Renin-Angiotensin-Aldosterone System

TASK-3 Channel Deletion in Mice Recapitulates Low-Renin Essential Hypertension

Nick A. Guagliardo, Junlan Yao, Changlong Hu, Elaine M. Schertz, David A. Tyson, Robert M. Carey, Douglas A. Bayliss, Paula Q. Barrett

Abstract—Idiopathic primary hyperaldosteronism (IHA) and low-renin essential hypertension (LREH) are common forms of hypertension, characterized by an elevated aldosterone-renin ratio and hypersensitivity to angiotensin II. They are suggested to be 2 states within a disease spectrum that progresses from LREH to IHA as the control of aldosterone production by the renin-angiotensin system is weakened. The mechanism(s) that drives this progression remains unknown. Deletion of Twik-related acid-sensitive K⁺ channels (TASK) subunits, TASK-1 and TASK-3, in mice (T1T3KO) produces a model of human IHA. Here, we determine the effect of deleting only TASK-3 (T3KO) on the control of aldosterone production and blood pressure. We find that T3KO mice recapitulate key characteristics of human LREH, salt-sensitive hypertension, mild overproduction of aldosterone, decreased plasma-renin concentration with elevated aldosterone:renin ratio, hypersensitivity to endogenous and exogenous angiotensin II, and failure to suppress aldosterone production with dietary sodium loading. The relative differences in levels of aldosterone output and aldosterone:renin ratio and in autonomy of aldosterone production between T1T3KO and T3KO mice are reminiscent of differences in human hypertensive patients with LREH and IHA. Our studies establish a model of LREH and suggest that loss of TASK channel activity may be one mechanism that advances the syndrome of low renin hypertension. (Hypertension. 2012;59:999-1005.)  ● Online Data Supplement

Key Words: TASK channels ▶ aldosterone ▶ hyperaldosteronism ▶ low renin essential hypertension

Aldosterone plays an important role in the regulation of blood pressure (BP). When overtly dysregulated it causes primary aldosteronism (PA), a syndrome characterized by hypertension, high plasma aldosterone concentration, decreased plasma renin activity and varying degrees of hypokalemia, and metabolic alkalosis. In PA, aldosterone overproduction is relatively independent of the renin-angiotensin system (RAS) and, thus, not suppressed by sodium loading. PA is the result of both known (aldosterone-producing adenomas and glucocorticoid-remediable hyperaldosteronism) and unknown causes (idiopathic hyperaldosteronism) and is suggested to be 2 states within a disease spectrum that progresses from LREH to IHA as the control of aldosterone production by the renin-angiotensin system is weakened. The mechanism(s) that drives this progression remains unknown. Deletion of Twik-related acid-sensitive K⁺ channels (TASK) subunits, TASK-1 and TASK-3, in mice (T1T3KO) produces a model of human IHA. Here, we determine the effect of deleting only TASK-3 (T3KO) on the control of aldosterone production and blood pressure. We find that T3KO mice recapitulate key characteristics of human LREH, salt-sensitive hypertension, mild overproduction of aldosterone, decreased plasma-renin concentration with elevated aldosterone:renin ratio, hypersensitivity to endogenous and exogenous angiotensin II, and failure to suppress aldosterone production with dietary sodium loading. The relative differences in levels of aldosterone output and aldosterone:renin ratio and in autonomy of aldosterone production between T1T3KO and T3KO mice are reminiscent of differences in human hypertensive patients with LREH and IHA. Our studies establish a model of LREH and suggest that loss of TASK channel activity may be one mechanism that advances the syndrome of low renin hypertension. (Hypertension. 2012;59:999-1005.)  ● Online Data Supplement

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also supported the importance of K channel activity in the control of aldosterone production. For example, deletion of 2 mouse genes that encode 2-pore domain Twik-related acid-sensitive K⁺ channels (TASK) channel subunits yields hyperaldosteronism. Ablation of Kcnk3 (TASK-1) causes an adrenal zonation defect and a novel form of glucocorticoid-remediable hyperaldosteronism, whereas deletion of both Kcnk3 and Kcnk9 (TASK-3) produces a syndrome that closely resembles human IHA, hypertension, increased urinary excretion of aldosterone, decreased levels of plasma renin, exaggerated ARR, and failure to suppress aldosterone production with Na⁺ loading or to normalize production with angiotensin II (Ang II) type 1 receptor blockade. Both TASK-1 and TASK-3 subunits are prominently expressed in adrenal ZG cells. By forming homodimeric or heterodimeric “leak” K⁺ channels, these subunits provide a background hyperpolarizing conductance that contributes to setting the negative membrane potential of ZG cells. ZG cells lacking both TASK-1 and TASK-3 are depolarized (by ≈20 mV), which permits autonomous overproduction of aldosterone characteristic of the IHA phenotype. Here, we show that deletion of only TASK-3 recapitulates key features of the milder LREH syndrome, salt-sensitive hypertension, mild overproduction of aldosterone, decreased levels of plasma renin, greater sensitivity to Ang II, and maintained resistance to Na⁺ suppression. We conclude that the progressive loss of TASK channel activity maybe a mechanism that advances the syndrome of low-renin hypertension.

