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Genetic mutation of *Kcnj16* identifies Kir5.1-containing channels as key regulators of acute and chronic pH homeostasis

Madeleine M. Puissant,* Clarissa Muere,* Vladislav Levchenko,* Anna D. Manis,* Paul Martino,*,† Hubert V. Forster,* Oleg Palygin,*,‡ Alexander Staruschenko,* and Matthew R. Hodges*,‡,1

*Department of Physiology and ‡Neuroscience Research Center, Medical College of Wisconsin, Milwaukee, Wisconsin, USA; and †Department of Biology, Carthage College, Kenosha, Wisconsin, USA

ABSTRACT: Acute and chronic homeostatic pH regulation is critical for the maintenance of optimal cellular function. Renal mechanisms dominate global pH regulation over longer time frames, and rapid adjustments in ventilation compensate for acute pH and CO_2 changes. Ventilatory CO_2 and pH chemoreflexes are primarily determined by brain chemoreceptors with intrinsic pH sensitivity likely driven by K^+ channels. Here, we studied acute and chronic pH regulation in *Kcnj16* mutant Dahl salt-sensitive ($SS^{Kcnj16-/-}$) rats; *Kcnj16* encodes the pH-sensitive inwardly rectifying K^+ 5.1 (Kir5.1) channel. $SS^{Kcnj16-/-}$ rats hyperventilated at rest, likely compensating for a chronic metabolic acidosis. Despite their resting hyperventilation, $SS^{Kcnj16-/-}$ rats showed up to 45% reduction in the ventilatory response to graded hypercapnic acidosis *vs.* controls. $SS^{Kcnj16-/-}$ rats chronically treated with bicarbonate or the carbonic anhydrase inhibitor hydrochlorothiazide had partial restoration of arterial pH, but there was a further reduction in the ventilatory response to hypercapnic acidosis. $SS^{Kcnj16-/-}$ rats also had a nearly absent hypoxic ventilatory response, suggesting major contributions of Kir5.1 to O_2- and CO_2-dependent chemoreflexes. Although previous studies demonstrated beneficial effects of a high-K^+ diet (HKD) on cardiorenal phenotypes in $SS^{Kcnj16-/-}$ rats, HKD failed to restore the observed ventilatory phenotypes. We conclude that Kir5.1 is a key regulator of renal H^+ handling and essential for acute and chronic regulation of arterial pH as determinants of the ventilatory CO_2 chemoreflex.—Puissant, M. M., Muere, C., Levchenko, V., Manis, A. D., Martino, P., Forster, H. V., Palygin, O., Staruschenko, A., Hodges, M. R. Genetic mutation of *Kcnj16* identifies Kir5.1-containing channels as key regulators of acute and chronic pH homeostasis. FASEB J. 33, 000–000 (2019). www.fasebj.org

KEY WORDS: pH regulation · control of breathing · ion channels

Tissue or plasma H^+ is among the most tightly controlled ions in mammals. Proton concentrations determine protein charge and function and drive electrochemical gradients through which dysregulation of pH acutely or chronically has detrimental effects on fundamental cellular processes. Globally, pH is regulated through multiple mechanisms, including intrinsic buffering mechanisms in bodily fluids [*e.g.*, bicarbonate (HCO_3^-), proteins] and feedback mechanisms in the ventilatory control system and kidneys. Renal mechanisms that contribute to chronic pH homeostasis ultimately drive the reuptake of HCO_3^- or secretion of H^+ into the tubular lumen along the nephron. Acutely, acidosis or alkalosis can be compensated for by rapid changes in ventilation, which can partially or completely restore pH to within the normal physiologic range through increases (hypoventilation) or decreases (hyperventilation) in arterial CO_2 levels. Thus, pulmonary ventilation is tightly coupled to arterial CO_2 and pH (1), a process thought to be largely driven by the activity of specialized sensory cells throughout the brainstem (2). These specialized sensory cells, also known as central respiratory chemoreceptors, are thought to be intrinsically sensitive to local changes in pH, CO_2, or both (3), which consequently alter their firing rate and input into the neural circuitry controlling breathing. This sequence of events reestablishes pH and CO_2 homeostasis through the ventilatory CO_2 and pH chemoreflexes (4, 5).

We previously identified genes that encode pH-sensitive ion channels in serotonin neurons (6), a subpopulation of central respiratory chemoreceptors with an intrinsic pH sensitivity (7, 8). Among other genes we identified was *Kcnj16*, which encodes the inwardly rectifying K^+ (Kir) 5.1 channel. The Kir5.1 subunit is thought to primarily form heterotetrameric channels with other Kir family members,

ABBREVIATIONS: Bal., balance; HCO_3^-, bicarbonate; HCTZ, hydrochlorothiazide; HKD, high-K^+ diet; Kir, inwardly rectifying K^+; $Kir5.1^{-/-}$, Kir5.1 knockout; NaCl, sodium chloride; ND, normal diet; $PaCO_2$, partial pressure of arterial CO_2; SS, Dahl salt sensitive; $SS^{Kcnj16-/-}$, *Kcnj16* mutant Dahl salt sensitive; V_E, total ventilation; V_T, tidal volume

[1] Correspondence: Department of Physiology, BSB-504, Medical College of Wisconsin, 8701 Watertown Plank Rd., Milwaukee, WI 53226, USA. E-mail: mhodges@mcw.edu

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particularly with Kir4.1 and Kir2.1 channels (9, 10). Atypically, they may function independently as a homomeric Kir5.1 channel (11). Heterotetrameric Kir channels pass K^+ currents that contribute to resting membrane potential, and those containing Kir5.1 subunits are inhibited as pH drops within a physiologic range (3, 12, 13). Kir2.1, Kir4.1, and Kir5.1 channels are highly expressed in astrocytes, oligodendrocytes, and select neurons in the brain (6, 9, 14). These channels are also highly expressed in epithelial cells in the distal nephron (15, 16), where they modulate membrane potential in a pH-dependent manner and participate in K^+ recycling, Na^+ and Cl^- resorption, and acid secretion (17).

