Published in final edited form as:

Clin Endocrinol (Oxf). 2010 July ; 73(1): 22–29. doi:10.1111/j.1365-2265.2009.03738.x.

The role of TASK1 in aldosterone production and its expression in normal adrenal and aldosterone-producing adenomas

Edson F. Nogueira¹, Daniel Gerry¹, Franco Mantero², Barbara Mariniello², and William E. Rainey¹

¹Department of Physiology, Medical College of Georgia, Augusta, GA – USA

²Division of Endocrinology, University of Padua, Padua – Italy

Summary

Objectives—Aldosterone production in the adrenal glomerulosa is mainly regulated by angiotensin II and K⁺. Adrenal glomerulosa cells are uniquely sensitive to extracellular K⁺. Genetic deletion of subunits of K⁺-selective leak-channels (KCNK), TASK1 and/or TASK3, in mice generates animals with hyperaldosteronism and histological changes in the adrenal cortex. Herein, we studied the expression of TASK1 in human adrenocortical cells, as well as its role in aldosterone production in H295R cells.

Design—TASK1 expression was investigated by comparative microarray analysis of aldosterone-producing adenomas (APA) and normal adrenals (NA). The effects of TASK1 knockdown by siRNA transfection were investigated in H295R cells. Fluo-4 fluorescent measurements of intracellular Ca²⁺ and pharmacological inhibition of Ca²⁺-dependent calmodulin kinases (CaMK) were performed to better define the effects of TASK1 on Ca²⁺ signaling pathways.

Results—Microarray analysis of APA and NA showed similar expression of TASK1 between these two groups. However, in APA, NA, and H295R cells the expression of TASK1 was predominant when compared to other KCNK family members. Knockdown of TASK1 (with siRNA) induced the expression of steroidogenic acute regulatory (StAR) protein and aldosterone synthase (CYP11B2), and also stimulated pregnenolone and aldosterone production. Cells transfected with siTASK1 had increased intracellular Ca²⁺, leading to activation of CaMK and increased expression of CYP11B2.

Conclusions—Our study reveals the predominant expression of TASK1 over other KCNK family genes in the human adrenal cortex. Herein, we also described the role of TASK1 in the regulation of human aldosterone production through regulation of intracellular Ca²⁺ and CaMK signaling pathways.

Keywords

adrenal; potassium; aldosterone; TASK1; CYP11B2; Ang II

Corresponding author: William E. Rainey, Ph.D., Department of Physiology, Medical College of Georgia, 1120 15th Street, CA Building – Room 3094, Augusta, GA 30912. USA, Phone: (+1) (706) 721-7665, Fax: (+1) (706) 721-8360, wrainey@mcg.edu.

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

Introduction

Primary hyperaldosteronism (PA) is the most common cause of secondary hypertension. Its prevalence is estimated to be approximately 10% within the hypertensive population ^{1, 2}, and even higher among patients with resistant hypertension ³. Elevated aldosterone levels produce structural and functional changes over and above those produced by high blood pressure ^{4, 56, 7}. In patients with primary hyperaldosteronism, aldosterone levels are not suppressed by dietary sodium loading, volume expansion, or antagonism of the renin-angiotensin system ^{8, 9}. The molecular mechanisms underlying the elevated levels of aldosterone secretion by adrenocortical cells in PA are still not characterized, and specific therapies to reduce aldosterone production are not clinically available.

Aldosterone biosynthesis in the zona glomerulosa of the adrenal cortex is physiologically regulated by angiotensin II (Ang II), K⁺, and ACTH ^{10, 11}. Binding of Ang II to its type 1 receptor (AT1R) stimulates a variety of signaling cascades, leading to the release of Ca²⁺ from the endoplasmic reticulum (ER) and subsequent cell membrane depolarization with additional flow of extracellular Ca²⁺ into the cytoplasm ¹². These events stimulate the early and the late regulatory steps in aldosterone production, StAR protein and CYP11B2 respectively, culminating with elevated aldosterone production ¹³⁻¹⁷.

