C, Germain A, Torrealba F, Valenzuela GJ, Seron-Ferre M.* Maternal melatonin effects on clock gene expression in a nonhuman primate fetus. Endocrinology 2006; 147: 4618–4626
16 *Morgan PJ, Ross AW, Graham ES, Adam C, Messager S, Barrett P.* oPer1 is an early response gene under photoperiodic regulation in the ovine pars tuberalis. J Neuroendocrinol 1998; 10: 319–323
17 *Lomax MA, Sadiq F, Karamanlidis G, Karamitri A, Trayhurn P, Hazlerigg DG.* Ontogenic loss of brown adipose tissue sensitivity to beta-adrenergic stimulation in the ovine. Endocrinology 2007; 148: 461–468
18 *Torres-Farfan C, Valenzuela FJ, Germain AM, Viale ML, Campino C, Torrealba F, Valenzuela GJ, Richter HG, Serón-Ferré M.* Maternal melatonin stimulates growth and prevents maturation of the capuchin monkey fetal adrenal gland. J Pineal Res 2006; 41: 58–66
19 *Lemos DR, Downs JL, Urbanski HF.* Twenty-four-hour rhythmic gene expression in the rhesus macaque adrenal gland. Mol Endocrinol 2006; 20: 1164–1176
20 *Fahrenkrug J, Hannibal J, Georg B.* Diurnal rhythmicity of the canonical clock genes Per1, Per2 and Bmal1 in the rat adrenal gland is unaltered after hypophysectomy. J Neuroendocrinol 2008; 20: 323–329
21 *Kume K, Zylka MJ, Sriram S, Shearman LP, Weaver DR, Jin X, Maywood ES, Hastings MH, Reppert SM.* mCRY1 and mCRY2 are essential components of the negative limb of the circadian clock feedback loop. Cell 1999; 98: 193–205
22 *Gekakis N, Staknis D, Nguyen HB, Davis FC, Wilsbacher LD, King DP, Takahashi JS, Weitz CJ.* Role of the CLOCK protein in the mammalian circadian mechanism. Science 1998; 280: 1564–1569
23 *Yu W, Nomura M, Ikeda M.* Interactivating feedback loops within the mammalian clock: BMAL1 is negatively autoregulated and upregulated by CRY1, CRY2, and PER2. Biochem Biophys Res Commun 2002; 290: 933–941
24 *Rahman SA, Kollara A, Brown TJ, Casper RF.* Selectively filtering short wavelengths attenuates the disruptive effects of nocturnal light on endocrine and molecular circadian phase markers in rats. Endocrinology 2008; 149: 6125–6135
25 *Ratajczak CK, Boehle KL, Muglia LJ.* Impaired steroidogenesis and implantation failure in Bmal1-/-mice. Endocrinology 2009; 150: 1879–1885
26 *Alvarez JD, Hansen A, Ord T, Bebas P, Chappell PE, Giebultowicz JM, Williams C, Moss S, Sehgal A.* The circadian clock protein BMAL1 is necessary for fertility and proper testosterone production in mice. J Biol Rhythms 2008; 23: 26–36
27 *Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, Emoto N, Okuno Y, Tsujimoto G, Kanematsu A, Ogawa O, Todo T, Tsutsui K, van der Horst GT, Okamura H.* Salt-sensitive hypertension in circadian clock-deficient Cry-null mice involves dysregulated adrenal Hsd3b6. Nat Med 2010; 16: 67–74
28 *Imbesi M, Arslan AD, Yildiz S, Sharma R, Gavin D, Tun N, Manev H, Uz T.* The melatonin receptor MT1 is required for the differential regulatory actions of melatonin on neuronal 'clock' gene expression in striatal neurons in vitro. J Pineal Res 2009; 46: 87–94
29 *Ansurudeen I, Kopprasch S, Ehrhart-Bornstein M, Willenberg HS, Krug AW, Funk RH, Bornstein SR.* Vascular-adrenal niche–endothelial cell-mediated sensitization of human adrenocortical cells to angiotensin II. Horm Metab Res 2006; 38: 476–480
30 *Dickmeis T.* Glucocorticoids and the circadian clock. J Endocrinol 2009; 200: 3–22
31 *Engeland WC, Arnhold MM.* Neural circuitry in the regulation of adrenal corticosterone rhythmicity. Endocrine 2005; 28: 325–332