Life Sciences 346 (2024) 122633

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Ammonium chloride-induced hypothermia is attenuated by transient receptor potential channel vanilloid-1, but augmented by ankyrin-1 in rodents

Zoltan Rumbus [a], Kata Fekete [a], Leonardo Kelava [a], Bibor Gardos [a], Krisztian Klonfar [a], Patrik Keringer [a], Erika Pinter [b], Eszter Pakai [a], Andras Garami [a,*]

[a] Department of Thermophysiology, Institute for Translational Medicine, Medical School, University of Pecs, Pecs H-7624, Hungary
[b] Department of Pharmacology and Pharmacotherapy, Medical School, University of Pecs, Pecs H-7624, Hungary

ARTICLE INFO

Keywords:
Hypothermia
Thermoregulation
TRPV1
TRPA1
Thermophysiology
NH_4Cl

ABSTRACT

Aims: Systemic administration of ammonium chloride (NH_4Cl), an acidifying agent used in human patients and experimental conditions, causes hypothermia in mice, however, the mechanisms of the thermoregulatory response to NH_4Cl and whether it develops in other species remained unknown.
Main methods: We studied body temperature (T_b) changes in rats and mice induced by intraperitoneal administration of NH_4Cl after blockade of transient receptor potential vanilloid-1 (TRPV1) or ankyrin-1 (TRPA1) channels.
Key findings: In rats, NH_4Cl decreased T_b by 0.4–0.8°C ($p < 0.05$). The NH_4Cl-induced hypothermia also developed in *Trpv1* knockout (*Trpv1*$^{-/-}$) and wild-type (*Trpv1*$^{+/+}$) mice, however, the T_b drop was exaggerated in *Trpv1*$^{-/-}$ mice compared to *Trpv1*$^{+/+}$ controls with maximal decreases of 4.0 vs. 2.1°C, respectively ($p < 0.05$). Pharmacological blockade of TRPV1 channels with AMG 517 augmented the hypothermic response to NH_4Cl in genetically unmodified mice and rats ($p < 0.05$ for both). In contrast, when NH_4Cl was infused to mice genetically lacking the TRPA1 channel, the hypothermic response was significantly attenuated compared to wild-type controls with maximal mean T_b difference of 1.0°C between the genotypes ($p = 0.008$). Pretreatment of rats with a TRPA1 antagonist (A967079) also attenuated the NH_4Cl-induced T_b drop with a maximal difference of 0.7°C between the pretreatment groups ($p = 0.003$).
Significance: TRPV1 channels limit, whereas TRPA1 channels exaggerate the development of NH_4Cl-induced hypothermia in rats and mice, but other mechanisms are also involved. Our results warrant for regular T_b control and careful consideration of NH_4Cl treatment in patients with TRPA1 and TRPV1 channel dysfunctions.

1. Introduction

Ammonium chloride (NH_4Cl) is a systemic and urinary acidifying agent that can be used in the treatment of metabolic alkalosis [1–3]. As an expectorant, it is a common ingredient of many cough mixtures, which explains why the excessive consumption of such over-the-counter medications can lead to metabolic acidosis [4,5] that was also observed in subjects receiving NH_4Cl in other types of medicines [6–8]. Extending its application areas, more recently, the use of NH_4Cl was implicated in COVID-19 [9,10].

The oral and/or parenteral administration of NH_4Cl is often used to induce systemic (extracellular) acidosis in animal models [11–15] and in human studies [16–19]. Although interspecies differences, for example, between rats and mice, were demonstrated in the response to the same NH_4Cl-loading protocol [13,20], NH_4Cl administration has been widely used to study the effect of acidosis associated with different conditions, such as physical exercise [16,21], kidney functions [22,23], iron metabolism [24], or parathyroid functions [25].

In 1988, Gordon showed that the systemic administration of NH_4Cl leads to hypothermia in mice [26]. This finding can explain why its overconsumption was also associated with hypothermia in case of a human patient [5]. However, the molecular mediators of the NH_4Cl-induced hypothermia have remained largely unknown. Our literature search did not reveal any studies that aimed to clarify the underlying

* Corresponding author.
E-mail address: andras.garami@aok.pte.hu (A. Garami).

https://doi.org/10.1016/j.lfs.2024.122633
Received 2 January 2024; Received in revised form 29 March 2024; Accepted 10 April 2024
Available online 12 April 2024
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mechanisms of this thermoregulatory phenomenon.

