Task3 Potassium Channel Gene Invalidation Causes Low Renin and Salt-Sensitive Arterial Hypertension

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Task1 and Task3 potassium channels (Task: tandem of P domains in a weak inward rectifying K⁺ channel-related acid-sensitive K⁺ channel) are believed to control the membrane voltage of aldosterone-producing adrenal glomerulosa cells. This study aimed at understanding the role of Task3 for the control of aldosterone secretion. The adrenal phenotype of Task3⁻/⁻ mice was investigated using electrophysiology, adrenal slices, and blood pressure measurements. Primary adrenocortical cells of Task3⁻/⁻ mice were strongly depolarized compared with wild-type (−52 vs. −79 mV), and in fresh adrenal slices Ca²⁺ signaling of Task3⁻/⁻ glomerulosa cells was abnormal. In living Task3⁻/⁻ mice, the regulation of aldosterone secretion showed specific deficits: Under low Na⁺ and high K⁺ diets, protocols known to increase aldosterone, and under standard diet, Task3 inactivation was compensated and aldosterone was normal. However, high Na⁺ and low K⁺ diets, two protocols known to lower aldosterone, failed to lower aldosterone in Task3⁻/⁻ mice. The physiological regulation of aldosterone was disturbed: aldosterone-renin ratio, an indicator of autonomous aldosterone secretion, was 3-fold elevated at standard and high Na⁺ diets. Isolated adrenal glands of Task3⁻/⁻ mice produced 2-fold more aldosterone. As a consequence, Task3⁻/⁻ mice showed salt-sensitive arterial hypertension (plus 10 mm Hg). In conclusion, Task3 plays an important role in the adaptation of aldosterone secretion to dietary salt intake. (Endocrinology 153: 4740–4748, 2012)

H igh blood pressure is one of the major cardiovascular risk factors (1). The pathogenesis of arterial hypertension, however, is very complex and encompasses environmental, genetic, vascular, and endocrine factors. Among the latter, inappropriately high aldosterone secretion is a common cause of salt and water retention resulting in hypertension. Recently, data from human genetics (2–4) pointed to a critical role of K⁺ channel defects as a cause of hyperaldosteronism.

In adrenal glomerulosa cells, depolarization is considered to be the first step of a chain of events leading to aldosterone secretion (5). Glomerulosa cells have a very high K⁺ conductance leading to a hyperpolarized membrane potential close to the K⁺ equilibrium potential. Thus, the membrane voltage closely follows the changes of the K⁺ equilibrium potential, and even small increases of plasma K⁺ can be sensed and depolarize the cells. Hyperkalemia or inhibition of the K⁺ conductance by angioten-
sin II results in membrane depolarization, which in turn activates voltage-sensitive T-type Ca\(^{2+}\) channels (5). By this mechanism, depolarization is transduced into a Ca\(^{2+}\) signal that induces aldosterone synthesis.

Recently, two pore domain K\(^+\) channels (K\(_{2p}\)) from the TASK family (TASK stands for TWIK-related acid-sensing K\(^+\) channel; TWIK stands for tandem of P domains in a weak inward rectifying K\(^+\) channel") have been reported to be important in the physiology of glomerulosa cells (6–14). The functional relevance of Task channels in adrenal glands has been highlighted by three different mouse models: Task1\(^{-/-}\) single-mutant, Task1\(^{-/-}\)/Task3\(^{-/-}\) double-mutant, and Task3\(^{-/-}\) single-mutant mice. All models showed hyperaldosteronism albeit with important pathophysiological differences. Deletion of Task1 led to depolarization of adrenocortical cells and to a sex-specific ectopic expression of aldosterone synthase in the zona fasciculata of female mice. As a result, female Task1\(^{-/-}\) mice displayed a diet-independent, low renin, and glucocorticoid-remediable form of hyperaldosteronism (15). Interestingly, TASK1 has also been shown to regulate aldosterone secretion in human cells (16). Like male Task1\(^{-/-}\) mice, Task1\(^{-/-}\)/Task3\(^{-/-}\) double-mutant male mice did not exhibit aberrant localization of aldosterone synthase in the zona fasciculata. But in contrast to the phenotypically normal male Task1\(^{-/-}\) mice, Task1\(^{-/-}\)/Task3\(^{-/-}\) mice also displayed low renin hyperaldosteronism. The phenotype of Task1\(^{-/-}\)/Task3\(^{-/-}\) male mice was considered to be remarkably similar to the clinical presentation of patients with idiopathic primary hyperaldosteronism (9). Very recently, Guagliardo et al. (17) described hyperaldosteronism and hypertension of single Task3\(^{-/-}\) mice. Based mainly on pharmacological in vivo experiments, the authors proposed angiotensin II hypersensitivity as cause of the hyperaldosteronism in Task3\(^{-/-}\) mice.