Adrenal glomerulosa cells are uniquely sensitive to small increases in extracellular K⁺ due to the presence of very high background K⁺ conductance, which makes the membrane potential very dependent on the K⁺ equilibrium potential. Interestingly, this distinctive characteristic is associated with the expression of high levels of KCNK channels, including the TWIK-related acid sensitive K⁺ 1 and 3 (TASK1 and TASK3) ¹⁸⁻²⁰. Small changes in extracellular K⁺ increase the sensitivity of adrenocortical cells to stimulation by Ang II ²¹⁻²³. Moreover, Ang II-induced cell membrane depolarization is suggested to involve the inhibition of background (leak) K⁺ channels ^{20, 24}. TASK channels are inhibited by extracellular protons (acidification), by some anesthetic agents, and by hormones that activate G_q-coupled receptors ^{19, 25, 26}. Recent data in the literature showed the establishment of an *in vivo* model of primary hyperaldosteronism following the deletion of subunits of K⁺ channels termed TASK1 and TASK3 ^{27, 28}. The adrenocortical cells in these animals were more depolarized than their controls, leading to increased aldosterone production. The observation that TASK1- and TASK1/TASK3-deficient mice have primary aldosteronism makes these genes potential candidates for causing human adrenal disease.

In the present study, we sought to better define the role of TASK1 in adrenal cell aldosterone production, as well as its potential role in PA. Herein, we demonstrate a key role for TASK1 in the regulation of adrenal cell Ca²⁺ levels which results in alterations in aldosterone production and CYP11B2 expression. In addition, we show that TASK1 mRNA expression is not altered in aldosterone-producing adenomas when compared to normal adrenal. In summary, our analysis confirmed the high expression of TASK1 in human adrenocortical tissue and cells, and its role in regulating aldosterone synthesis.

Materials and Methods

Subjects and tissues

Normal human adult adrenals were obtained through the Cooperative Human Tissue Network (Philadelphia, PA, USA) and Clontech (Mountain View, CA). Ten normal adrenal samples came from patients who each underwent adrenalectomy, secondary to renalectomy due to renal carcinoma. The APA samples were obtained from the Division of Endocrinology at the University of Padua. All APA samples were taken from Conn's syndrome patients with significantly elevated circulating aldosterone levels that returned to the normal range after surgical removal of the tumor. Fourteen adenomas were collected from patients with PA. These samples had levels of CYP11B2 that were two S.D.s greater than levels seen in normal adrenal glands. The use of these tissues was approved by the Institutional Review Boards of the Medical College of Georgia (Augusta, GA, USA), and the University of Padua (Padua, Italy). In addition, this study was approved by the ethical committee at the University of Padua, and informed consent was obtained from every patient.

Microarray analysis

RNA isolated from 14 APA and 10 NA was hybridized to an Affymetrix human HG_U133C2 oligonucleotide microarray set containing 54,675 probe sets representing approximately 40,500 independent human genes. Results were analyzed using GeneSpring software version 7.3 (Silicon Genetics, Redwood City, CA, USA) to identify differences in expression of genes in the two groups.

Cell culture and treatments

H295R human adrenocortical cells were cultured in DME/Ham's F12 medium (Invitrogen, Grand Island, NY) supplemented with 2.5% Ultrosor G (Pall Biosepra, Cergy, Saint-Christophe, France), 1% penicillin/streptomycin (Invitrogen), 0.01% gentamicin (Invitrogen), and 1% ITSTM + Premix (BD Biosciences, Bedford, MA). Cells were maintained in a 37°C humidified atmosphere (5% CO₂). For analysis of TASK1 transcriptional regulation by aldosterone secretagogues, H295R cells were sub-cultured onto 24-well dishes at a density of 2×10^5 cells/well for subsequent treatment with Ang II (10 nM) or K⁺ (18 mM) for the following time points: 30 min, 1, 2, 3, 6, 12, and 24 h. Intracellular Ca²⁺ signaling in H295R cells transfected with siTASK1 was analyzed by blocking Ca²⁺-dependent calmodulin kinases (CaMK) with 3 μM KN93 for 12 h, followed by quantitative real time PCR (qPCR) analysis of CYP11B2 mRNA.

For analysis of pregnenolone production by siControl versus siTASK1 cells, both groups were incubated with 15 μM trilostane (4α, 5 epoxy-17β-hydroxy-3-keto-5α-androstane-2α-carbonitrile), an inhibitor of 3β-hydroxysteroid dehydrogenase ²⁹, and 19 μM SU-10603 (7-chloro-3,4-dihydro-2-(3-pyridyl) naphthalene-1-(2H)-one), an inhibitor of steroid 17α-hydroxylase ³⁰ for 6 h to arrest pregnenolone metabolism. These compounds were generously supplied by Sterling-Winthrop Inc. (Rensselaer, NY) and Ciba Geigy Inc. (Summit, NJ), respectively.