Human mutations in *KCNJ16* have been identified in patients with nonfamilial Brugada syndrome, which is characterized by cardiac arrhythmias and sudden death (18). Human *KCNJ10* (Kir4.1) mutations cause epilepsy, ataxia, sensorineural deafness, and tubulopathy syndrome (19, 20), characterized by renal salt wasting, metabolic alkalosis, and hypokalemia as predicted by the physiologic roles of homomeric Kir4.1 or heteromeric Kir4.1/Kir5.1 channels. We recently showed that *Kcnj16* mutant Dahl salt-sensitive ($SS^{Kcnj16-/-}$) rats with Dahl salt-sensitive (SS) hypertension had reduced body weight and lower arterial blood pressure, hypokalemia, and 100% fatality when fed a high-salt diet (15). However, $SS^{Kcnj16-/-}$ rats showed improved body weights and survival during high-salt dietary challenges when their diet was supplemented with increased K^+ in a high-K^+ diet (HKD) (15). Phenotypes of Kir5.1 knockout ($Kir5.1^{-/-}$) mice do not appear to induce sudden cardiac death (21, 22) like that in human Brugada syndrome and differ from many of the established phenotypes in the $SS^{Kcnj16-/-}$ rat (15). In addition, $Kir5.1^{-/-}$ mice have been shown to have metabolic acidosis (22) and blunted neuronal pH responses (23). However, whereas ventilatory responses to hypoxia and hypercapnia were reduced in $Kir5.1^{-/-}$ mice, phrenic nerve responses to hypercapnic acidosis in reduced preparations lacking peripheral chemoreceptor inputs were unaffected, leading to the conclusion that "…Kir5.1 channels are dispensable for functional central and peripheral respiratory chemosensitivity" (21). Thus, the specific role of Kir5.1 channels in the acute and chronic regulation of pH remains unclear.

Here, we tested the hypothesis that Kir5.1 mutation in rats ($SS^{Kcnj16-/-}$ rats) would lead to acute respiratory and chronic renal dysregulation of arterial pH. $SS^{Kcnj16-/-}$ rats showed a chronic hyperchloremic acidosis, which was partially mitigated by inherent hyperventilation while breathing room air. In addition, acute ventilatory responses to increases in partial pressure of arterial CO_2 ($PaCO_2$) (decreases in pH) were significantly reduced in $SS^{Kcnj16-/-}$ rats, despite the resting hyperventilation and chronic metabolic acidosis. We then queried whether the correction of the chronic acidosis would rectify the resting hyperventilation or reduced CO_2 sensitivity. Supplemental HCO_3^- in the drinking water or inhibition of carbonic anhydrase with hydrochlorothiazide (HCTZ) significantly increased arterial pH but led to further reductions in the ventilatory CO_2 chemoreflex in $SS^{Kcnj16-/-}$ rats. Countering the chronic hypokalemia in $SS^{Kcnj16-/-}$ rats with an HKD, however, failed to normalize resting breathing or hypercapnic ventilatory responses. We conclude that Kir5.1 subunit–containing channels play major roles in global physiologic pH regulation through chronic renal and acute neural respiratory control mechanisms *in vivo* and that mitigating the resulting metabolic acidosis in $SS^{Kcnj16-/-}$ rats unmasks an essential role for Kir5.1 channels in the hypercapnic ventilatory response.

MATERIALS AND METHODS

Ethical approval

All experimental approaches were approved by the Medical College of Wisconsin Institutional Animal Use and Care Committee prior to the initiation of studies.

Animals

Adult (>8 wk of age) male control SS and experimental $SS^{Kcnj16-/-}$ rats (15) were acquired from internal colonies maintained at the Medical College of Wisconsin Biomedical Resource Center, housed under standard 12-h light/dark cycles, and provided food and water *ad libitum*.

Surgical implantation of arterial cannulae (catheters)

The surgical procedures for chronic implantation of femoral arterial catheters have been previously described in Mouradian *et al.* (24). Under isoflurane anesthesia (2–3%), a small incision was made on the medial side of the thigh, and the femoral artery was isolated for insertion of a sterile custom-made catheter. The catheter was affixed with suture and tunneled subcutaneously for exteriorization through the skin above the scapula, where it was anchored with additional suture. The catheters were trimmed to length and capped, all surgical sites were closed with suture, and triple antibiotic ointment was applied. Rats received intraoperative injections of carprofen (5 mg/kg, i.p.) and additional analgesia *bis in die* for 2 d postsurgery. Rats also received antibiotics (1 mg/100 ml Baytril; Bayer, Berlin, Germany) in their drinking water throughout the study to prevent infection. Studies commenced after ≥7 d postsurgery.

Ventilatory and blood gas measurements

All ventilatory measurements were in awake animals and made using a custom-made 10 L flow through a plethysmographic chamber as previously described in refs. 24–26. Inflow gases were supplied by compressed gas cylinders filled with premixed medical-grade gases for room air [21% O_2, balance (Bal.) N_2], hypoxia (12% O_2, Bal. N_2), or graded hypercapnia (3, 5, or 7% CO_2 in 21% O_2, Bal. N_2), continuously monitored by a calibrated O_2 capnograph (07-0193; Oxigraf, Sunnyvale, CA, USA). Analog signals for ventilation and chamber O_2, CO_2, temperature, and relative humidity were continuously recorded with an 8-channel analog-to-digital converter and data acquisition software (ADInstruments, Sydney, NSW, Australia), and the data were analyzed offline. Arterial blood samples (0.4 ml) were obtained during the final 5 min of room air conditions and each gas exposure. Each gas challenge was performed on separate days, and the order of gas exposure was randomized in each animal. Rectal temperatures were measured following each study with a thermocouple probe and reader, and arterial blood samples were analyzed with a calibrated blood gas analyzer (Rapidlab 248; Bayer).

Terminal procedures

Rats were anesthetized with inhaled isoflurane (2–3%), and arterial blood was drawn (0.4 ml) for radiometric analyses (ABL800

Flex; Radiometer Medical, Copenhagen, Denmark) of blood electrolytes and blood gases prior to euthanasia by the combination of isoflurane overdose and thoracotomy.

Treatments

Rats were fed standard low-sodium chow [0.4% sodium chloride (NaCl), 0.36% K; D113755; Dyets, Bethlehem, PA, USA] or HKD (0.4% NaCl, 1.41% K by supplementing potassium chloride with base diet) (15). We refer to these diets as normal diet (ND) or HKD throughout this paper. HCTZ (H4759; MilliporeSigma, Burlington, MA, USA) was added to drinking water (75 mg/L) from wean (~3 wk of age) until 8 wk of age at the time of study. Alternatively, HCO_3^- (0.1 or 0.2 M) water was continuously provided for at least 7 d after completion of pretreatment control studies.