The aim of present study was to gain further insights into the pathophysiological mechanisms underlying the adrenal phenotype of Task3\(^{-/-}\) mice. Similar to Guagliardo et al. (17), we observed in our independent Task3 knockout mouse model a strong increase of the plasma aldosterone/renin ratio (ARR), a clinical parameter that is classically considered as a robust index for inappropriate aldosterone secretion and salt sensitivity (18). For a more detailed phenotypic analysis, we performed measurements of Ca\(^{2+}\) transients in ex vivo tissue using a novel fluorescence-based adrenal slice preparation, and aldosterone secretion was determined in ex vivo perfused adrenal glands. These ex vivo approaches revealed that a significant part of the aldosterone secretion of Task3\(^{-/-}\) mice is autonomous (independent from angiotensin II) and not due to angiotensin II hypersensitivity.

### Materials and Methods

#### Animal experiments

Task3\(^{-/-}\) mice were generated as described elsewhere (19). The animals were backcrossed for 11 generations into the C57 Bl/6j genetic background. Mice (3–6 months of age) were chronically maintained on a normal diet (chow, R03T-25; SAFE, 0.75% K\(^+\), 0.27% Na\(^+\)) or on an experimental diet (Ssniff Spezialdiäten GmbH, Soest, Germany): high Na\(^+\) (8% NaCl) or low Na\(^+\) (<0.03% NaCl) diets for 2 wk; high K\(^+\) (3% K\(^+\)) or low (<0.05% K\(^+\)) K\(^+\) diets for 1 wk. The animals had free access to food and water. The experimental protocols were approved by the local councils for animal care and were conducted according to the German and French laws for animal care and the NIH Guide for the Care and Use of Laboratory Animals. Mouse anesthesia was carried out with 1.5–3% isoflurane (Baxter Deutschland GmbH, Unterschleißheim, Germany).

#### Primary cell culture

Under isoflurane anesthesia, adult male mice were perfused with a collagenase-containing Ringer-type solution [0.5 mg/ml collagenase II (Biochrome, Berlin, Germany) and 0.5 mg/ml collagenase IV (Sigma-Aldrich, Munich, Germany)]. Adrenal glands were harvested, cut into small pieces, and digested for another 10 min at 37 C. Single cells and cell clusters were seeded on culture dishes (Falcon, Heidelberg, Germany) in DMEM/F-12 (1:1) (Life Technologies, Inc., Darmstadt, Germany) supplemented with 2% heat-inactivated fetal calf serum (Life Technologies, Inc.), 0.1 mM ascorbic acid (Sigma-Aldrich), 1 μmol/liter (+)-α-Tocopherol (Sigma-Aldrich), 1 mg/liter human insulin solution (Sigma-Aldrich), and 0.5% Penicillin/Streptomycin (Life Technologies, Inc.). Cells were used for measurements 16–24 h after seeding.

#### Patch clamp of primary adrenocortical cells

Whole-cell recordings at room temperature were performed on primary cells using an EPC-10 amplifier (Heka, Lambrecht, Pfalz, Germany). Patch pipettes (8–12) MΩ were used for the recordings. The patch pipette solution contained (in mmol/liter) 95 K-gluconate, 30 KCl, 4.8 Na\(_2\)HPO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 5 glucose, 2.38 MgCl\(_2\), 0.726 CaCl\(_2\), 1 EGTA, 3 ATP, pH 7.2. The extracellular Ringer-type control solution contained (in millimoles/liter) 142.5 NaCl, 0.4 Na\(_2\)HPO\(_4\), 1.6 Na\(_2\)HPO\(_4\), 5 glucose, 1 MgCl\(_2\), 1.3 CaCl\(_2\), 5 HEPES, and 3.8 KCl, pH 7.4.