RNA extraction, cDNA synthesis, and real-time RT-PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA) according to protocols from the manufacturer. Purity and integrity of the RNA were checked spectroscopically using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE). Total RNA was reverse transcribed using the High-capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) following the manufacturer protocols. Primer and probe mixtures for the amplification of the TASK1 (cat # HS00605529_M1) and TASK3 (cat # HS00363153_m1) target sequences were purchased from Applied Biosystems. PCR amplifications were performed using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems) following the reaction parameters recommended by the manufacturer, using 20 μ l total volumes which consisted of TaqMan Fast Universal PCR Master Mix (Applied Biosystems), primers/probes mix, and cDNA. 18S rRNA (TaqMan Ribosomal Control Reagents, Applied Biosystems) was used as the normalization gene. Negative controls contained water instead of cDNA.

In all experiments, the relative gene expression was calculated by the C_t method. Briefly, the resultant mRNA was normalized to a calibrator; in each case, the calibrator chosen was the basal sample. Final results were expressed as n -fold difference in gene expression relative to 18s rRNA and calibrator as follows: $n\text{-fold} = 2^{-(C_t \text{ sample} - C_t \text{ calibrator})}$, where C_t values of the sample and calibrator were determined by subtracting the average C_t value of the 18s rRNA gene from the average C_t value of the transcript under investigation for each sample.

Analysis of intracellular Ca^{2+} concentration

H295R cells transfected with siTASK1 or siControl were plated in a clear-bottom/dark-wall 96 well plate (Corning Costar, Corning, NY) and allowed to recover in growth medium for 48 h. The medium was then substituted with a dye loading solution provided in the Fluo-4 NW Ca^{2+} assay kit (Molecular Probes, Eugene, OR), followed by a 30 min incubation at 37°C. Changes in intracellular Ca^{2+} following stimulation with 10 nM Ang II were measured by variations in fluorescence captured by the FLUOstar Optima instrument (BMG Labtech, Durham, NC). Experiments were repeated a minimum of 3 times.

Adrenal cell transfection

H295-R cells were electroporated using the Amaxa System (Gaithersburg, MD), and transfected with siRNA for TASK1 (cat # L-006262-00) or control siRNA (cat # D-001810-10-05), both purchased from Dharmacon Inc. (Chicago, IL). Transfected cells were plated at a density of 2 million cells/well onto 6-well dishes. In addition, we measured the effect of agonists (10 nM Ang II and 18 mM K^+) on siRNA transfected cells. Experiments were repeated a minimum of 3 times.

Protein assay and Western analysis

H295R cells transfected with siTASK and siControl were lysed in 1× RIPA buffer (Pierce Thermo Scientific, Rockford, IL) + 1 % protease inhibitor solution (Pierce Thermo Scientific). The protein content of the samples was then determined using the BCA assay kit

(Pierce Thermo Scientific). Twenty five micrograms of protein from each sample was electrophoresed in a 12% Bis-Tris gel (Invitrogen) and transferred to a nitrocellulose membrane. After transfer, the membranes were blocked for 1 h at room temperature with a 5% BSA solution in a 1× Tris-Buffered Saline with 0.01% Tween-20 (TBS-T) solution. Then the membranes were incubated overnight at 4° C with mouse anti-StAR primary antibody (1:5000 dilution in 5% BSA/TSB-T) which was generously provided by Dr. Dale Buchanan Hales (University of Illinois, Chicago, IL), or rabbit anti-TASK1 primary antibody (Aviva Systems biology, San Diego, CA) at 1:1000 dilution in 5% BSA/TSB-T. Membranes were washed (3 × 5 min washes) with TBS-T before incubation with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, Santa Cruz – CA) at 1:5000 dilution in 5% BSA/TSB-T. Then membranes were washed with TBS-T, and immunoreactive bands were visualized using ECL Western Analysis Substrate (Pierce Thermo Scientific). Membranes were re-probed with mouse β -actin primary antibodies for normalization.

Steroid measurement

Aldosterone content of the medium recovered from each well was determined with aldosterone radioimmunoassays (Siemens, Los Angeles, CA) following the protocol from the manufacturer, using aldosterone standards prepared in low-serum cell culture medium. Results of aldosterone assays were normalized to cellular protein and expressed as pmol per mg cell protein.