Data analysis and statistics

All ventilatory signals were processed offline using LabChart software (ADInstruments), in which a minimum of 15 s of continuous, uninterrupted breathing was used from the final 5 min of room air (10–20 min total) or gas challenges (10 min total) as previously described in Mouradian *et al.* (24). Tidal volume (V_T) was calculated and multiplied by breathing frequency (f; breaths/minute) to obtain total ventilation (V_E). Data are expressed as means ± SEM (along with individual datapoints). A 1-way ANOVA and Tukey's *post hoc* analysis (Prism; GraphPad Software, La Jolla, CA, USA) was applied to test all specific hypotheses among control SS and $SS^{Kcnj16-/-}$ rats (with ND and HKD or HCO_3^- and HCTZ treatments). An unpaired Student's t test was used to test the hypothesis that HCTZ affected resting breathing in groups of treated and untreated $SS^{Kcnj16-/-}$ rats. For all hypothesis testing, a significance threshold of $P < 0.05$ was used.

RESULTS

$SS^{Kcnj16-/-}$ rats hyperventilate at rest in compensation for a chronic hyperchloremic metabolic acidosis

A previous study by Paulais *et al.* showed that Kir5.1$^{-/-}$ mice had renal electrolyte mishandling, including modest hypokalemia and hyperchloremic metabolic acidosis (22). However, it was not determined in these previous studies whether there was any ventilatory compensation for the chronic acidosis in Kir5.1$^{-/-}$ mice. In order to measure whether ventilatory compensation occurred, simultaneous measurements of breathing and arterial blood gases (through indwelling catheters) in unanesthetized SS (control) and $SS^{Kcnj16-/-}$ rats were performed. Kir5.1 mutant rats had an increased V_E at baseline relative to SS control rats (**Fig. 1*A***), driven by increased f and not V_T. This hyperpnea represented hyperventilation because $PaCO_2$ was decreased in $SS^{Kcnj16-/-}$ rats (29.0 ± 0.7 mmHg) compared with SS controls (37.0 ± 0.7 mmHg; Fig. 1*B*). The hyperventilation at rest in $SS^{Kcnj16-/-}$ rats was likely due to a chronic metabolic acidosis, evident from decreased arterial pH and HCO_3^- (Fig. 1*B*). These chronic shifts in arterial pH, $PaCO_2$, and ventilation in $SS^{Kcnj16-/-}$ rats compared with SS control rats demonstrate

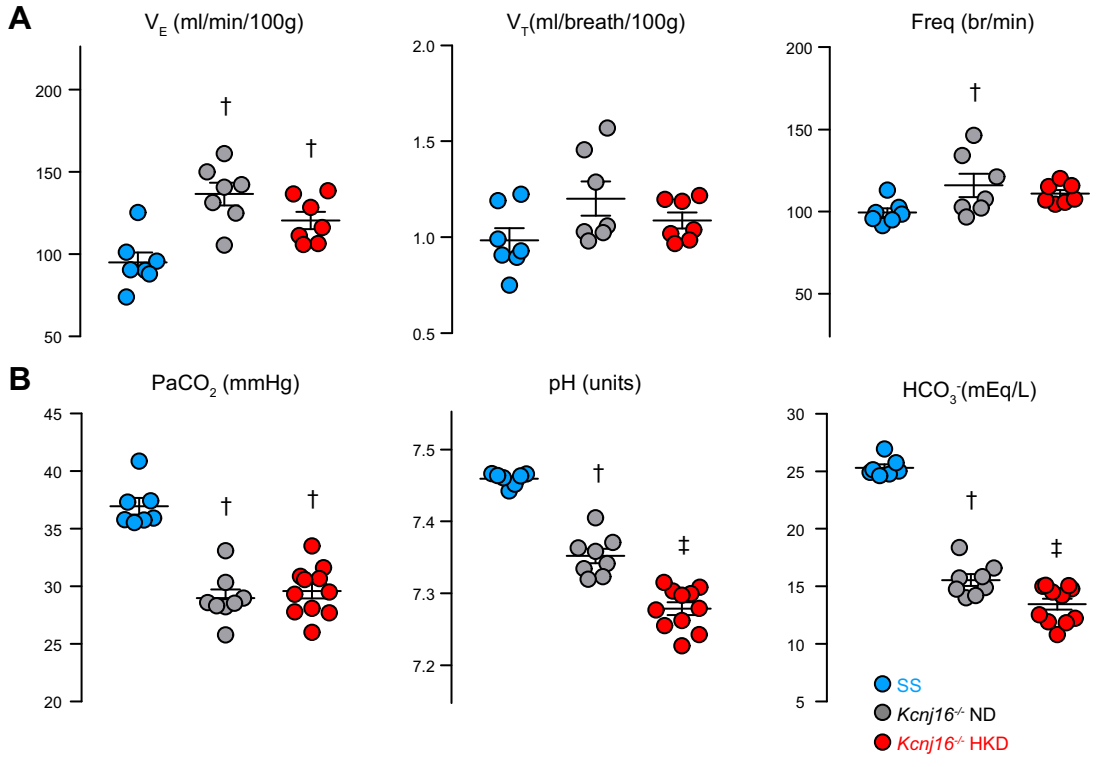

Figure 1. $SS^{Kcnj16-/-}$ rats hyperventilate because of chronic metabolic acidosis at rest. *A*) Individual and mean ± SEM data for V_E (ml/min/100 g), V_T (ml/breath/100 g), and f (breaths/min) from SS control rats (blue; $n = 7$), $SS^{Kcnj16-/-}$ rats on an ND (gray; $n = 7$), and $SS^{Kcnj16-/-}$ rats fed the HKD (red; $n = 7$) while breathing room air. *B*) Individual and mean ± SEM data for $PaCO_2$ (mmHg), arterial pH, and HCO_3^- (mEq/L) from SS controls ($n = 7$) and $SS^{Kcnj16-/-}$ rats on an ND ($n = 8$) and HKD ($n = 11$) while breathing room air. Br, breath; Freq, frequency. $^{\dagger}P < 0.05$ from SS controls, $^{\ddagger}P < 0.05$ from SS controls and $SS^{Kcnj16-/-}$ rats fed an ND.

that the mutation of Kir5.1 channels resulted in a partially compensated metabolic acidosis.