Methods

Mice

TASK knockout (KO) mice were generated as described previously (please see the online-only Data Supplement). Male mice were used to remove the potential confounds of hormonal surges associated with the estrus cycle. All of the experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Virginia Animal Care and Use Committee.

Metabolic Cage Experiments

Salt Diets

Metabolic cage experiments and blood analysis were conducted as described previously using 1 of 4 experimental protocols (please see Figure S1 and expanded Methods section in the online-only Data Supplement for details). Diets contained normal Na⁺ (NS; 0.3% Na⁺, 0.8% K⁺), low Na⁺ (LS; 0.05% Na⁺, 0.8% K⁺), high Na⁺ (HS; 4.0% Na⁺, 0.8% K⁺), or high K⁺ (4.0% K⁺, 0.3% Na⁺).

Ang II Delivery

After NS urine collection (days 4–7), ALZET osmotic minipumps (Durect Corporation, Cupertino, CA) containing Ang II (0.040/0.4/0.800/1.200/4.000 μg of Ang II per kg/min) or 0.9% saline vehicle were implanted SC. Mice were allowed 1 day of surgical recovery before collection of 24-hour urine samples (days 10–13; please see the online-only Data Supplement).

Telemetric BP

BP was recorded from conscious, freely behaving mice. Pressure transmitters (Data Sciences International telemetry system; DSI, St Paul, MN) were threaded through the left carotid artery and implanted in the aortic arch (please see the online-only Data Supplement). After 7 days of surgical recovery, BP and heart rate were recorded (NS, days 8–11) before challenge with HS (days 12–18) or with candesartan (10 mg/kg per day) delivered in the drinking water (days 12–15).

ZG Isolation, RT-PCR, and Western Blot Analysis

The ZG layer was isolated from mouse adrenal glands using laser capture microdissection (Leica Microsystems, Inc.). RNA was isolated for quantitative RT-PCR analysis of TASK-1, Kcnj5, Ang II type 1A receptor, Ang II type 1B receptor, and Cyp11b2 expression. For Western blot analysis of CYP11b2 protein, adrenal lysates from a single mouse (2 adrenals) were combined and equivalent total protein diluted with SDS sample buffer, resolved by 10% SDS-PAGE, and analyzed by immunoblot using anti-Cyp11b2 antibody (please see the online-only Data Supplement).

Electrophysiology

Thinly sectioned adrenal sections (80 μm) were prepared from 35- to 55-day-old mice, and ZG cells were identified by their location in subcapsular cell rosettes. Electrophysiological recordings were obtained at room temperature using patch electrodes (3–5 MΩ), an Axopatch 200B amplifier, and a pCLAMP 10.3 (Molecular Devices). Baseline membrane voltages were recorded in current clamp from ZG cells after 2 to 4 minutes of perfusion with standard external solution (please see the online-only Data Supplement).

Data Analysis

For each experimental protocol, T1T3KO or T3KO mice were run in parallel with wild-type (WT) mice to control for potential environmental differences among cohorts. Individual animals were run on only 1 experimental protocol, and data were collapsed by genotype across cohorts. Mean urine aldosterone/creatinine and plasma renin concentration were log transformed because of unequal variances and analyzed using a 2-way ANOVA. Plasma K⁺ and Cyp11b2 were compared using a 2-way ANOVA. BP and heart rate were analyzed using a 1-way (NS) or repeated-measures 2-way ANOVA (before and after candesartan, HS). If the overall ANOVA reported a statistical significance (P<0.05), group means were compared using a Bonferroni post hoc test and significance determined if P<0.05. Dose-response curves for Ang II sensitivity were generated and analyzed with Origin Pro software. An independent t test compared TASK-1 and Kcnj5 mRNA expression and membrane potential, with significance at P<0.05.