Pregnenolone and cortisol content of the medium recovered from each well was determined with pregnenolone and cortisol enzyme immunoassays (EIA) (Alpco Diagnostics, Salem, NH), following the protocol from the manufacturer, using standards provided with the kits. Results of pregnenolone and cortisol assays were normalized to cellular protein and expressed as nmol/ μ g and nmol/mg cell protein, respectively.

Statistical analysis

All values were expressed as a mean $\pm$ S.E.M. One-way ANOVA was used to compare groups. P-values lower than 0.05 were considered statistically significant.

Results

Regulation of aldosterone production by TASK1 channels

The inhibition of TASK channels by Ang II is one of the molecular mechanisms through which Ang II increases intracellular Ca^{2+} concentration and subsequently induces aldosterone production³¹. Transfection of H295R cells with siTASK1 decreased TASK mRNA expression by approximately 80% in both basal and treated conditions (Fig.1, panel A), and the knockdown was confirmed by a decrease of 70% in TASK1 protein expression (Fig.1, panel B).

Decreased TASK1 expression in H295R cells increased aldosterone production by 2.5-fold under basal conditions, and by 1.5-fold in cells treated with Ang II or K^+ (Fig.2, panel A), whereas cortisol production was not significantly affected by siTASK1 transfection (Fig. 2,

panel B). The increase in aldosterone production was explained by the induction of the early regulatory step in aldosterone synthesis, that is, the increase in StAR protein by 2.5-, 2-, and 1.5-fold, in basal, Ang II- and K⁺-treated cells, respectively (comparing siControl versus siTASK1 cells in Fig.3, panel A). Confirming the up-regulation of the early steps in aldosterone biosynthesis, the production of pregnenolone was 1.7-fold higher in siTASK1 compared to siControl cells (Fig.3, panel B).

The expression of CYP11B2, which is the late regulatory step, was elevated by 4-fold in basal conditions and approximately 2-fold in treated (Ang II or K⁺) conditions (Fig.4, panel A). The expression of CYP11B1, which is responsible for the final steps in cortisol production by adrenocortical cells, was not significantly altered by siTASK1 transfection in basal or treated conditions (Fig. 4, panel B).

As seen in panel C of Fig.4, H295R cells gradually recovered the expression of TASK1 from 48 h to 96 h after siRNA transfection. Consistently, the increase in CYP11B2 expression caused by TASK1 knockdown was gradually diminished by TASK1 recovery (Fig.4, panel D).

Regulation of Ca²⁺ and CaMK signaling in siTASK1 cells

Increased intracellular Ca²⁺ in adrenocortical cells leads to activation of CaMK and subsequent regulation of gene transcription, culminating with increased aldosterone production ^{31, 32}. Herein, we studied changes in Ca²⁺ signaling in siTASK1-transfected cells by comparing their Fluo-4-mediated fluorescence with control cells. SiTASK1 cells have a higher level of fluorescent measurements throughout the time of measurement in both basal and Ang II-stimulated conditions, which is suggestive of a more depolarized membrane potential in the siTASK1-transfected adrenocortical cells (Fig.5, panel A).

As seen in panel B of Fig.5, inhibition of CaMK by KN93 caused a 50 % decrease in CYP11B2 expression in control cells. Also, the augmented expression of CYP11B2 was totally abolished by CaMK inhibition in siTASK1 cells. These results confirm the expected role for Ca²⁺-activated pathways on the induction of aldosterone secretion by siTASK1 transfection.

Effects of aldosterone secretagogues on TASK1 expression

Although the functional inhibition of TASK1 by Ang II has been previously reported, the transcriptional regulation of TASK1 channels by aldosterone secretagogues has not yet been defined. In the present study, H295R cells were treated for various time points (30 min, 1 h, 3 h, 6 h, 12 h, 24 h) with Ang II or K⁺, followed by RNA isolation and qPCR analysis of TASK1 mRNA expression. TASK1 mRNA expression was not modified by these agonists in any of the time points studied (data not shown). These results are confirmed by the similar levels of TASK1 protein in basal versus treated (Ang II or K⁺) conditions (Fig.1, panel B).