Kir5.1 mutation resulted in a reduced ventilatory pH and CO_2 sensitivity

Chronic metabolic acidosis led to the chronic resting hyperventilation in $SS^{Kcnj16-/-}$ rats, but experimental hypercapnic acidosis elicited by increasing inspired CO_2 levels (7% CO_2) failed to stimulate ventilation equal to control SS rats. When expressed relative to resting breathing (% control), hypercapnia-induced V_E levels increased to 297% in SS control rats. This robust response to hypercapnia was as great or greater than those reported for Sprague-Dawley rats and was similar to previous studies (24, 26). However, ventilation only increased to 231% in $SS^{Kcnj16-/-}$ rats (**Fig. 2*A***), suggesting a ~25% reduction in the hypercapnic ventilatory response to 7% CO_2. Ventilatory responses to decreases in inspired O_2 (12% O_2, Bal. N_2) in $SS^{Kcnj16-/-}$ rats were also significantly reduced compared with SS control rats (Fig. 2*B*), whereas V_E during hypoxia in $SS^{Kcnj16-/-}$ rats was not changed from room air breathing conditions. The lack of increase in V_E during hypoxia was due to decreased frequency and V_T responses in $SS^{Kcnj16-/-}$ rats compared with SS control rats.

A significant advantage in studying rats as compared with mice is the ability to obtain simultaneous arterial blood gases and ventilatory measurements during wakefulness. To further examine the role of Kir5.1 in the acute regulation of pH through the control of breathing, arterial blood samples were drawn from control and $SS^{Kcnj16-/-}$ rats while breathing room air or progressively increased CO_2 levels (3, 5, or 7% CO_2 in air; obtained on separate days) to progressively decrease arterial pH. Total ventilation plotted against arterial pH (**Fig. 3*A***) or arterial PCO_2 (Fig. 3*B*) allowed for the direct determination of a CO_2 sensitivity (slope) measure in SS control and $SS^{Kcnj16-/-}$ rats. Ventilation relative to arterial pH was right shifted in $SS^{Kcnj16-/-}$ rats because of the chronic metabolic acidosis, whereas the ventilation-to-$PaCO_2$ relationship was left shifted because of the resting hyperventilation (Fig. 3). However, in all cases, the slope of the relationship of ventilation to pH or $PaCO_2$ was reduced, and when quantified for each individual $SS^{Kcnj16-/-}$ rat and averaged for the group (Fig. 3*C*), the slope was significantly reduced by up to 45% ($\Delta V_E/\Delta pH$) and 36% ($\Delta V_E/\Delta PaCO_2$) in $SS^{Kcnj16-/-}$ rats relative to SS controls. Thus, the data suggest that in addition to a renal role in maintaining chronic pH, Kir5.1 subunit–containing channels appear to be required for the acute regulation of pH by facilitating the ventilatory CO_2 chemoreflex.

Correction of arterial pH in $SS^{Kcnj16-/-}$ rats further reduced ventilatory CO_2 sensitivity

The underlying chronic metabolic acidosis in $SS^{Kcnj16-/-}$ rats shifted the relationship of ventilation to pH and CO_2 in part through the stimulation of ventilation at rest. Thus, we sought

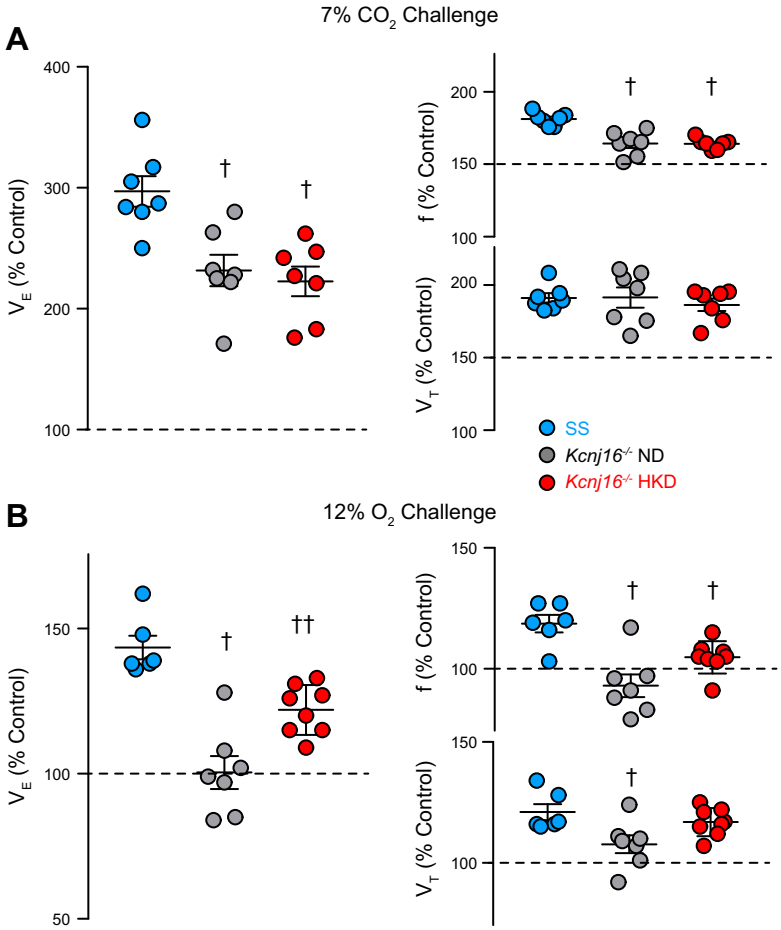

Figure 2. Ventilatory chemoreflexes are reduced in $SS^{Kcnj16-/-}$ rats. *A*) Individual and mean ± SEM data for V_E, *f*, and V_T expressed as a percentage of room air breathing during hypercapnic acidosis (7% inspired CO_2) in SS controls (blue; $n = 7$), $SS^{Kcnj16-/-}$ rats on an ND (gray; $n = 7$), and $SS^{Kcnj16-/-}$ rats fed the HKD (red; $n = 7$). *B*) Individual and mean ± SEM data for V_E, *f*, and V_T expressed as a percentage of room air breathing during hypoxia (12% inspired O_2) in SS controls ($n = 6$), $SS^{Kcnj16-/-}$ rats on an ND ($n = 7$), and HKD ($n = 8$). $^{\dagger}P < 0.05$ from SS controls, $^{\ddagger}P < 0.05$ from SS controls and $SS^{Kcnj16-/-}$ rats fed an ND.