#### Ca\(^{2+}\) measurements

Adrenal glands from adult male mice were placed into chilled storage solution containing (in millimoles/liter) 26 NaHCO\(_3\), 116.5 NaCl, 1.25 NaH\(_2\)PO\(_4\), 10 glucose, 2 MgCl\(_2\), 1 CaCl\(_2\), 2.5 KCl, pregazed with medical carbogen. Glands were cut into 150-μm thick slices using a vibratome (VT 1200 S; Leica, Wet­zlar, Germany). Slices were kept for a maximum of 5 h in storage solution gazed with carbogen. The slices were loaded with 2.5 μmol/liter Fluo-4 AM in the presence of 1× Power Load permeabilizing reagent (Invitrogen GmbH, Darmstadt, Germany).

#### Immunofluorescence

Anesthetized mice (3% isoflurane) were killed by replacement of blood by 0.9% NaCl solution containing 10 IU/ml...
heparin via a catheter placed into the abdominal aorta. For tissue fixation, mice were then perfused with a fixative containing (mm): 3% paraformaldehyde, 100 sucrose, 90 NaCl, 1.5 K2HPO4, 1 EGTA, 2 MgCl2, pH 7.4. Adrenals were removed, incubated in 17% sucrose solution for 30 min, and frozen in isomethylbutane (−30 °C). Cryosections (5 μm) were mounted on Polysine slides (Kindler, Freiburg, Germany). Before incubation with the primary antibodies, sections were incubated in SDS 0.1% (5 min), and rinsed again with PBS. Primary and secondary antibodies were diluted in PBS (pH 7.4) containing 0.04% Triton X-100 and 0.5% BSA (Sigma). Primary antibodies were applied overnight at 4°C: a polyclonal rabbit antialdosterone synthase antibody [kindly provided by Dr. Celso Gomez Sanchez (20)] and a polyclonal rabbit anti-Task3 antibody (Alomone Laboratories, Jerusalem, Israel). As secondary antibodies, appropriate CY2 and Alexa555-labeled antibodies were used (Invitrogen, Germany).

Plasma renin concentration

For the measurement of the plasma renin concentration (PRC) of mice, blood samples were taken from a facial vein and incubated for 1.5 h at 37°C with plasma of bilaterally nephrectomized male rats as renin substrate. The production of Angiotensin I (ng/ml-h) was measured by [125I] RIA (Byk and DiAsorin Diagnostics, Germany) to determine the PRC.

Ex vivo perfused adrenal glands

Adult mice were anesthetized with isoflurane 3%, and both adrenal glands were removed. Single adrenals were cut into four pieces, placed into a Pasteur pipette containing 1 ml Sephadex G50 (Sigma-Aldrich), suspended in control solution, and perfused (0.15 ml/min for 90 min at 37°C) with low K+ (2.5 mM) washing solution. The low K+ washing solution contained 46.9 ml of DMEM Low Glucose 31885 (Life Technologies, Inc.) and 53.1 ml of Krebs-HEPES solution (in millimolar concentration: NaCl, 90, CaCl2, 1.8, MgSO4, 0.8, NaHCO3, 1 Na2HPO4, 20 HEPES, 14.5 glucose). Finally, 100 mg/liter of BSA was added to the washing solution and, after gazing with medical carbogen (5% CO2 and 95% O2), the pH was adjusted to 7.4. After washing, adrenals were perfused for 40 min with control solution. For the control solution (containing 3.8 mm K+), 2.5 mm of NaCl in the Krebs-HEPES solution was replaced by KCl. At the end of the perfusion period, an aliquot of the flowthrough was collected, and the aldosterone concentration was measured.

Real-time RT-PCR

Real-time RT-PCR was performed on a LightCycler 480 device (Roche, Basel, Switzerland) using an annealing temperature of 57°C and the following primer pairs: Task3 sense primer: ACATCAGCTCCGATGACTACC; Task3 antisense primer: CAGGTTGACCATGTCCTACA; β-actin sense primer: CCA CGG ATC CAC ACA GAG TAC TT; β-actin antisense primer: GAC AGG ATG CAG AAG GAG ATT ACT G.