Analysis of TASK1 gene expression in APA versus NA

The correlation of TASK1 channels with increased production of aldosterone has highlighted the importance of this gene in adrenal research. In Table 1 we compared the

expression of TASK1 and other members of the KNCK family in APA versus NA using DNA microarray analyses. The KNCK family gene expression was not significantly different between the two groups. Microarray results for TASK1 (KCNK3), TASK3 (KCNK9), and TREK1 (KCNK2) were confirmed by qPCR analysis and no differences in expression were observed between NA and APA (data not shown). It is worth noting that the expression comparisons were made between a predominantly cortisol producing tissue (whole adrenal) with a predominantly aldosterone producing tissue (APA). Till the present day, there are no reports of differential expression of TASK family members in different zones of the human adrenal cortex. Thus, changes in KCNK channel expression in the adrenal zones cannot be adequately evaluated by the analyses used in the current study.

Interestingly, TASK1 (KCNK3) expression in the adrenal cortex was higher than all of the other genes in its family (about 15-fold higher than KCNK2, and over 40-fold higher than the other members), including TASK3, which has been previously described to be important in setting the glomerulosa cell membrane potential and in regulating their background currents in rats¹⁸. The greater expression of TASK1 over TREK1 and TASK3 was confirmed by qPCR analysis of normal adrenal tissue and H295R cells (Fig.6, panels A and B). TASK1 had 13- and 53-fold higher expression in normal adrenal tissue than TREK1 and TASK3, respectively. In H295R cells TASK1 had 21- and 171-fold higher expression than TREK1 and TASK3, respectively.

Discussion

Ang II increases the capacity for aldosterone biosynthesis in the adrenal cortex through the activation of multiple signaling pathways which are responsible for sustained aldosterone production^{31,33}. Previous studies have shown that Ang II is able to inhibit TASK channels through the activation of phospholipase C (PLC) and its subsequent hydrolysis of PIP2 from the membrane^{25,34-36}. However, there is no clear evidence of which product of this hydrolysis is involved in such a process. TASK channel inhibition culminates with membrane depolarization and entry of Ca^{2+} into the cytoplasm. The KCNK family of K^{+} channels contributes to K^{+} currents in multiple cell types^{37,38}. When members of this family of two-pore domain K^{+} channels are functionally expressed, they give rise to unique K^{+} -selective currents that are open at all voltages, in contrast to other types of K^{+} channels whose activities are controlled by voltage or metabolic regulation. A decrease in leak K^{+} channels would lead to a depolarization that enhances the open probability of both T-type and L-type Ca^{2+} channels, causing periodic Ca^{2+} entry and the stimulation of aldosterone synthesis in adrenal cells (Fig.7). Czirjak and colleagues reported the importance of KCNK channels in adrenal glomerulosa cells¹⁹, specifically in rat glomerulosa cells, where TASK1 and TASK3 seem to be the predominant background channels^{18,19}. Recently two studies described the generation of a mouse model for primary hyperaldosteronism by deletion of TASK1 alone²⁸ or by TASK1/TASK3 double knockout²⁷.

TASK1 knockout mice presented histological and functional changes in the adrenal cortex leading to increased aldosterone levels in these animals²⁸. The authors described the expression of CYP11B2 in the inner zones of adrenal cortex of these animals, whereas the outer layer of cells, corresponding to the adrenal zona glomerulosa, did not express

CYP11B2. The disruption of adrenocortical zonation by TASK1 knockout suggests a role for TASK channels in the steroidogenic enzyme profile of the adrenal cortex. The results suggest yet that, physiologically, the expression of TASK1 is required for CYP11B2 expression in the zona glomerulosa, whereas in the inner zones of the adrenal cortex (fasciculata and reticularis) TASK1 seems to impair the expression of CYP11B2. Taken together these results highlight the importance of TASK1 in the development of the adrenal cortex. Interestingly, in adulthood the phenotype was observed only in females and in castrated males²⁸. In addition, TASK1 knockout females had normal aldosterone levels after treatment with testosterone. Therefore it is likely that androgens play a role in the compensatory mechanism described in males. The authors also reported an increase in TASK3 gene expression in male mice, and suggested that it could restore the KCNK-channel function in the adrenal cortex. TASK3 is known to dimerize with TASK1, setting the membrane potential in the rat adrenal cortex³⁹. Importantly, in mice, TASK3 expression is restricted to the zona glomerulosa, whereas TASK1 is widespread throughout the adrenal cortex²⁷.