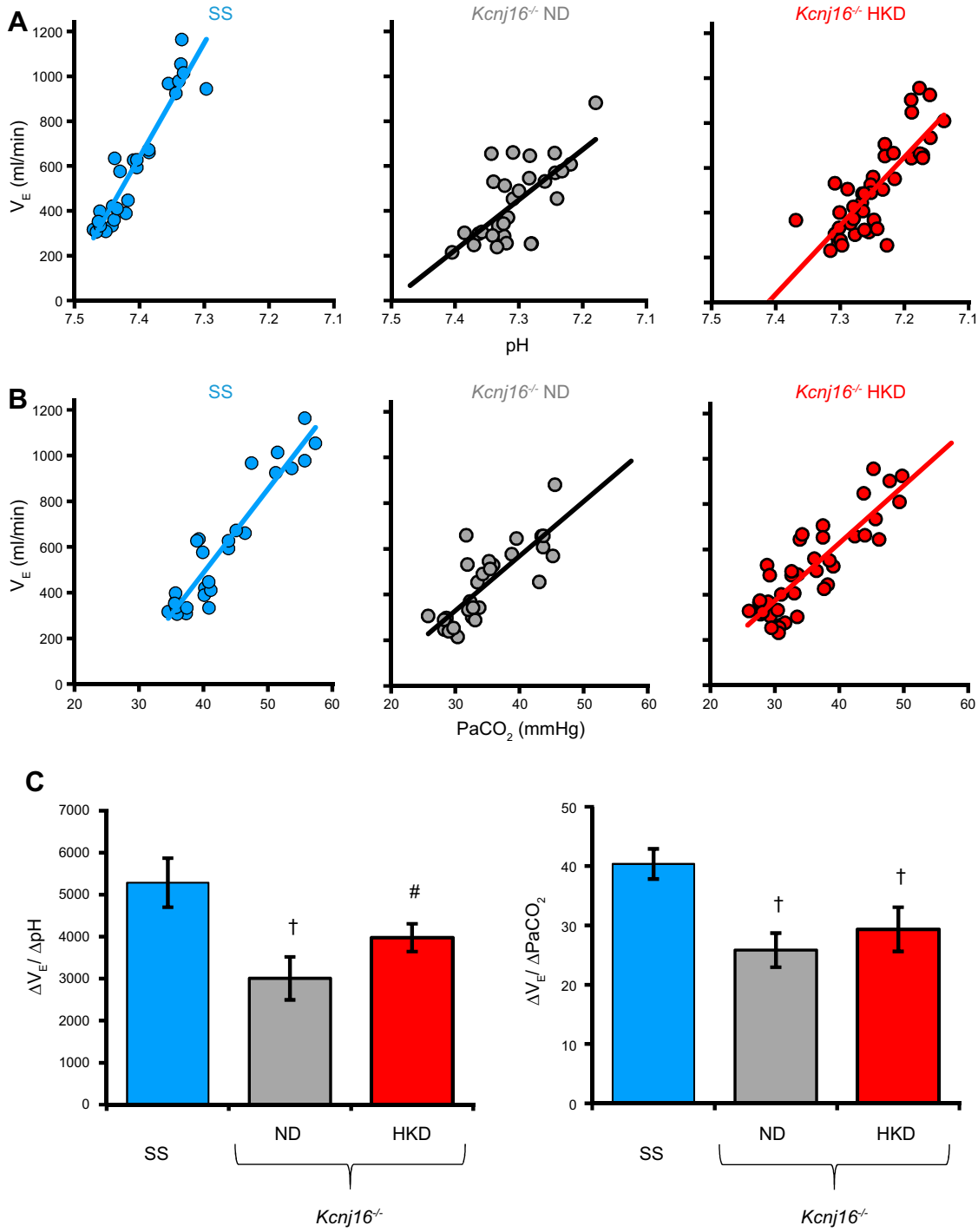

Figure 3. Ventilatory CO_2 and pH sensitivity is reduced in $SS^{Kcnj16-/-}$ rats. *A*, *B*) Individual data and best-fit plots for the relationship between arterial pH (*A*) or $PaCO_2$ (*B*) and V_E during room air breathing or 3, 5 or 7% inspired CO_2 in SS controls (blue; $n = 7$), $SS^{Kcnj16-/-}$ rats on an ND (gray; $n = 8$), and $SS^{Kcnj16-/-}$ rats fed the HKD (red; $n = 9$). *C*) Mean ± SEM data for slope of the ventilatory responses from individual animals presented in *A* and *B*. $^{\dagger}P < 0.05$ from SS controls, $^{\#}P < 0.05$ from SS control and $SS^{Kcnj16-/-}$ rats.

to restore arterial pH in $SS^{Kcnj16-/-}$ rats to determine whether the ventilatory CO_2 chemoreflex was further affected. $SS^{Kcnj16-/-}$ rats had either 0.1 or 0.2 M HCO_3^- added to their drinking water for 7 d, and the ventilatory response to 7% CO_2 was tested before and after 1, 4, or 7 d of treatment. Treatment of $SS^{Kcnj16-/-}$ rats with 0.1 M HCO_3^- significantly increased arterial HCO_3^- and pH relative to untreated $SS^{Kcnj16-/-}$ rats, but these measures remained below those in control SS rats (**Fig. 4**), suggesting only a partial restoration of acid/base status. Both doses of 0.1 or 0.2 M HCO_3^- lead to a further reduction in the hypercapnic ventilatory responses in $SS^{Kcnj16-/-}$ rats by d 7 (**Fig. 5*A***).

Additional groups of SS control and $SS^{Kcnj16-/-}$ rats were treated for up to 6 wk with HCTZ (75 mg/L in drinking water), a carbonic anhydrase inhibitor and loop diuretic that acts to alkalinize the blood. There was little or no effect on ventilation after 1 wk of treatment (unpublished results), but chronic (6 wk) HCTZ treatment in $SS^{Kcnj16-/-}$ rats reduced