Statistics

Data from n observations are shown as mean values ± SEM as indicated. Paired as well as unpaired Student’s t test was used as appropriate. Differences were considered significant if P < 0.05.

Results

Cellular localization of Task3 in mouse adrenal gland

The Task3 K+ channel was strongly expressed in mouse adrenal glands and the central nervous system, whereas Task3 mRNA was almost absent in the other tissues examined (Fig. 1). Previous in situ hybridization studies demonstrated high levels of Task3 mRNA in glomerulosa cells and in the adrenal medulla (9, 21). By immunofluorescence, the Task3 protein was found in the plasma membrane of glomerulosa cells and in the outer portion of zona fasciculata with the latter only observed in adult male mice (Fig. 1A). The sex-dependent expression was under the control of testosterone because castration diminished the Task3 signal and testosterone restored Task3 mRNA and protein expression in castrated mice (our unpublished data). In contrast to the ectopic expression of aldosterone synthase [Cyp11b2 (name of the aldosterone synthase gene)] observed in fasciculata cells of female Task1+/− mice (15), the zona glomerulosa-restricted expression of the aldosterone synthase was preserved in Task3−/− mice from both sex (Fig. 1B).

Task3 channel disruption depolarized adrenocortical cells

To evaluate the contribution of Task3 channels to background K+ currents, primary cultured adrenocortical cells of adult male Task3−/− and wild-type mice were examined by the patch-clamp technique. At resting conditions, cells from Task3−/− displayed a strongly reduced K+ current (reduced steepness of the I/V curve in Fig. 1D) and were strongly depolarized (Fig. 1E). These data indicate that Task3 is a major component of the K+ conductance in mouse adrenocortical cells.

Ca2+ signaling of glomerulosa cells in adrenal slices

To investigate glomerulosa cell function in a more native environment, Fluo-4 Ca2+ measurements were performed in fresh adrenal slice preparations. In glomerulosa cells from wild-type animals, spontaneous Ca2+ oscillations were rarely observed under control conditions. Upon angiotensin II (40 nm) stimulation, cytosolic Ca2+ was increased in wild-type glomerulosa cells, and almost all of the responsive cells displayed oscillatory activity (Fig. 2A; Supplemental Video 1 published on The Endocrine Society’s Journals Online web site at http://endo.endojournals.org). In slices from Task3−/− mice, glomerulosa cells behaved differently. Already under control conditions, oscillations of cytosolic Ca2+ activity were observed frequently. Angiotensin II elicited a heterogeneous response:
some cells kept oscillating as before, other cells stopped their oscillatory activity after an initial Ca^{2+} peak, and some cells responded in a way similar to wild-type cells (Fig. 2B and Supplemental Video 2). The summary of slices from three wild-type and three Task3^{-/-} animals showed a robust and sustained increase of cytosolic Ca^{2+} by an-

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**FIG. 2.** Fluo-4 Ca^{2+} measurements in fresh adrenal slices. A and B, Original traces of the angiotensin II (AngII) response of three typical Task3^{+/+} (A) and Task3^{-/-} (B) glomerulosa cells. C and D, Mean values ± SEM of Fluo-4 fluorescence in six independent slices (three animals each genotype). Values are expressed in arbitrary units (au). E and F, Ca^{2+} measurement in fresh adrenal slices after stimulation with high extracellular K^{+}. Traces represent mean values ± SEM of at least three independent experiments. Values are expressed in arbitrary units (au).
data (Fig. 1, D and E, and Fig. 2) that Task3 deletion has a strong effect on aldosterone secretion. On a normal salt diet, however, Task3−/− mice did not present with apparent hyperaldosteronism (Fig. 3). In agreement with normal aldosterone levels at control conditions, Task3−/− mice exhibited grossly normal serum and urine electrolytes (Tables 1 and 2). Given the high Task3 expression in zona fasciculata of males, fasciculata-derived corticosterone was measured under basal unstressed conditions. No differences in plasma corticosterone between wild-type and knockout animals were observed (our unpublished data).