Results

T3KO Mice Display Mild Hyperaldosteronism

In previous work, we found that TASK-1⁻/⁻:TASK-3⁻/⁻ double KO mice on a mixed genetic background (SV129/ C57BL/6) produced more aldosterone than age-matched control littermates despite lower levels of plasma renin concentration (PRC). Here, we measured urinary aldosterone excretion (24-hour, normalized to creatinine) and PRC in TASK-1⁻/⁻:TASK-3⁻/⁻ mice on a congenic C57BL/6 background (T1T3KO) to determine whether the reported phenotypic difference between genotypes was attributable to deletion of the TASK genetic loci. Consistent with our previous observations, T1T3KO mice on an NS-diet displayed overt hyperaldosteronism, producing >4-fold more aldosterone than C57BL/6 WT mice (P<0.001) despite plasma renin levels that were only 20% of that of WT mice (P<0.001; Figure 1A, left). This dysregulation resulted in an ARR that was 30-fold greater than WT, consistent with the previously characterized mouse IHA phenotype (Figure 1B, left). Thus, the PA phenotype is associated with the deletion of TASK-1 and TASK-3 and is not dependent on a particular genetic background.
The expression of both TASK-1 and TASK-3 subunits in ZG cells allows the potential for homodimeric and heterodimeric TASK channel conformations. To determine whether TASK-3 deletion is sufficient to recapitulate the IHA phenotype of double KO mice, we studied congenic T3KO mice in parallel. Urinary aldosterone excretion in T3KO mice was elevated modestly but significantly (37%; \( P < 0.017 \)) above that of WT mice (Figure 1A, top right) and was accompanied by reduced levels (\( P < 0.001 \)) of plasma renin (Figure 1A, bottom right). These opposing changes in aldosterone output and renin status in T3KO mice produced an elevated ARR that was 2.6-fold greater than that of WT mice (Figure 2A). T3KO mice also displayed an elevated modestly but significantly (37%; \( P < 0.017 \)) above that of WT mice (Figure 1A, top right) and was accompanied by reduced levels (\( P < 0.001 \)) of plasma renin (Figure 1A, bottom right). These opposing changes in aldosterone output and renin status in T3KO mice produced an elevated ARR that was 2.6-fold greater than that of WT mice but was, nonetheless, an order of magnitude less than that of T1T3KO mice (Figure 1B, right). These comparative differences in the level of aldosterone output and ARR are reminiscent of observations in human hypertensive patients with LREH and IHA. To determine whether these mice were hypertensive, we placed pressure transmitters in the aortic arch to determine BP in conscious freely behaving mice by telemetry. We found that both T1T3KO and T3KO mice have significantly higher 24-hour ambulatory systolic (SBP; \( P < 0.001 \)) and diastolic (DBP; \( P < 0.01 \)) BPs than WT mice (Figure 1C). Thus, we conclude that both T1T3KO and T3KO mice are hypertensive, the former because of IHA and the latter because of low-renin primary hypertension.

**T3KO Mice Display Hyperaldosteronism That Is Resistant to Salt Suppression**

Ang II and extracellular K\(^+\) are the 2 major regulators of aldosterone production in vivo. Consistent with the stimulation of the RAS, limiting dietary Na\(^+\) (LS) increased urinary aldosterone excretion above that produced on an NS diet in both WT and T3KO mice (\( P = 0.011 \)). Nevertheless, urinary aldosterone excretion in T3KO mice remained 1.6-fold that of WT (Figure 2A). T3KO mice also displayed an augmented response to dietary K\(^+\) feeding (high K\(^+\)) that was 1.7-fold that of WT. However, the dysregulation of aldosterone production in T3KO mice was most striking in mice fed an HS diet, when the activity of RAS is decreased. Unlike WT mice, T3KO mice failed to suppress aldosterone output with HS but maintained excretion at NS feeding levels. This resistance to HS inhibition was shared by T1T3KO mice (data not shown). Thus, HS challenge revealed a component of aldosterone output in T3KO mice that is independent of the RAS.