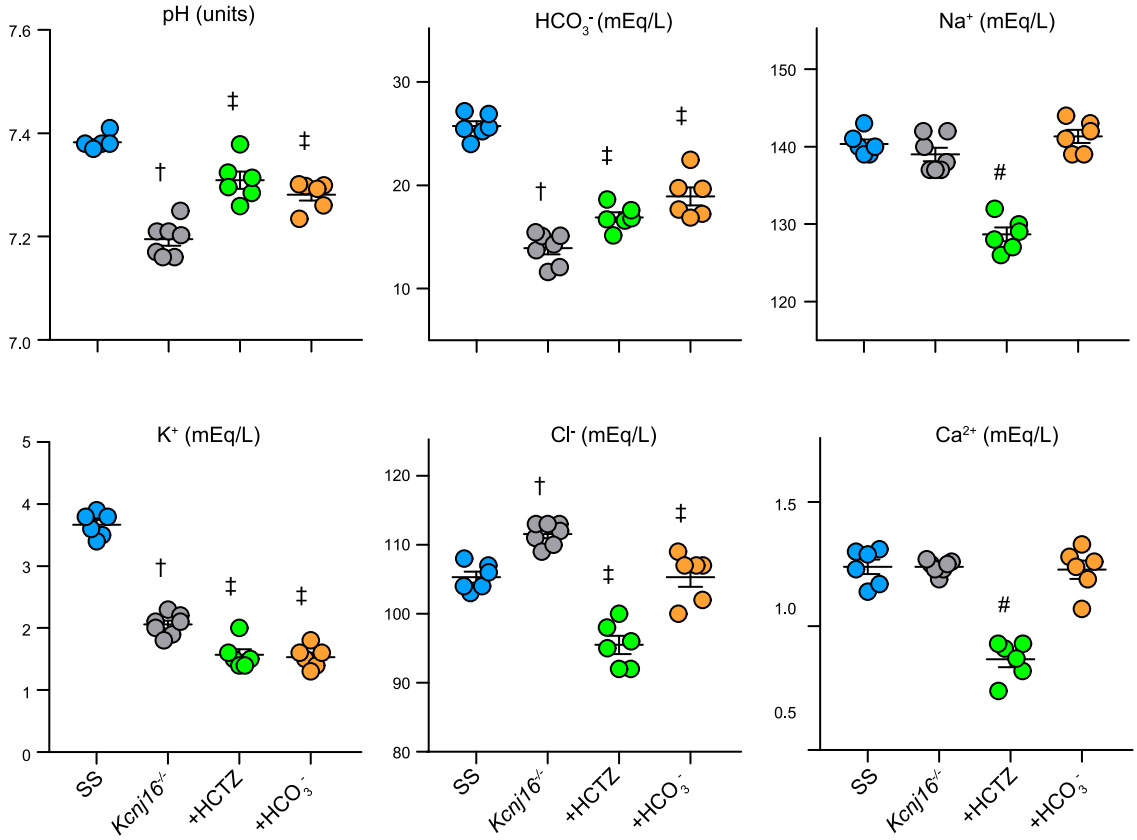

Figure 4. Effects on blood electrolytes of HCTZ and HCO_3^- treatments in $SS^{Kcnj16-/-}$ rats. Electrolytes were measured *via* radiometer in arterial blood samples obtained during anesthesia from SS control rats (blue; $n = 6$), $SS^{Kcnj16-/-}$ rats (gray; $n = 7$), and $SS^{Kcnj16-/-}$ rats after chronic HCTZ [6 wk in drinking water (75 mg/L); green; $n = 6$] or HCO_3^- treatment [14 d in drinking water (0.1 or 0.2 M); orange; $n = 6$] to determine effects of HCTZ and HCO_3^- treatments. $^{\dagger}P < 0.01$ from SS controls, $^{\ddagger}P < 0.05$ from untreated $SS^{Kcnj16-/-}$ rats, $^{\#}P < 0.0001$ between HCTZ and HCO_3^- treatments.

arterial Na^+, Cl^-, Ca^{2+}, and K^+ while increasing HCO_3^- and pH (Fig. 4). However, the increased HCO_3^- and pH in the HCTZ-treated $SS^{Kcnj16-/-}$ rats were not sufficient to completely restore pH homeostasis because these measures remained low relative to SS control rats. Compared with age-matched $SS^{Kcnj16-/-}$ rats, the HCTZ-treated $SS^{Kcnj16-/-}$ rats had increased f (95.6 ± 4.7 *vs.* 118.1 ± 1.2 breaths/min; $P = 0.0006$) but decreased V_T (2.7 ± 0.1 *vs.* 1.9 ± 0.1 ml/breath; $P = 0.0002$), respectively, which led to no differences in resting V_E (264.6 ± 22.1 *vs.* 225.0 ± 8.8 ml/min; $P = 0.12$). However, ventilatory responses to 5 and 7% inspired CO_2 were significantly reduced in HCTZ-treated $SS^{Kcnj16-/-}$ rats after 6 wk of treatment (Fig. 5*B*). In contrast, chronic HCTZ treatment in SS control rats had no significant effects on the ventilatory response to 7% CO_2 despite the similar trend in the response ($P = 0.093$). Overall, these data indicate that partial restoration of arterial pH in $SS^{Kcnj16-/-}$ rats mitigated the increased ventilation breathing room air and led to a further reduction in ventilatory sensitivity to hypercapnic acidosis.

Correction of hypokalemia with increased dietary K^+ failed to alter respiratory phenotypes in $SS^{Kcnj16-/-}$ rats

A consequence of Kir5.1 mutation in mice and rats is hypokalemia (15, 22). We had previously demonstrated that $SS^{Kcnj16-/-}$ rats fed high-NaCl diets experience 100% mortality within 1–2 d, but this could be completely prevented by increasing potassium chloride in the diet (15). Thus, we next tested whether HKD could correct the observed ventilatory phenotypes in $SS^{Kcnj16-/-}$ rats. $SS^{Kcnj16-/-}$ rats continued to show increased resting V_E and reduced arterial PCO_2, pH, and HCO_3^- when chronically fed an HKD (Fig. 1*A, B*). In addition, ventilatory responses to 7% CO_2 remained below control levels (Fig. 2*A*), and the slope of the relationships between ventilation and arterial pH or PCO_2 was reduced 29% ($\Delta V_E/\Delta$pH) and 27% ($\Delta V_E/\Delta PaCO_2$) in $SS^{Kcnj16-/-}$ rats fed the HKD, similar to $SS^{Kcnj16-/-}$ rats fed an ND (Fig. 3). Finally, the hypoxic ventilatory responses in $SS^{Kcnj16-/-}$ rats fed an HKD were slightly increased compared with those fed an ND, but they remained reduced relative to control SS rats (Fig. 2*B*). Thus, there were little or no effects on respiratory phenotypes in $SS^{Kcnj16-/-}$ rats fed an HKD, suggesting that hypokalemia does not likely contribute to the blunted chemoreflexes and pH dysregulation in $SS^{Kcnj16-/-}$ rats.