To assess the physiological regulation of aldosterone secretion by salt intake, mice were subjected to various diets. At high K+ and low Na+ diets, the increases of plasma aldosterone levels were largely similar in Task3+/+ and Task3−/− mice. However, under diets expected to reduce the aldosterone secretion, i.e., low K+ and high Na+ diets, knockout animals showed disturbed responses. Under low K+ diet, glomerulosa cells of wild-type mice hyperpolarized (due to the high equilibrium potential of K+ at conditions with low plasmatic K+). This hyperpolarization reduced Ca2+ influx through depolarization-activated Ca2+ channels and, as a consequence, decreased aldosterone secretion of wild-type mice (Fig. 3). By contrast, female Task3−/− mice failed to lower aldosterone secretion significantly at low K+ diet, probably because Task3−/− cells were unable to hyperpolarize normally (Fig. 3).

Under high Na+ diet, Task3+/+ animals exhibited the physiological reduction of plasma aldosterone, whereas Task3−/− animals were totally unresponsive (Fig. 4A). The normal control of aldosterone by dietary Na+ occurs via the renin-angiotensin axis. Despite the normal aldosterone levels under control conditions, PRC was decreased in Task3−/− mice [PRC (ng AngI/ml): wild-type males: 213 ± 22, n = 8; Task3−/− males: 72 ± 14, n = 9; wild-type females: 175 ± 46, n = 8; Task3−/− females: 51 ± 16, n = 5]. Accordingly, plasma ARR, a clinical indicator for autonomous aldosterone production, was more than doubled in Task3−/− mice both under control conditions and high Na+ diet (Fig. 4B). Under low Na+ intake, PRC was increased similarly in both genotypes, indicating that the functional integrity of

TABLE 1. Serum parameters (in mM) in Task3−/− and Task3+/+ mice

<table>
<thead>
<tr>
<th></th>
<th>Na+</th>
<th>K+</th>
<th>Cl−</th>
<th>Phosphate</th>
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</thead>
<tbody>
<tr>
<td>Female Task3+/+ (n = 9)</td>
<td>137.2±2.6</td>
<td>4.9±0.2</td>
<td>110.6±2.3</td>
<td>2.0±0.1</td>
</tr>
<tr>
<td>Female Task3−/− (n = 8)</td>
<td>139.2±2.3</td>
<td>4.8±0.2</td>
<td>111.5±2.1</td>
<td>2.1±0.1</td>
</tr>
<tr>
<td>Male Task3+/+ (n = 7)</td>
<td>139.2±2.7</td>
<td>5.4±0.3</td>
<td>110.1±2.2</td>
<td>2.2±0.1</td>
</tr>
<tr>
<td>Male Task3−/− (n = 6)</td>
<td>141.3±1.3</td>
<td>5.2±0.2</td>
<td>112.8±2.3</td>
<td>2.4±0.1</td>
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</table>

No statistically significant difference between the genotypes. Mean values ± SEM.
renin-secretory mechanism was still maintained after Task3 gene invalidation [PRC (ng AngI/h/ml): Task3+/+ males: 597 ± 84, n = 10; Task3−/− males: 676 ± 184, n = 10; Task3++/+ females: 724 ± 107, n = 9; Task3−/− females: 592 ± 90, n = 8].

Aldosterone secretion in in vitro perfused glands

The high ARR of Task3−/− mice suggested that their aldosterone secretion was 1) partially autonomous (independent from physiological stimuli) and 2) induced suppression of the renin-angiotensin system, thereby compensating for the partial autonomy. To test this hypothesis, measurements of aldosterone secretion were performed in vitro on freshly isolated and perfused adrenal gland tissue. In the absence of angiotensin II, perfused adrenal gland tissue of Task3−/− mice produced 2-fold more aldosterone as wild-type tissue (Fig. 4C). These data on ex vivo tissue corroborated the concept that suppression of the renin-angiotensin axis compensated for partially autonomous aldosterone production of Task3−/− mice.

Effect of dietary salt intake on blood pressure

Arterial systolic blood pressure (SBP) was assessed in wild-type and knockout animals by tail cuff measurements. Under a normal-salt diet, no differences were observed between the genotypes. Increasing the NaCl concentration to 8% in the diet for 1 wk had no effect on SBP in wild-type mice, whereas an increase of approximately 10 mm Hg was observed in Task3−/− mice of both sexes (Fig. 4D).