Transient receptor potential (TRP) vanilloid-1 (V1) and ankyrin-1 (A1) channels are temperature-sensitive members of the TRP channel family, for the reason that *in vitro* they can be activated by noxious heat and cold, respectively [27,28]. In addition to thermal signals, they can be both activated by ligand agonists and by changes in pH, hence they function as polymodal receptors [29,30], especially on primary afferent neurons where they are often co-expressed [31,32]. Their thermosensor function has been well established in pain sensation [29,33,34], however, neither of them was found to serve as a thermosensor for the thermoregulation system in rodents [35,36]. It was proposed that their activation by agonists other than temperature (by protons for TRPV1 and by sulfides for TRPA1) contributes to the regulation of deep body temperature (T_b) [37,38]. Interestingly, both channels can be activated by NH_4Cl [39,40], and also by low pH [41–44]. However, to our best knowledge, the contribution of the TRPV1 or TRPA1 channel to the thermal response to NH_4Cl has not been investigated yet.

In the present work, we studied whether NH_4Cl induces hypothermia also in rats in addition to mice to exclude the possibility of a mouse-specific effect, and whether the blockade of TRPV1 and TRPA1 channels influences the NH_4Cl-induced hypothermia by using genetic deletion of the channels. To avoid chronic compensatory mechanisms, which may develop for the function of a knocked out gene, we also used pharmacological inhibition of TRPV1 and TRPA1 in genetically unaltered animals.

2. Methods

2.1. Animals

Experiments were conducted in adult male rats and mice under protocols approved by the Institutional Animal Use and Care Committee of the University of Pecs (registration no.: BA02/2000–13/2021) and in accordance with the directives of the National Ethical Council for Animal Research and those of the European Communities Council (86/609/EEC). One hundred and ten Wistar rats and thirty-seven C57BL/6 mice were obtained from the Laboratory Animal Centre of the University of Pecs. In addition, mice with ($^{-/-}$ aka knockout, KO) or without ($^{+/+}$ aka wild type, WT) a homozygous targeted mutation in the *Trpv1* gene (*Trpv1* KO: n = 21; *Trpv1* WT: n = 14) or in the *Trpa1* gene (*Trpa1* KO: n = 24; *Trpa1* WT: n = 16) were also obtained from the Laboratory Animal Centre of the University of Pecs, where they were bred and kept as described in details elsewhere [35,45]. Mice from these strains were also used in previous studies aiming to investigate the thermoregulatory function of TRPA1 [35,37] and TRPV1 [38,46]. The animals were housed in polycarbonate cages kept in temperature-controlled rooms on a 12 h light/dark cycle (lights on at 5:00 A.M.). The ambient temperature was maintained at 24–25°C and humidity at 30–40%. Standard rodent chow and tap water were available *ad libitum*. Animals were extensively habituated to the experimental setup, as described elsewhere [47,48]. At the time of the experiments, the mice and rats weighed 25 ± 1 and 328 ± 3 g, respectively.

2.2. Surgeries

2.2.1. Anesthesia and perioperative care

Animals were anesthetized with intraperitoneal (i.p.) administration of a ketamine–xylazine cocktail (81.7 and 9.3 mg/kg for mice, 55.6 and 5.5 mg/kg for rats, respectively), and they received antibiotic protection intramuscularly (gentamycin, 6 mg/kg). To prevent intra- and postoperative hypothermia, during the surgery mice were kept on a heating pad (PECO Services Ltd., Brough, United Kingdom), and then they were allowed to recover from anesthesia in a temperature-controlled chamber (model MIDI F230S; PL Maschine Ltd., Tarnok, Hungary) set to 31°C. Each rat and mouse was implanted with an i.p. catheter, additionally rats assigned to experiments with pharmacological antagonists were also implanted with an intravenous (i.v.) catheter during the same surgery. The i.p. and i.v. catheter implantations have been widely used in thermoregulation experiments, and the animals tolerated these interventions well [37,38,46,49]. These preimplanted catheters were used for the non-stressful administration of substances to conscious animals in our experiments. In case of a bolus injection (i.p. or i.v.), the handling of the animal and the puncture of the abdominal wall with the needle would have resulted in pain and stress-induced hyperthermia (for review, see [50]), which could interfere with our results. All experiments were performed 3–5 days after the surgery.