DISCUSSION

Homomeric Kir5.1 channels are not typically considered functional (27), and the addition of Kir5.1 subunits to other members of the Kir family to form heteromeric channels

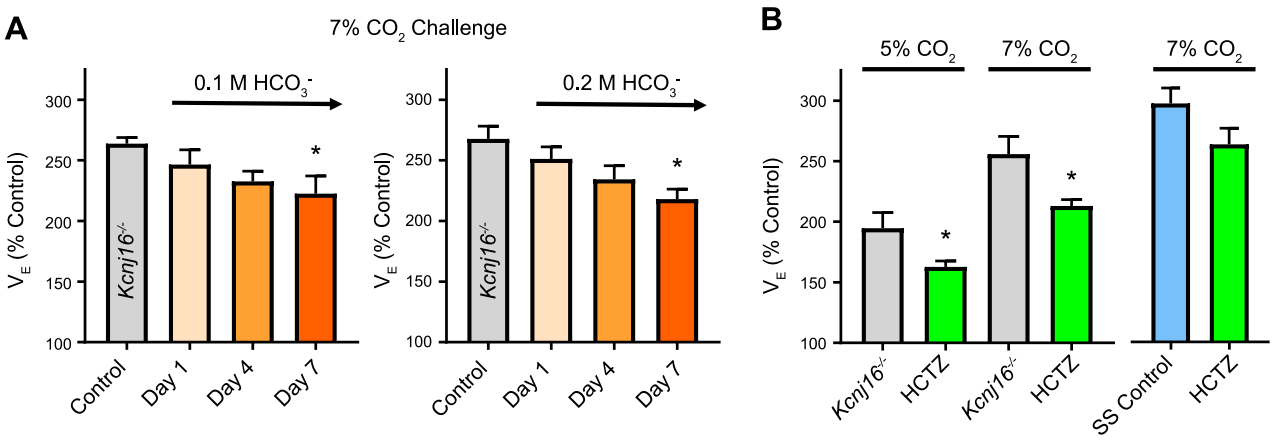

Figure 5. Correction of metabolic acidosis in $SS^{Kcnj16-/-}$ rats further blunts the ventilatory response to hypercapnic acidosis. *A*) Ventilation (expressed relative to room air breathing) during a 7% inspired CO_2 challenge in $SS^{Kcnj16-/-}$ rats before or after 7 d of 0.1 M ($n = 6$) or 0.2 M ($n = 6$) HCO_3^- in the drinking water. *B*) Ventilation (expressed relative to room air breathing) during a 5 or 7% inspired CO_2 challenge in $SS^{Kcnj16-/-}$ rats ($n = 6$; gray) or 7% inspired CO_2 challenges in SS control rats ($n = 6$; blue) treated with HCTZ (75 mg/L in drinking water for 6 wk; green). $*P < 0.05$ from pretreatment control.

modulates single-channel conductance and pH sensitivity (28). The data herein obtained from rats with a mutation in Kir5.1 demonstrate an essential role for Kir5.1 in acute and chronic (renal and respiratory) pH regulation *in vivo*. $SS^{Kcnj16-/-}$ rats showed a highly unique combination of renal and respiratory phenotypes, including a chronic hyperchloremic metabolic acidosis and hyperventilation while breathing room air. Acute hypercapnic acidosis created by graded increases in inspired CO_2 failed to elicit robust ventilatory responses in $SS^{Kcnj16-/-}$ rats relative to SS control rats, in which ventilatory CO_2 sensitivity was reduced as much as 45%. In contrast to a previous study in which HKD improved cardiorenal phenotypes (15), treatment of $SS^{Kcnj16-/-}$ rats with HKDs failed to correct or in some cases exacerbated dysfunction in pH regulation. Importantly, correction of arterial pH in $SS^{Kcnj16-/-}$ rats with HCTZ or HCO_3^--rich drinking water led to normalization of resting breathing, indicating that H^+ chemoreceptors were functional and contributing to the resting hyperventilation. However, correction of arterial pH led to further reductions in the hypercapnic ventilatory responses in $SS^{Kcnj16-/-}$ rats, unmasking an even greater contribution from Kir5.1 subunit–containing channels to this chemoreflex. Remarkably, ventilatory responses to hypoxia were also dramatically reduced in $SS^{Kcnj16-/-}$ rats, suggesting that both hypoxic and hypercapnic ventilatory chemoreflexes require functional Kir5.1 channels. Overall, the data support the conclusion that Kir5.1-containing Kir channels play a major role in the mechanisms that regulate acute and chronic pH, including the hypercapnic ventilatory response, and significantly contribute to hypoxic ventilatory chemoreflexes.

Physiologic pH is regulated through mechanisms at the molecular, cellular, and systems levels. Acute disturbances in arterial pH trigger a ventilatory chemoreflex that drives alveolar ventilation in opposition to the change in pH (decreased pH stimulates ventilation), whereas the renal regulation of ions play a dominant role in chronic pH regulation. The pH and CO_2 sensors within the brain that tightly couple ventilation to arterial pH and CO_2 levels (central respiratory chemoreceptors) have long been thought to derive an intrinsic cellular sensitivity to intracellular and extracellular pH shifts from the gating of pH-sensitive ion channels (3, 29). Among others, Kir family channels modulate the resting membrane potential and the electrical activity of cells through modulating K^+ flux, and several Kir family channels have inherent pH sensitivity [reviewed in Putnam *et al.* (3)]. Homomeric Kir4.1 channels are open at physiologic pH but close when pH nears pH ~6.5. In contrast, the addition of Kir5.1 to Kir4.1 subunits to form a heteromultimeric Kir4.1/Kir5.1 channel shifts the half-maximal channel activity to near a pH of 7.4. Similar effects are observed when Kir5.1 subunits combine with other Kir channels (3), suggesting Kir5.1 subunit–containing Kir channels may be ideal candidates to detect pH within the physiologic range within central respiratory chemoreceptors. This intrinsic and physiologic pH sensitivity, along with expression within brainstem neurons shown to contribute to the hypercapnic CO_2 chemoreflex (6, 14), has led to the hypothesis that Kir5.1-containing Kir channels play a role in pH regulation, particularly in the ventilatory CO_2 chemoreflex.