The study of TASK1 and TASK3 double knockout mice added crucial information regarding the role of TASK genes in the adrenal. The animals in this study exhibited hyperaldosteronism, which was associated with a rise of 20 mV in glomerulosa cell membrane potential²⁷. The double knockout animals did not express changes in adrenocortical zonation, suggesting that the sex difference observed in TASK1 knockout animals may not be explained by a raise in TASK3 expression. Unfortunately the effects of knocking out TASK3 alone in aldosterone production and adrenocortical zonation are not reported. A recent article reported the expression of two members of the KCNK family, TASK3 (KCNK9) and TREK1 (KCNK2), in H295R cells⁴⁰. These channels are suggested to have a role in setting the membrane potential in these cells, however the production of aldosterone was not significantly altered by transfection with dominant-negative vectors for TASK3 or TREK1⁴⁰. In fact, in this last study, Ang II-stimulated aldosterone production was not affected, and the K⁺ induction was unexpectedly blunted by the dominant-negative constructs.

In our study, no difference was observed in the expression of TASK1 and TASK3 genes between APA and NA. Interestingly, the *in vivo* studies mentioned above with TASK1 or TASK1/TASK3 depletion were not associated with tumorigenic forms of primary hyperaldosteronism. TASK1 knockout animals had altered adrenal zonation and a glucocorticoid-remediable form of hyperaldosteronism. On the other hand, TASK1/TASK3 knockout animals had no difference in the zonation process and displayed a form of hyperaldosteronism that resembles the idiopathic form of hyperaldosteronism observed in humans. Herein, we also demonstrated a much higher expression of TASK1 compared to other members of the KCNK family in human adrenocortical tissue, as well as in H295R cells.

Our functional studies with siTASK1-treated cells showed an increase in aldosterone production in basal conditions, as well as in the presence of aldosterone secretagogues, corroborating the previous studies with knockout mice. Moreover, transfection of H295R cells with siTASK1 was not associated with an elevation in TASK3 mRNA expression (data

not shown) as occurred in the male TASK1 knockout mice²⁸. Importantly, both channels are suggested to be important as background K⁺ channels in rats²⁷. The predominant expression of TASK1 channels in human adrenocortical cells described here might partially explain its major role in regulating human adrenal aldosterone production.

The sustained secretion of aldosterone by adrenal glomerulosa cells is dependent on membrane depolarization and activation of T- and L-type Ca²⁺ channels⁴¹. In agreement with previous studies in adrenocortical cell models^{25, 27, 28}, the knockdown of TASK1 generated H295R cells with a more depolarized membrane potential, which is suggested by the increased Ca²⁺ flux through the plasma membrane. Furthermore, the induction of CYP11B2 expression in siTASK1 cells was totally dependent on the activation of CaMK signaling, which has been reported to be critical for Ang II and K⁺ induction of aldosterone synthesis^{42, 43}.

In conclusion, we have demonstrated for the first time that TASK1 is the predominant KCNK family member expressed in the human adrenal cortex, and that TASK1 knockdown stimulated aldosterone production through augmentation of Ca²⁺ flux and activation of CaMK in human adrenocortical cells (Fig.7). These intracellular signaling events culminated with the activation of early (StAR) and late (CYP11B2) rate-limiting steps in aldosterone production. Further studies are necessary to evaluate functional changes in TASK1 that could potentially contribute to disease conditions associated with increased aldosterone production.

Acknowledgments

This work was supported by National Institutes of Health grant DK43140 to WER.

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Fig.1.

Knockdown of TASK1 expression in H295R cells by siRNA transfection. Real time PCR analysis (qPCR) showed the knockdown of TASK1 mRNA expression in H295R cells transfected with siTASK1 (A). Western analysis confirmed the knockdown of TASK1 protein in H295R cells transfected with siTASK1 in both basal and treated conditions (B). * = $p < 0.05$ (in siTASK1 transfected cells versus control). Results represent the mean $\pm$ S.E.M. for three independent experiments. $P < 0.05$ was considered significant.

Fig.2.

Induction of aldosterone production in siTASK1 transfected H295R cells. Analysis of aldosterone production by RIA showed that knockdown of TASK1 expression caused an increase in aldosterone production under basal, Ang II, and K⁺ treated cells (A). Cortisol production measured by EIA was not altered by siTASK1 (B). * = $p < 0.05$ (in siTASK1 transfected cells versus control). Results represent the mean $\pm$ S.E.M. for five independent experiments. $P < 0.05$ was considered significant.

Fig.3.