Discussion

Recent findings from knockout mice as well as human genetics underlined the importance of glomerulosa cell K+ channels for the control of aldosterone secretion, adrenocortical zonation, and adenoma formation (3, 9, 12, 15). Here we took advantage of the Task3−/− single-knockout mouse to investigate the specific contribution of this K+ channel to mineralocorticoid homeostasis. In vitro analysis of adrenocortical cells and adrenal slices disclosed Task3 as an important determinant of the membrane resting potential and Ca2+ signaling. Even though glomerulosa cells of Task3−/− mice showed profound depolarization at resting conditions and disturbed Ca2+ signaling, the animals still managed to have grossly normal plasma aldosterone levels. However, the high ARR and salt-sensitive hypertension pointed to substantial alterations in the hormonal control of aldosterone secretion in Task3−/− mice.

Electrophysiology and Ca2+ signaling in Task3-expressing glomerulosa cells

Task3 is a K2P channel almost exclusively expressed in the glomerulosa layer from adrenal cortex in female mice whereas in males it is also found in the outer part of zona fasciculata. The presence of Task3 in zona fasciculata in males might contribute to the compensation observed in male Task1−/− mice, which recovered normal adrenal zonation of the aldosterone synthase and normal aldosterone production after puberty (15).

In agreement with previous reports (6, 22) and disagreement with a very recent report (17), our experiments on primary cultured adrenocortical cells showed that the K+ current carried by Task3 channels is a major determinant of the resting membrane potential. Surprisingly, expression of the aldosterone synthase remained restricted to the zona glomerulosa in Task3−/− mice of both sexes and did not mimic the ectopic fasciculata expression found in Task1−/− mice (15). Clearly, the depolarization of glomerulosa and fasciculata cells may contribute to the loss of functional zonation in female Task1−/− mice but is not sufficient to explain the spectacular phenotype of female Task3−/− mice.

Ca2+ imaging on brain slice preparations has proved to be a powerful technique with which to investigate neuronal activity in complex tissue networks (23). We have adapted the slice technique for adrenal tissue to circumvent possible artifacts in primary cultured dispersed cells and to unambiguously identify subcapsular glomerulosa cells. Similar to dispersed cells, slices from Task3−/− mice behaved very differently from wild-type under basal conditions. Task3−/− adrenocortical slices frequently displayed spontaneous Ca2+ oscillations in the absence of angiotensin II, a phenomenon rarely found in wild-type slices. These oscillatory changes of cytosolic Ca2+ are in

### TABLE 2. Urine parameters (in mM) in Task3−/− and Task3+/+ mice

<table>
<thead>
<tr>
<th></th>
<th>Na+</th>
<th>K+</th>
<th>Cl−</th>
<th>Phosphate</th>
<th>Creatinine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Task3+/+ (n = 9)</td>
<td>175±29</td>
<td>265±25</td>
<td>149±16</td>
<td>37± 9</td>
<td>3.3±0.4</td>
</tr>
<tr>
<td>Female Task3−/− (n = 8)</td>
<td>201±29</td>
<td>208±30</td>
<td>146±20</td>
<td>10±2‡</td>
<td>2.7±0.3</td>
</tr>
<tr>
<td>Male Task3+/+ (n = 7)</td>
<td>140±28</td>
<td>205±22</td>
<td>157±20</td>
<td>21±11</td>
<td>2.8±0.3</td>
</tr>
<tr>
<td>Male Task3−/− (n = 6)</td>
<td>120±35</td>
<td>296±49</td>
<td>150±41</td>
<td>28± 6</td>
<td>3.3±0.5</td>
</tr>
</tbody>
</table>

*a* Significant difference between the genotypes. Mean values ± SEM.
line with a very recent patch-clamp study by Hu et al. (24) in which oscillations of the membrane voltage have been observed, probably caused by oscillatory activity of Cav3.2 Ca\(^{2+}\) channels. In this beautiful study, oscillations were already present at control conditions, and their frequency was increased by high K\(^+\) or angiotensin. Although we rarely found Ca\(^{2+}\) oscillations under control conditions in wild-type slices (probably due to differences in experimental conditions), Ca\(^{2+}\) oscillations of high frequency occurred after angiotensin II stimulation and in constitutively depolarized cells in Task3\(^{-/-}\) slices. In contrast to the measurement of an individual cell per experiment using the patch-clamp technique (24), our fluorescence-based approach allowed the parallel measurements of the cytosolic Ca\(^{2+}\) activity in many cells of a whole area of the adrenal cortex. The apparently concerted changes of Ca\(^{2+}\) activity of neighboring cells suggests that adrenocortical cells might be coupled (Supplemental Videos 1 and 2).

