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TASK channels are not required to mount an aldosterone secretory response to metabolic acidosis in mice

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ABSTRACT

The stimulation of aldosterone production by acidosis enhances proton excretion and serves to limit disturbances in systemic acid–base equilibrium. Yet, the mechanisms by which protons stimulate aldosterone production from cells of the adrenal cortex remain largely unknown. TWIK-related acid sensitive K channels (TASK) are inhibited by extracellular protons within the physiological range and have emerged as important regulators of aldosterone production in the adrenal cortex. Here we show that congenic C57BL/6J mice with genetic deletion of TASK-1 ($K_{2P}3.1$) and TASK-3 ($K_{2P}9.1$) channel subunits overproduce aldosterone and display an enhanced sensitivity to steroidogenic stimuli, including a more pronounced steroidogenic response to chronic NH_4Cl loading. Thus, we conclude that TASK channels are not required for the stimulation of aldosterone production by protons but their inhibition by physiological acidosis may contribute to full expression of the steroidogenic response.

1. Introduction

In man, changes in aldosterone production are associated with acid–base disturbances (Jones et al., 1992; Perez et al., 1977; Schambelan et al., 1987; Sicuro et al., 1998; Yamauchi et al., 1998). For example, patients with primary hyperaldosteronism, especially those with Conn's syndrome, frequently present with hypokalemia and a mild metabolic alkalosis, whereas aldosterone deficiency disorders, whether primary (Addisons disease, selective aldosterone synthase deficiency) or secondary (hyporeninemia, mineralocorticoid blockage), are associated with hyperkalemia and metabolic acidosis (DuBose, 2000). Conversely, the regulation of aldosterone production by H ions can be considered part of a physiological feedback loop to maintain acid–base balance. Supraphysiological elevations in plasma H ion concentration imposed by acute or chronic ammonium chloride dietary loading (Perez et al., 1977; Schambelan et al., 1987), or less extreme acid–base changes produced by hemodialysis with dialysates of varied bicarbonate concentration (Jones et al., 1992), evoke increases in plasma aldosterone that are independent of changes in systemic factors known to regulate aldosterone production (e.g., Ang II, K and ACTH). Yet, although aldosterone production is regulated by blood acid–base status *in vivo* and *in vitro*, the molecular targets that mediate this action have remained largely unexplored.

Twik-related acid-sensitive potassium (TASK) channels have emerged as prominent candidate molecular substrates underlying regulation of aldosterone production. Aldosterone producing zona glomerulosa (zG) cells in rodents robustly express mRNA for two members of the KCNK gene family of 2-pore domain K channels, TASK-1 and TASK-3 (Bayliss et al., 2003; Czirjak et al., 2000; Czirjak and Enyedi, 2002a,b). These subunits can form homo- or hetero-meric channels (Czirjak and Enyedi, 2002a,b) and by generating leak or background K currents they play a major role in setting the resting membrane voltage of cells in which they are expressed. Importantly, these channels are inhibited by extracellular protons within the physiological range (Duprat et al., 1997).

Our laboratory has recently characterized TASK-1 and TASK-3 ($TASK^{-/-}$) double knockout mice (Davies et al., 2008) on a mixed genetic background. Deletion of TASK subunits removes greater than 90% of recorded depolarization-elicited current from zG cells and is associated with an approximate 20 mV depolarization of the resting membrane potential. Such membrane depolarization would be expected to result in excessive aldosterone production. Indeed, $TASK^{-/-}$ mice phenocopy key features of nontumorous idiopathic primary hyperaldosteronism: increased urinary aldosterone, low plasma renin, high aldosterone to renin ratios, a failure to suppress excess aldosterone production on a high salt diet or to normalize it

Abbreviations: Ang II, Angiotensin II; ACTH, Adrenocorticotropic hormone; TASK, Twik-related acid-sensitive potassium channels; zG, Zona glomerulosa; AT1R, Type 1 Angiontensin II receptor; WT, Wild-type; RIA, Radioimmunoassay.