T3KO mice maintained a low renin status on all of the Na\(^+\) diets compared with WT mice (\( P < 0.002 \); Figure 2B). However, high K\(^+\) feeding reduced renin concentration levels of WT mice to that of T3KO mice, implying a direct role for TASK-3 in the regulation of renin secretion. Nevertheless, treatment with candesartan, an insurmountable Ang II type 1 receptor blocker that removes Ang II type 1 receptor–activated feedback inhibition of renin secretion, restored plasma levels of renin in T3KO mice to values that were indistinguishable from those of WT mice (\( P = 0.63 \); Figure 2B inset). Thus, we conclude that the low renin status of T3KO mice on all Na\(^+\) diets is not likely the result of reduced renin stores or impaired vesicular secretion but rather an enhanced sensitivity of JG cells to Ang II inhibition, a consequence of TASK-3 subunit deletion.

**Conventional Determinants of Autonomous Aldosterone Production Are Not Changed in T3KO Mice**

The deletion of both TASK-1 and TASK-3 results in a \( \approx 20\)-mV membrane depolarization of ZG cells and provides a cellular explanation for the overt autonomous overproduction of aldosterone in the T1T3KO mouse strain. By using current clamp recordings, we found that baseline membrane potential in ZG cells from T3KO mice was not different from WT mice (\( P = 0.868 \)), remaining at the hyperpolarized level.
characteristic of these cells (Figure 3A). Moreover, T3KO mice displayed a mild but significant hypokalemia ($P<0.001$; Figure 3B) that would be predicted to further hyperpolarize the ZG cell in vivo. Neither the mRNA expression of Figure 3B) that would be predicted to further hyperpolarize the ZG cell in vivo. Neither the mRNA expression of inwardly rectifying K^+ channel (Kcnj5) TASK-1 subunits ($P<0.001$). Thus, T3KO mice displayed a hypersensitivity to Ang II. To isolate the Ang II–sensitive component of aldosterone output and to corroborate and extend these findings across the diets, we measured urinary aldosterone excretion before and after candesartan was delivered in the drinking water (10 mg/kg per day). As expected during dietary Na^+ restriction, stimulation of the RAS markedly increased the Ang II–evoked component of aldosterone output in mice of both genotypes ($P<0.001$; Figure 4B, left), but, in T3KO mice, this component was nearly twice that seen in WT mice ($P<0.001$). In fact, there was a significant effect of genotype across diets; aldosterone production in T3KO mice was approximately double that of WT mice ($P<0.001$). Thus, we conclude that, as in humans with LREH,21,23 T3KO mice are uncommonly sensitive to the aldosterone-stimulating action of exogenous or endogenous Ang II.

Lack of aldosterone suppression after salt loading is used as a screening test for PA and is a hallmark of autonomous aldosterone production that is independent of the RAS.3 A relative resistance to salt suppression has also been noted in some patients with LREH20,21 underscoring a possible continuum between the 2 disease states.7,8 We used aldosterone output in the presence of candesartan, as a measure of an Ang II–independent component of aldosterone production. We found that candesartan normalized urinary aldosterone excretion between T3KO and WT mice on LS and NS diets, whereas there was an Ang II–independent component of aldosterone output on HS ($P<0.001$; Figure 4B, right). These results differ from those seen with T1T3KO mice in which, relative to WT mice, an enhanced Ang II–independent component of aldosterone output was evident on all of the salt diets.15 Thus, we observe that T1T3KO and T3KO mice differ in the degree to which the RAS axis is imbalanced and aldosterone output is autonomous.

Ang II–Dependent and Salt-Sensitive Hypertension in T3KO Mice

Relative aldosterone excess, as indicated by an elevated ARR, is the strongest predictor of DBP and the second-most
important predictor of SBP. We used candesartan to normalized both aldosterone output and plasma renin concentration between genotypes on an NS diet to determine whether the hypertension of T3KO mice could be corrected. In T3KO mice maintained on an NS diet, 24-hour ambulatory DBP and SBP were elevated, =13 mm Hg (P = 0.01) and 17 mm Hg (P < 0.001), respectively. Candesartan decreased DBP and SBP in mice of both genotypes but normalized only DBP (Figure 5A). By contrast, SBP of T3KO mice, although corrected to normotensive values, remained elevated 17 mm Hg above that of WT mice (P < 0.001) indicating that, in T3KO mice, factors other than the RAS control SBP.

Salt-sensitive hypertension is a frequent observation in LREH and a hallmark of volume-dependent hypertension. We found that normotensive WT mice were able to adjust to a salt load maintaining normal DBP and SBP during HS challenge (Figure 5B). In T3KO mice, DBP also remained stable during Na⁺ loading, whereas SBP rose by 9 mm Hg (P < 0.001). This suggests that autonomy of aldosterone production imparts salt sensitivity that weakens the control of SBP while DBP remains under RAS control.