2.2.2. I.p. catheter implantation

For the non-stressful i.p. administration of the substances during the experiment, a polyethylene (PE)-50 catheter filled with pyrogen-free saline was implanted into the peritoneal cavity of each mouse and rat, similarly as in previous studies [38,46]. In brief, through a small midline incision on the abdomen, the internal end of the catheter was fixed to the left side of the abdominal wall with a suture, while the external end of the catheter was tunneled under the skin to the nape, where it was exteriorized and heat-sealed. The surgical wound was sutured in layers. The catheter was flushed with 0.1 ml of saline on the day after the surgery and every other day thereafter.

2.2.3. I.v. catheter implantation

This procedure was performed in rats assigned to treatment with a TRPV1 or a TRPA1 antagonist. As described before [46], the i.v. catheter was implanted during the same surgery as the i.p. catheter. A small longitudinal incision was made on the ventral surface of the neck, left of the trachea. The left jugular vein was exposed, freed from its surrounding connective tissue, and ligated. A silicone catheter (with inner and outer diameter of 0.5 and 0.9 mm, respectively) was filled with heparinized (10 U/ml) saline, then it was inserted into the left jugular vein, passed into the superior vena cava, and secured in place with ligatures. The free end of the catheter was knotted and exteriorized at the nape. The skin wound was sutured. The catheter was flushed with heparinized saline on the day following the surgery and every other day thereafter.

2.3. Thermocouple thermometry

The mice and the rats were placed in cylindrical confiners and equipped with copper-constantan thermocouples (Omega Engineering, Stamford, CT, USA) to measure colonic temperature (a form of deep T_b). The colonic thermocouple was inserted beyond the anal sphincter (10 and 3 cm deep for rats and mice, respectively); fixed to the base of the tail with adhesive tape; and plugged into a data logger device (Cole-Palmer, Vernon Hills, IL, USA) connected to a computer. Animals in their confiners were then placed into a biochemistry incubator (model BJPX-Newark; Biobase; Jinan, China). As the expected T_b change was hypothermia, the ambient temperature was set to 25°C, which is slightly subneutral for rats and mice in this setup. The preimplanted i.p. and i.v. catheter (when present) was connected to a PE-50 extension, which was prefilled with the substance of interest and connected to a syringe placed in an infusion pump (model 975; Harvard Apparatus Inc., Holliston, MA, USA).

2.4. Drugs and drug administration

2.4.1. I.p. NH_4Cl to mice and rats

NH_4Cl was purchased from VWR Chemicals (Leuven, Belgium). On the day of an experiment, NH_4Cl was freshly dissolved in sterile water to achieve final concentrations of 32.1, 220 or 280 mg/ml. For the i.p. administration of NH_4Cl to mice, the working solution (32.1 mg/ml) was infused (10 ml/kg) over 16 min to deliver NH_4Cl at 321 mg/kg (~6 mmol/kg). In rats, the working solutions (220 and 280 mg/ml) were infused (1 ml/kg) over 5 min to deliver NH_4Cl at 220 and 280 mg/kg (ca.

4 and 5 mmol/kg), respectively. The decreased dose of NH_4Cl in the rats compared to mice was necessary, because rats were more sensitive to the effect of the same NH_4Cl-loading protocol on protein expression than mice [13]. Control animals were infused with sterile water. All infusion rates and volumes were selected with the goal to minimize the stress and discomfort of the animals that may originate from the substance administration procedure itself. Similar i.p. infusion rates as in the present experiments were successfully applied in our previous studies for non-stressful administration of substances to mice [51] and rats [46].

2.4.2. I.v. AMG 517 and A967079 to rats

AMG 517, a highly potent TRPV1 antagonist [52], and A967079, a highly potent TRPA1 antagonist [53], were purchased from Tocris (Bristol, UK). A stock solution of AMG 517 (1 mg/ml) was prepared with 10% dimethyl sulfoxide (DMSO) and 10% Tween-80 in saline, aliquoted, and stored at −80°C. On the day of the experiment, the stock was diluted to give a working solution of AMG 517 at 210 μg/ml in 10% DMSO and 10% Tween-80 in saline. A stock solution of A967079 (10 mg/ml) was prepared with polyethylene glycol 400 (PEG 400), aliquoted, and stored at −80°C. On the day of the experiment, the stock was diluted with PEG 400 and saline to give a working solution of A967079 at 5 mg/ml in 80% PEG 400 in saline. For the i.v. administration, the working solution of AMG 517 or A967079 (210 μg/ml or 5 mg/ml, respectively) was infused (1 ml/kg) over 10 min to deliver AMG 517 and A967079 at 210 μg/kg and 5 mg/kg, respectively. Both antagonists were infused i.v. to the rats 20 min before the i.p. infusion of NH_4Cl. Control rats were infused with the vehicle of the antagonist of interest. The efficacies of AMG 517 and A967079 administered i.v. at similar doses and rates as in the present experiments were shown in previous studies [54,55].