Our current data validate this hypothesis, demonstrating critical roles for Kir5.1-containing Kir channels in long-term pH regulation and in facilitating ventilatory responses to acute pH disturbances. Respiratory challenges with increased inspired CO_2 levels increase $PaCO_2$ and decrease arterial pH, but the slope of the ventilatory response to these changes was reduced from ~30 to 45% in $SS^{Kcnj16-/-}$ rats, indicating that the ventilatory system (and possibly pH and CO_2 chemoreceptors themselves) cannot elicit a robust response to a given change in arterial pH. Based on this observation, the reduction in ventilatory CO_2 sensitivity should lead to hypoventilation at rest and $PaCO_2$ would be expected to increase, but unexpectedly, $SS^{Kcnj16-/-}$ rats hyperventilated during room air breathing. The hyperventilation likely resulted from the reduced blood HCO_3^- and consequent metabolic acidosis, which would drive ventilation through the carotid body and, to a lesser extent, brain chemoreceptor stimulation. This conclusion is strengthened by the observations that

HCO_3^--rich drinking water and chronic HCTZ treatment, which partially normalized the metabolic acidosis, led to a normalization of room air breathing but further reduced the hypercapnic ventilatory response in $SS^{Kcnj16-/-}$ rats.

The reduction in the slope of the relationship between arterial CO_2 and pH and ventilation, however, would be most consistent with an impaired pH sensitivity of respiratory chemoreceptors, suggesting Kir5.1-containing Kir channels may be a determinant of cellular pH and CO_2 sensitivity in peripheral and central respiratory chemoreceptor cells. This mechanism would be at least in part driven by decreased pH sensitivity of Kir channel currents and consequent decreases in excitatory drive to the neural respiratory control network. Defining the slope between arterial CO_2 and pH and ventilation, which is the gold standard in assessing ventilatory CO_2 sensitivity, cannot be accomplished in mice because of the required amount of arterial blood collected under conditions of wakefulness, highlighting the value of physiologic studies in (genetically modified) rats. The most parsimonious explanation of our data is that pH-sensitive Kir5.1-containing channels directly contribute to cellular pH sensitivity in respiratory chemoreceptors, as previously suggested in refs. 3, 6, and 23. This conclusion is based on the current data and additional observations of pH-sensitive astrocytes within the retrotrapezoid nucleus, which depend in part on a Kir5.1-containing current (10); the dependence of pH sensitivity in locus coeruleus neurons upon Kir currents (30); and reduced locus coeruleus neuronal responses to intracellular acidification in $Kir5.1^{-/-}$ mice (23). Irrespective of the mechanism, our data demonstrate that Kir5.1-containing channels are obligatory for chronic and acute pH regulatory mechanisms and are particularly important in coupling ventilation to changes in arterial CO_2 and pH.

Both $Kir5.1^{-/-}$ mice (21) and $SS^{Kcnj16-/-}$ rats also exhibit reduced responses to hypoxia. This is an intriguing result given that Kir5.1-containing Kir channels have not been implicated in hypoxia-sensing mechanisms within the glomus cells of the carotid bodies or the recently proposed astrocyte-mediated hypoxia-sensing mechanisms in the brain (31–33). However, Kir5.1 expression has been detected in the carotid bodies (34) and brainstem astrocytes and glia (6, 9), and both have known pH sensitivity (10, 35). Despite the loss of the hypoxic ventilatory response in $SS^{Kcnj16-/-}$ rats, it is possible that the chronic metabolic acidosis leads to hyperventilation at rest through a carotid body–mediated mechanism given that the blood-brain barrier protects the brain to a large extent during systemic metabolic acidosis (36). In this case, it would be concluded that the carotid bodies are functional but lack or have severely reduced O_2 sensitivity. However, this hypothesized reduction in carotid body function would not likely contribute to the reduced ventilatory response to hypercapnic acidosis because carotid body denervation does not affect this response in rats (24). Alternatively, the hypoxic and hypercapnic ventilatory responses in $SS^{Kcnj16-/-}$ rats had in common a blunted *f* response, which could mark a more generalized failure of the neural respiratory network to respond to an O_2-dependent or CO_2-dependent chemoreflex drive. Although these possible mechanisms are not distinguishable based on the current data, it is clear that Kir5.1-containing Kir channels critically contribute to both hypoxic and hypercapnic chemoreflex drives *in vivo*.

We are only beginning to understand the importance of Kir family channels in human physiology and the resulting pathophysiology in various Kir channelopathies. For example, rare missense mutations in the *KCNJ10* (Kir4.1) gene in humans cause a panoply of complex and severe phenotypes in epilepsy, ataxia, sensorineural deafness, and tubulopathy syndrome (19), highlighting the functional importance of Kir4.1-containing Kir channels in the regulation of electrolytes and pH (17). Type I Andersen-Tawil syndrome, which is characterized by physical abnormalities (short stature) and cardiac arrhythmia, is caused by mutations in another Kir family gene, *KNCJ2* (Kir2.1) (37), whereas *de novo* genetic mutations in *KCNJ16* in humans are associated with life-threatening cardiac arrhythmias in nonfamilial Brugada syndrome (18). We previously demonstrated the effects of *Kcnj16* mutation in rats, which led to reduced somatic growth, lower blood pressure, hypokalemia, and salt wasting, leading to mortality when fed a high-salt diet (15). The data herein further establish additional, unique physiologic features resulting from *Kcnj16* mutations in rats, including a chronic hyperchloremic acidosis and acute dysregulation in ventilatory mechanisms of regulating arterial pH, CO_2, and O_2. Given that Kir2.1, Kir4.1, and Kir5.1 channels can bind to one another to form various combinations of functional heteromeric Kir channels within the kidney and brain, it follows that the collection of phenotypes exhibited by the $SS^{Kcnj16-/-}$ rat resembles aspects of human Kir channelopathies.

CONCLUSIONS

Kcnj16 mutation results in several distinct respiratory, renal, and cardiovascular phenotypes, suggesting that Kir5.1 in particular has an essential role in physiologic pH regulation over both short and long time scales. Although previous investigations led to the general conclusion that Kir5.1 channels are not necessary for coupling ventilation with pH and CO_2 changes, the results of *Kcnj16* mutation in rats suggest Kir5.1 may critically modify several homeostatic mechanisms, either independently or, more likely, through heterodimerization with other Kir family members. We predict that yet-to-be-discovered genetic mutations in *KCNJ16* in humans may ultimately be linked to additional diseases and syndromes characterized by pH, electrolyte, and ventilatory chemoreflex abnormalities and other neural phenotypes, adding to a growing list of known channelopathies in humans. FJ

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AUTHOR CONTRIBUTIONS

M. M. Puissant, C. Muere, V. Levchenko, A. D. Manis, and O. Palygin contributed to study design, data acquisition

and analysis, and manuscript preparation; and P. Martino, H. V. Forster, A. Staruschenko, and M. R. Hodges contributed to study design, interpretation of the data, and manuscript preparation.

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