Discussion

Our studies show that the genetic deletion of TASK-3 subunits produces a phenotype that duplicates key features of human LREH. First, as in LREH, levels of aldosterone output in T3KO mice are mildly elevated but inappropriate for the low levels of plasma renin, resulting in an ARR that is greater than twice that of normotensive WT mice. Second, on LS and NS, aldosterone production remains under the control of RAS with a demonstrated hypersensitivity to both endoge-

ous and exogenous Ang II. An enhanced responsiveness to the steroiodogenic actions of Ang II is a well-described characteristic of LREH. Third, on HS, RAS control is weakened, revealing a component of aldosterone output that is relatively autonomous, consistent with the relative resistance to salt suppression observed in some patients with LREH. Finally, SBP is salt sensitive in T3KO mice, mimicking yet another established feature of LREH.

Our mouse model of LREH displays enhanced responsiveness to the actions of Ang II. Candesartan normalized aldosterone production (LS and NS), restored suppressed levels of plasma renin (LS, NS, and HS), and corrected the elevation in DBP (NS and HS) between genotypes. This subtle but uniform abnormality in the RAS was unexpected. The adrenal hypersensitivity to Ang II is likely not the result of an increase in Ang II type 1 receptor expression, because message levels for Ang II type 1A receptor and Ang II type 1B receptor in ZG microdissected from T3KO mice and WT adrenals were equivalent (Figure S2), conclusions that are supported by observations in the Lyon hypertensive rat where neither changes in the affinity nor regulation of Ang II receptor subtypes in the ZG accounted for enhanced adrenal sensitivity to Ang II. The adrenal hypersensitivity to Ang II is also not likely the result of an increase in sympathetic nervous system activity (eg, driving adrenal hyperplasia and/or potentiating aldosterone release), because heart rates in T3KO mice were reduced significantly from those of WT mice (Figure S3). In addition, we found no evidence of ZG hyperplasia. Our data also show that neither an increase in the synthetic capacity of the ZG cell to produce aldosterone, as

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**Figure 3.** Membrane potential, K⁺ channels and Cyp11β2 expression in the adrenal zona glomerulosa cells (ZGs). T3KO hyperaldosteronism is not explained by differences in ZG basal Vm, plasma K⁺, or Twik-related acid-sensitive K⁺ channels (TASK) 1 expression. **A**. Baseline membrane potential (Vm) (mV) of ZG cells in adrenal sections from wild-type (WT; n = 16) and T3KO mice (n = 17) determined in current clamp. **B**. Plasma K⁺ (mmol/L) in WT and T3KO mice on salt diets (high K⁺ [HK], n = 11, normal Na⁺ [NS], low Na⁺ [LS], high Na⁺ [HS], n = 21–26). *Indicates main effect of genotype (P < 0.001). **C** and **D**, Expression of mRNA (C, TASK-1 and TASK-3) and **D** Kcnj5 in ZG layer isolated by laser microdissection, from WT (n = 6) and T3KO (n = 6) adrenal slices, measured by RT-PCR and expressed as fold-initial mRNA (2−ΔΔCt) relative to actin mRNA. **E**, mRNA expression of Cyp11β2 in ZG layer, NS (n = 6, *P < 0.001) or HS (n = 4, *P < 0.038). **F**, Western blot analysis of lysates (20 μg of total protein) prepared from mouse adrenals (2 adrenals per lane): NS (n = 3), HS (n = 5), LS (n = 4), detected with aldosterone synthase (Cyp11β2) antibody. Values represent mean ± SEM, *vs WT mice (P < 0.05).
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measured by protein levels of Cyp11β2, nor a significant reduction in baseline membrane potential can explain enhanced responsiveness to Ang II. In this respect, our data suggest that either TASK-3 channels are not important in setting the baseline membrane potential of ZG cells (in disagreement with previous observations) or that compensatory expression of other unidentified conductance(s) maintains membrane potential in TASK-3–deficient ZG cells.