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with Ang II Receptor (AT_1R) blockade, and hypertension (Davies et al., 2008).

The present study was undertaken to determine if modulation of TASK channels by H ions contributes to elevated aldosterone production in response to metabolic acidosis. The results demonstrate that NH_4Cl acid loading in WT mice increases urinary aldosterone excretion by a mechanism that depends principally on enhanced renin levels and Ang II activity. Notably, renin is not increased by acidosis in TASK$^{-/-}$ mice. Nevertheless, a smaller Ang II-independent component of the aldosterone secretory response to H ions is shared between genotypes and, surprisingly, is even augmented in TASK$^{-/-}$ mice. These data indicate that physiological inhibition of TASK channels by H ions is not required to evoke an increase in aldosterone production; nevertheless, TASK channel inhibition may permit development of enhanced steroidogenic responses to multiple stimuli.

2. Materials and methods

2.1. Animals

All experiments were approved by the University of Virginia's Animal Care and Use Committee in accordance with *The Guide for the Care and Use of Laboratory Animals*. C57BL/6J (WT) and TASK$^{-/-}$ mice were 2–4 months of age and housed on a 12:12 light:dark cycle in a temperature and humidity controlled environment. Male mice were used in order to remove potential confounds of hormonal surges associated with the estrus cycle and zonation defects observed in female TASK knockout mice (Heitzmann et al., 2008). TASK-1 and TASK-3 double KO mice were generated as previously described (Lazarenko et al., 2010). For this work, mouse lines with 'floxed' alleles for TASK-1 (TASK-1$^{f/f}$) and TASK-3 (TASK-3$^{f/f}$) were backcrossed onto a C57BL/6J background by using 'speed congenics' (University of Virginia Transgenic Core). The floxed exon of each TASK channel gene was excised by breeding with a Cre-deleter strain, also on a C57BL/6J background (EIIa-Cre; Jackson Labs Stock #003724), and the resulting knockout lines were intercrossed to generate the double TASK-1$^{-/-}$:TASK-3$^{-/-}$ line (TASK$^{-/-}$). All knockout lines were maintained as homozygotes, with the parental C57BL/6J mouse line used as a control strain.

2.2. Metabolic cage experiments

All mice were housed in metabolic cages for a habituation period of 3–4 days and were fed a standard rodent chow and deionized drinking water before initiating an experimental protocol. After habituation, urine samples were collected daily and total urine volume and fluid intake over 24 h were recorded. Prior to acid loading, mice had free access to sucrose water (2%) for 3 days. Acid loading was imposed over a 3-day period by delivering 0.28 M NH_4Cl in sucrose water. In order to assess the impact of acid loading in the absence of AT_1R activity, a second group of mice received candesartan (10 mg/kg/day) in sucrose water for 2 days, prior to and then during a 3-day period of acid loading.

2.3. Urine analysis

Urine samples were analyzed for aldosterone concentration using an aldosterone ^{125}I radioimmunoassay kit (RIA, Diagnostic Products Corporation, Los Angeles, CA). Urinary Na and K concentrations were measured by Flame Photometry (IL943 Automatic Flame Photometer, Instrumentation Laboratory Inc., Lexington, MA). To correct for differences in urine volume, aldosterone, Na, and K concentrations were standardized to urinary creatinine concentration, measured by Jaffes' colorimetric detection with a Creatinine Assay Kit (Cayman Chemical Company, Ann Arbor, MI).

2.4. Plasma renin concentration and blood chemistry

Blood samples were taken for each mouse before the habituation period and after the 3 consecutive days of acid loading. For renin analysis, mice were put into a restraint and blood was quickly drawn from a nicked tail vein using a capillary tube, separated by centrifugation, and stored at −20 °C until analysis. Plasma Renin Concentration was measured by RIA kit (Diasorin, Stillwater, MN). After tail vein sampling, mice were anesthetized with ketamine (70 mg/kg) and dexmedetomidine (0.5 mg/kg) (ip) and blood was drawn quickly from the retro-orbital sinus in a heparinized capillary tube. Samples were analyzed immediately for blood chemistries with an iStat hand held monitor configured with an EC8+ or CG8+ cartridge (Heska, Fort Collins, CO).