Taken together, we assume that the depolarized state of Task3\(^{-/-}\) cells is responsible for the entry of Ca\(^{2+}\) through voltage-dependent Ca\(^{2+}\) channels. This rise in Ca\(^{2+}\) probably causes activation of Ca\(^{2+}\)-dependent K\(^+\) channels that transiently repolarizes the cells, thus leading, in concert with transient activation of Cav3.2 channels (24), to the oscillatory pattern of intracellular Ca\(^{2+}\) activity. Ca\(^{2+}\) oscillations were also observed in zona fasciculata cells, but this spontaneous activity did not translate in aldosterone secretion because the Cyp11b2 is not expressed in these cells. Interestingly, angiotensin II often led to silencing of previously active cells in Task3\(^{-/-}\) slices. Further studies will be needed to investigate why the canonical increase of cytosolic Ca\(^{2+}\) by angiotensin II was diminished in Task3\(^{-/-}\) adrenal slices.

**Task3\(^{-/-}\) mice present with high ARR and salt-sensitive hypertension**

Given the striking genotype-related differences in the in vitro preparations, we were surprised that the phenotype of Task3\(^{-/-}\) mice was largely normal at first sight. Not only were plasma aldosterone levels not altered at basal conditions, they also showed adaptation to classical challenges such as high K\(^+\) as well as low Na\(^+\) diets. However, the physiological suppression of plasma aldosterone levels by high Na\(^+\) intake was virtually absent in Task3\(^{-/-}\) mice. These data point to defective control of aldosterone secretion by the renin-angiotensin axis. Indeed, the ARR was clearly increased. Characteristically, increases in the ARR are observed in states in which aldosterone secretion is partially autonomous, i.e. independent from its key regulator renin-angiotensin system (18, 25, 26). Therefore, high ARR is a hallmark of latent and apparent hyperaldosteronism. It is likely that the constitutively depolarized state of glomerulosa cells in Task3\(^{-/-}\) animals produced a partially autonomous aldosterone release that is counterbalanced by a decreased activity of the renin-angiotensin axis.
Very recently, Guagliardo et al. (17) also described low-renin hyperaldosteronism in another independent Task3−/− mouse model. They concluded, mainly from pharmacological in vivo data, that hypersensitivity toward angiotensin II is responsible for the hyperaldosteronism of Task3−/− mice. To test the relevance of angiotensin II for the hypersecretion in our Task3−/− mice, in vitro measurements on perifused adrenal tissue were performed. However, already in the complete absence of angiotensin in the perfusate, tissue of Task3−/− animals showed a 2-fold increase of the aldosterone secretion compared with wild-type tissue. Our data indicate that autonomous aldosterone secretion (independent from angiotensin II) substantially contributes to the hyperaldosteronism because it can no longer be suppressed by decreased amounts of circulating angiotensin II under a high Na+ or low K+ diets. We conclude that both TASK1 and TASK3 are major hyperpolarizing channels in cortical adrenal. Their activity is required to confer the physiological K+ and angiotensin II sensitivity to glomerulosa cells. TASK1 appears to have an additional function that is probably independent form its hyperpolarizing effect on the membrane voltage: it prevents expression of Cyp11b2 in fasciculata cells, thus restricting its expression to glomerulosa cells in which the aldosterone secretion is physiologically regulated.

Outlook: a role of TASK3 in the human adrenal gland?

Despite large efforts in genetic studies of hypertension (30, 31), the mechanisms underlying interindividual variations of blood pressure and salt sensitivity in the general population remain poorly understood. In mice, Task3 appears to be a critical component for the adaption of aldosterone secretion to dietary salt intake. Further studies will be needed to test whether Task3 has a similar function in humans and might contribute to salt sensitivity in certain patients with arterial hypertension.

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