At present, the precise cellular mechanism that underlies enhanced responsiveness to Ang II remains unanswered. Nevertheless, our mouse models of LREH and IHA suggest an evolution of aldosterone dysregulation, from normal to exaggerated to autonomous, that depends on the absence of TASK-3 (LREH) or both TASK-1 and TASK-3 (IHA). In this respect, it is noteworthy that extracellular K+ acts both alone and in concert with Ang II to regulate aldosterone production. K+ elevation depolarizes ZG cells, permitting the opening of voltage-dependent Ca2+ channels and the consequent increase in extracellular Ca2+ entry, a step that is critical for sustaining steroidogenesis. Thus, one could posit that, in LREH, a small change in K+ conductance that does not appreciably affect baseline membrane potential may, nevertheless, render the ZG cell more susceptible to depolarizing influences (eg, by Ang II) and, thus, exaggerate responses to submaximal concentrations of aldosterone secretagogues. On the other hand, a larger change in K+ conductance, as demonstrated in our mouse model of IHA, depolarizes the ZG cell and, thus, imparts autonomy to the production of aldosterone. We propose, therefore, that these mouse models of LREH and IHA stand as proof of principle that progressive loss of K+ channel activity can be a mechanism to advance the syndrome of low-renin hypertension.

Perspectives

IHA and LREH present with a high frequency in hypertension, promoting the development of cardiovascular and renal disease. Here, we demonstrate that disruption of the TASK-3 or TASK-3/TASK-1 genes results in phenotypic characteristics of LREH and IHA in C57BL/6 mice, low-renin hypertension with high ARR, hypersensitivity to Ang II, and autonomous aldosterone production. These mouse models provide the opportunity to identify the cellular basis for these phenotypic characteristics and suggest that variants in human TASK channel genes may contribute to the development of LREH and IHA. The development of pharmacological agents...
that increase TASK channel activity may be of therapeutic benefit in the treatment of these hypertensive disorders.

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**Disclosures**

None.

**References**


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In the *Hypertension* article by Guagliardo et al (Guagliardo NA, Yao J, Hu C, Schertz EM, Tyson DA, Carey RM, Bayliss DA, Barrett PQ. TASK-3 Channel Deletion in Mice Recapitulates Low-Renin Essential Hypertension. *Hypertension*. 2012;59:999–1005), corrections have been made.

In Figure 4, two corrections have been made. In the top panel (A), the x-axis label has been corrected to read “ng/kg/min” instead of “mg/kg/min.” In the bottom panel (B), for the graph on the right, the title has been corrected to read “Ang II-independent” instead of “Ang II-dependent.”

The authors regret these errors.

These corrections have been made to the current online version of the article, which is available at http://hyper.ahajournals.org/content/59/5/999.full.
ONLINE SUPPLEMENT

TASK-3 CHANNEL DELETION IN MICE RECAPITUATES LOW RENIN ESSENTIAL HYPERTENSION

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Expanded Methods

Mice
Mice were housed in a temperature and humidity controlled vivarium on a 12:12 light:dark cycle with free access to diet and drinking water. All diets used throughout these experiments were developed by and purchased from Harlan Teklad Laboratories (Madison, WI).

To generate T3KO mice, separate mouse lines with “floxed” TASK-1 and TASK-3 alleles were backcrossed to a C57Bl/6J background using “speed congenics” and bred with Cre-deleter mice (also on a C57Bl/6J) to generate TASK-1 KO and TASK-3 KO mice on a congenic background. These 2 mice strains were then crossed to generate TASK-1/3 double KO mice.

Metabolic Cage Experiments
Mice used in metabolic cages studies were male and between 60 and 100 days old at the start of the experiment. Each mouse participated in only one experimental protocol. A schematic of the experimental protocols used and number of WT and T3KO mice per experimental protocol is presented in Figure S1. T1T3KO mice (n=12) were studied using protocol 1.

Salt diets
Mice were habituated to metabolic cages prior to experiments, then placed on one of 4 experimental protocols (Figure S1). For the initial studies of aldosterone production (Protocol 1), mice received normal sodium (NS, 0.3% Na\(^+\)), low sodium (LS, 0.05% Na\(^+\)) and high sodium (HS, 4.0% Na\(^+\)) diets for one week, with a week of recovery (NS) between dietary testing. In addition, after LS, mice were given LS + candesartan (Astra Zeneca, 10 mg/kg/day, in drinking water) for 4 days. Fluid consumption was monitored previous to candesartan delivery to ensure accurate dosing. To further compare the effect of candesartan on aldosterone production between genotypes, mice were tested on one of 2 additional protocols; Protocol 2: one week of NS followed by NS + candesartan, or Protocol 3: HS followed by HS + candesartan. Mice studied under Protocol 4 received high potassium, normal sodium diet (HK, 4%K\(^+\), 0.3%Na\(^+\)) for 7 days. The mean aldosterone/creatinine of the last 4 days on each diet and the last 3 days of diet + candesartan treatment was calculated for each mouse and used to generate group means.