2.5. Statistics

Data were analyzed using repeated measures ANOVAs and Bonferroni post hoc analysis to determine significance between groups ($p < 0.05$).

3. Results

3.1. Hydration, acid–base status and urinary electrolytes

To assess whether TASK channels are required for the aldosterone secretory response to dietary acid loading, we studied age-matched WT and congenic TASK$^{-/-}$ male mice maintained on a diet of normal Na content (0.32%). In pilot studies, we noticed that addition of NH_4Cl to the drinking water significantly decreased fluid consumption and reduced body weight. Therefore, drinking water was supplemented with 2% sucrose to achieve adequate hydration prior to and during acid loading. During a 3-day hydration period, mice of both genotypes consumed more fluid (Fig. 1A, average of days 2–3) and produced more urine (Fig. 1B, average of days 2–3) than a cohort of mice of either genotype drinking regular deionized water (see dashed lines on Fig. 1A and B; consumption: 7.4 ± 0.5 ml/day; excretion: 2.0 ± 0.1 ml/day). In both genotypes, fluid consumption and urine volume decreased over 3 days of acid loading; however these values were not different from normal consumption and excretion values of mice drinking regular water. Thus in 3-day acidotic mice, body weight was stable, plasma blood urea nitrogen remained constant, and plasma hematocrit and hemoglobin were not increased, consistent with adequate hydration (Table 1). Nonetheless, in both genotypes, hypernatremia developed after 3 days of acid loading providing a conflicting indicator of volume status and the possibility of an early onset acid-induced volume depletion (Table 1).

Three days of acid loading produced a mild metabolic acidosis that was equivalent between genotypes (Fig. 1C): the pH decreased by approximately 0.12 units and was accompanied by a reduction in plasma HCO_3. Neither sinus PCO_2 nor PO_2 changed after 3 days of acidosis (Table 1) in WT or TASK$^{-/-}$ mice, suggesting little to no respiratory compensation. As also shown in Table 1, acidosis was accompanied by increased urinary excretion of K in both genotypes; this produced significant hypokalemia in WT mice while the existing hypokalemia already evident in TASK$^{-/-}$ mice was maintained. Also, in both genotypes, acidosis increased the urinary excretion of Na while producing a hypernatremia, consistent with acid-induced impairment of water reabsorption, likely in the collecting duct.

3.2. Aldosterone secretory response to acid loading

To evaluate the steroidogenic response to acidosis, we measured urinary aldosterone excretion (24 h, normalized to creatinine), as an indicator of integrated aldosterone secretory activity *in vivo*. This non-invasive measure minimizes variability arising from diurnal patterns and stress-induced changes in aldosterone production. Similar to the excessive aldosterone production previously observed for TASK$^{-/-}$ mice on a mixed genetic background (Davies et al., 2008), we found here that urinary aldosterone excretion in this congenic line of TASK$^{-/-}$ mice was elevated over that of C57BL/6 mice (by ~5-fold; Fig. 2A). In WT mice, 3 days of acid loading elicited a robust increase in urinary aldosterone excretion to levels ~2.5-fold of control; by comparison TASK$^{-/-}$ mice responded with a more modest increase (to ~1.25-fold of control), although the absolute magnitude of the increase in aldosterone produced between genotypes was similar.

To assess the participation of the renin–angiotensin system in the secretory response to NH_4Cl loading, we measured plasma renin concentration before and after inducing acidosis in WT and TASK$^{-/-}$ mice. In WT mice, acidosis was associated with a substantial increase in renin levels (~3-fold over control; Fig. 2B). In TASK$^{-/-}$ mice, as previously reported, renin concentration was lower than in WT mice (Fig. 2B, Davies et al., 2008); in response to acid loading renin levels were unaltered in TASK$^{-/-}$ mice despite systemic responses to acid that were equivalent to