Analysis of the early regulatory step in the pathway leading to aldosterone production. H295R cells transfected with siTASK1 had increased expression of StAR protein (A) and pregnenolone production (B). * = $p < 0.05$ (in siTASK1 transfected cells versus control). Results represent the mean $\pm$ S.E.M. from three independent experiments. $P < 0.05$ was considered significant.

Fig.4.

Analysis of the late regulatory steps in the pathway leading to aldosterone production. Transfection of H295R cells with siTASK1 increased CYP11B2 mRNA expression (A), while the expression of CYP11B1 was not altered (B). Time-dependent recovery of TASK1 expression (C) was associated with the gradual decrease in CYP11B2 transcript levels in siTASK1 cells (D). * = $p < 0.05$ (in siTASK1 transfected cells versus control). Results represent the mean $\pm$ S.E.M. for five independent experiments for A and B; and three independent experiments for C and D. $P < 0.05$ was considered significant.

Fig.5.

Analysis of intracellular Ca^{2+} signaling in siTASK1 transfected cells. Evaluation of changes in intracellular Ca^{2+} by Fluo-4 fluorescent measurements in siTASK1 cells showed an elevation in basal fluorescent signals; and this difference in comparison to control cells remained after stimulation with Ang II (A). Blockage of CaMK signaling by KN93 reduced siControl and siTASK1 cell expression of CYP11B2 (B). * = $p < 0.05$ (in siTASK1 transfected cells versus control). § = $p < 0.05$ (in siTASK1 transfected cells in the presence or absence of KN93). Results represent the mean $\pm$ S.E.M. for three independent experiments. $P < 0.05$ was considered significant.

Fig.6.

Comparison of TASK1, TASK3 and TREK1 mRNA levels by qPCR in normal adrenal tissue (A) and H295R cells (B). TASK3 expression levels were set to 1 for comparison of fold change. * = $p < 0.05$ (in comparison to TASK3). \$ = $p < 0.05$ (in comparison to TREK1).

Fig.7.

Schematic of the proposed TASK1 mediated signaling events leading to sustained production of aldosterone. The suppression of background K⁺ currents through functional inhibition of TASK1 by Ang II leads to membrane depolarization and entrance of Ca²⁺ into the cytoplasm, followed by activation of CaMK and induction of StAR and CYP11B2 expression. These events culminate with increased adrenocortical capacity to produce aldosterone.

Table 1
Microarray expression analysis of KCNK family members, CYP11B2, and StAR protein in APA and NA

Gene name	APA	NA	Ratio APA/NA	p Value
KCNK1	0.28 ± 0.01	0.26 ± 0.14	1.10	0.90
KCNK2 (TREK1)	3.57 ± 0.66	3.35 ± 0.24	1.06	0.75
KCNK3 (TASK1)	48.89 ± 4.32	45.03 ± 2.98	1.08	0.19
KCNK4	1.55 ± 2.80	1.69 ± 0.04	0.91	0.79
KCNK5	1.63 ± 0.11	1.81 ± 0.16	0.89	0.56
KCNK6	0.83 ± 0.05	0.70 ± 0.03	1.17	0.14
KCNK7	0.86 ± 0.06	0.92 ± 0.03	0.94	0.11
KCNK9 (TASK3)	1.47 ± 0.09	1.35 ± 0.04	1.09	0.23
KCNK10	0.81 ± 0.10	0.80 ± 0.13	1.01	0.09
KCNK12	0.57 ± 0.05	0.44 ± 0.03	1.29	0.14
KCNK13	0.49 ± 0.05	0.48 ± 0.01	1.01	0.21
KCNK15	0.44 ± 0.03	0.41 ± 0.01	1.07	0.40
KCNK16	1.34 ± 0.07	1.26 ± 0.01	1.06	0.09
KCNK17	1.14 ± 0.05	0.95 ± 0.01	1.19	0.12
CYP11B2	4.03 ± 0.57	0.15 ± 0.04	26.78	< 0.00001
StAR	7.41 ± 0.46	7.93 ± 0.33	0.93	0.41

Comparison of normalized signals for KCNK family members, CYP11B2, and StAR protein in APA versus NA. Microarray analysis of 10 NA and 14 APA revealed similar expression of KCNK family genes between these two groups. TASK1 (KCNK3) expression was predominant in comparison to other KCNK genes in human adrenocortical cells. CYP11B2 expression was significantly higher in APA, whereas StAR protein is similarly expressed in both tissues.