Angiotensin II Delivery (Protocol 5)
To obtain the correct dose, mice were weighed on the day before surgery to calculate the concentration of Ang II per osmotic minipump for each mouse. Pumps were filled and placed in 37 °C isotonic saline overnight to promote prompt delivery after implantation. On the day of surgery, mice were anesthetized with ketamine/dexmedetomidine (50-70 mg/kg/0.25-0.5 mg/kg IP, reversal agent antipamezole, 1mg/kg IP) and osmotic minipumps were implanted
subcutaneously. Mice were allowed one day of recovery in a home cage before returning to metabolic cages.

**Urine Analysis**
24-hr. urine samples from mice housed in metabolic cages were analyzed for aldosterone and creatinine. Urinary aldosterone concentration was determined using an aldosterone I\textsuperscript{125} radioimmunoassay (RIA, Diagnostic Products Corporation, Los Angeles, CA). Samples were standardized to urinary creatinine concentration as measured by Jaffe' colorimetric detection with a Creatinine Assay Kit (Cayman Chemical Company, Ann Arbor, MI).

**Blood Analysis**
For blood sample collection, mice were transported to the laboratory from the vivarium in the morning and allowed to habituate to the laboratory setting for ~5 hours before blood was sampled. Tail vein sampling was performed using a 50 um, non-heparin capillary tube containing 1uL of 0.125M EDTA; blood was separated by centrifugation and stored at -20 °C until analysis for plasma renin. Plasma renin concentration was measured using an RIA (Diasorin, Stillwater, MN). In addition, after each diet, mice were anesthetized with ketamine/dexmedetomidine (50-70 mg/kg/0.25-0.5 mg/kg IP, reversal agent antipamezole, 1mg/kg IP) and blood was sampled from the retro-orbital sinus using heparinized capillary tubes for analysis with an iStat hand held analyzer (EC8+ cartridge, Heska, Fort Collins, CO).

**Blood Pressure**
Blood pressure was monitored in conscious, freely moving mice using a radio-telemetry device consisting of a pressure-sensing catheter and telemetric transmitter (implant TA11PA-C10, Data Science International, St. Paul, MN). Mice were anesthetized with a combination of ketamine (50-70 mg/kg) and dexmedetomidine (0.25-0.5 mg/kg IP) and kept on a water-circulating heating pad. The left carotid artery was exposed with blunt dissection and occluded just caudal to the carotid bifurcation. The pressure-sensing catheter was inserted into the carotid and advanced until the tip was just inside the aortic arch. The catheter was ligated in place with silk suture and the arterial entry site was closed with tissue glue. Through the same incision site a subcutaneous pocket was formed on the right flank of the mouse and the transmitter/battery was placed within the pocket. The skin was closed with polypropylene sutures and the mouse was revived with the reversal agent antipamezole (1 mg/kg, i.p ). After surgery, mice were given the analgesic ketoprofen for two days (sc, 4mg/kg bw per day). Mice were individually housed in home cages positioned on the radio-telemetric receivers and given 7 days to recover before data collection. Twenty second pressure wave forms were collected and stored every 10 minutes using Dataquest A.R.T software (Data Sciences International, St. Paul, MN) and mean arterial pressure derived. Baseline data was collected for 4 days after recovery, followed by 4 days of candesartan (10mg/kg/day, in drinking water) or the last 4 days of HS diet. Mice whose transmitters failed were excluded from analysis.
**Electrophysiology**

Adrenal slices were prepared from adrenal glands freshly harvested from anesthetized mice (ketamine, 15mg, i.p.) and kept in ice-cold low-Ca\(^2\) bicarbonate buffered saline (BBS; mM: 10 glucose, 140 NaCl, 2 KCl, 5 MgCl\(_2\), 0.1 CaCl\(_2\), 26 NaHCO\(_3\) bubbled with 95% O\(_2\)/5% CO\(_2\)). The surrounding fat tissue from each adrenal was carefully removed under a dissecting microscope, embedded in low melting temperature agar (2.5% in BBS), and sectioned (80µm) using a DSK supermicroslicer (Ted Pella, Inc). The slices were incubated at 35 °C in BBS for 30 minutes, then kept at room temperature for the remainder of the experiment.

Adrenal slices were submerged in a recording chamber, secured with a slice anchor, and visualized using an Examinier A1 microscope (Zeiss) with 40x objective. Cells located near the surface of the slice just beneath the capsule were targeted for recording, based on anatomic location and characteristic shape. Electrophysiology recordings were obtained at room temperature using patch electrodes (2-5 MΩ) and an Axopatch 200B amplifier (Molecular Devices, Inc). Data acquisition was performed using pCLAMP 10.3 (Molecular Devices). Slices were perfused with an external solution that contained (mM): 140 NaCl, 3 KCl, 10 HEPES, 2 MgCl\(_2\), 2 CaCl\(_2\), 10 glucose, pH 7.3. The pipette (intracellular) solution contained (mM): 135 KMeSO\(_3\), 4 NaCl, 10 HEPES, 1 MgCl\(_2\), 0.5 EGTA, 3 Mg-ATP, 0.3 Tris-GTP, pH 7.2. Voltage traces were acquired at 2.5 kHz filtered at 1 kHz with Axopatch 200B integrated low pass Bessel filter. Baseline membrane voltages were recorded from ZG cells for 2-4 minutes after stabilization of the recording.

**ZG isolation and RT-PCR**

Adrenal glands were dissected from terminally anesthetized mice (ketamine, 15mg, i.p.), flash-frozen, sectioned on a cryostat at 8 µm, and mounted onto specialized microscope slides containing a polyethylene napthalate window. A laser capture system (AS/LMD, Leica Microsystems, Inc.) was used to visualize and carefully sample ZG tissue for subsequent qRT-PCR analysis. RNA was isolated with the PicoPure RNA isolation kit (Arcturus) and cDNA was generated with iScript reverse transcription kit (BioRad) and qRT-PCR performed in quadruplicate using an iCycler, with iQ SYBR Green SuperMix reagents (BioRad). In preliminary experiments, a dilution series of cDNA was used with each primer set to establish conditions (primer concentration, annealing temperature) to yield >90% efficiency; in addition, the PCR product was run on an agarose gel and sequenced to confirm its identity. Melt curve analysis and no-template controls were included with each run. qRT-PCR data was analyzed using a delta-Ct normalization procedure against expression of Actin and values compared between T3KO and WT mice.
### Primers used:

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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</thead>
<tbody>
<tr>
<td><strong>TASK-1 f</strong></td>
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<tr>
<td><strong>TASK-1 r</strong></td>
<td>GAACACCTTGCCCTCGTGCGTGC</td>
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<tr>
<td><strong>Cyp11β2 f</strong></td>
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<td><strong>Cyp11β2 r</strong></td>
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<td><strong>AT1b r</strong></td>
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### Cyp11β2 Protein Expression

Both adrenals from each mouse (~100 days old) were dissected, trimmed of fat, immediately frozen and stored at -80 until time of analysis. Adrenals were disrupted in 100µl RIPA buffer (PBS, 0.1% SDS, 1% NP40, deoxycholate 0.5%, protease inhibitor cocktail from sigma 1:100), homogenized with an insulin syringe (25 passes), and centrifuged (10,000g) for 10min at 4C. The supernatant was used as lysate. After protein analysis, 20µg total protein per sample was separated on 10% SDS-polyacrylamide, and transfered to PVDF membrane. Membranes were preblocked with 5% milk/TBST prior to antibody exposure. The Cyp11β2 antibody, a gift from Dr. Gomes-Sanchez, was used at 1:1000 in TBST with 5% milk, 0.1% BSA and 0.1 NaN3.
Figure S1. Schematic diagram of metabolic cage protocol and dietary manipulations.
Figure S2. AT$_{1A}$R and AT$_{1B}$R mRNA expression in ZG of mice. ZG was isolated using laser capture microdissection. AT$_{1A}$R and AT$_{1B}$R mRNA expression was measured using qRT-PCR and quantified relative to Actin mRNA. Both AT$_{1A}$R (A) and AT$_{1B}$R (B) mRNA levels were similar between WT mice and T3KO on both NS (n=6 per group) and HS (n=4 per group) diets.
Figure S3. Heart rate (HR, beats per minute) during day (sleeping) and night (active) phases on NS. During sleep HR between genotypes was equivalent but differed during active phase. *P=0.004; n=11 per genotype. Values represent mean ± S.E.M of 4 day accumulated measurements.