2.4.3. Subcutaneous (s.c.) AMG 517 to mice

To study the effect of AMG 517 on NH_4Cl-induced hypothermia in mice, we used a drug dose and administration model that was successfully applied earlier [56]. The stock and working solutions of AMG 517 were prepared as described above. The working solution (or its vehicle) was injected s.c. as a bolus to deliver AMG 517 at a dose of 210 μg/kg just before setting up the mice for the experiment (i.e., ~120 min before the administration of NH_4Cl). Then, the mice were allowed to accommodate to the experimental conditions for ~2 h before they received the non-stressful, i.p. infusion of NH_4Cl (321 mg/kg) through a pre-implanted catheter. The 2-h latency until the NH_4Cl infusion was needed to reduce the stress-induced hyperthermia resulting from the needle prick associated with the s.c. injection of AMG 517 (or its vehicle). Because of the long (31h) half-life of AMG 517 in rodents [57], the effect of the drug could be reasonably expected at the time of NH_4Cl infusion even with this latency.

The drug administration procedures in the different experiments are summarized in Table 1.

2.5. Blood pH measurements

One hour after the i.p. administration of NH_4Cl, the animals were anesthetized with a ketamine-xylazine cocktail, and then blood samples were collected by cardiac puncture with a heparinized syringe. The pH of the blood samples was measured by a pH meter (model OP-211/2; Radelkis Ltd., Budapest, Hungary) within 1 min after collection. After the blood withdrawal, the animals were euthanized with sodium pentobarbital (100 mg/kg, i.p.).

2.6. Data processing and analysis

Data on deep T_b and on blood pH were compared by ANOVA, as appropriate. ANOVA was followed by the Student-Newman-Keuls *post hoc* test as in our earlier study [49]. Sigmaplot 11.0 (Systat Software, San Jose, CA, USA) software was used for statistical analyses. Differences were considered significant when $p < 0.05$. Data are presented as mean ± SE.

3. Results

3.1. Systemic administration of NH_4Cl causes hypothermia in rats

First, we studied the thermal effect of NH_4Cl administered systemically (i.p.) to rats in order to exclude the possibility that the NH_4Cl-induced hypothermic response is specific only for mice. We found that the i.p. injection of NH_4Cl to the rats caused hypothermia, which was more pronounced at the higher dose (Fig. 1). The colonic temperature of the rats started to drop promptly, already at 10 min after the injection of NH_4Cl at both doses. Compared to the baseline, the NH_4Cl-induced maximal mean (± SE) decrease in deep T_b was −0.4 ± 0.1°C at 20 min in case of 220 mg/kg and −0.8 ± 0.2°C at 30 min in case of 280 mg/kg. From that timepoint, the T_b of the NH_4Cl-treated rats gradually increased and reached the T_b level of the control (sterile water-treated) group by the end of the experiment. The T_b of the sterile water-treated rats tended to increase, and then to decrease during the experiment after the i.p. infusion, but it did not differ significantly from the baseline at any timepoints. The initial increase in the T_b could be caused by an unwanted stress response to sterile water infusion despite all of our efforts to minimize the discomfort associated with substance administration, while the gradual fall might have reflected ultradian body temperature rhythms in rats maintained at an ambient temperature of ~25°C, which is below the thermoneutral zone. Statistically, both doses of NH_4Cl had a significant effect as compared to controls ($p < 0.001$ for both), and a statistical difference was also present between the lower and the higher dose groups ($p < 0.001$). At 220 mg/kg, the NH_4Cl-induced decrease in deep T_b was significant compared to the control group between 20 and 50 min, while at 280 mg/kg, the T_b was significantly lower than in controls between 20 and 90 min. The development of hypothermia in response to NH_4Cl in rats is a novel finding of our study, especially considering that the effect occurred already at an i.p. dose of 220 mg/kg, which is almost 50% smaller than the threshold dose predicted earlier in mice [26].

Table 1
Summary of drug administration procedures in the experiments.

Species (strain)	Pretreatment			NH_4Cl	Figure number
	Drug	Dose (mg/kg) and route	Time prior NH_4Cl (min)	I.p. dose (mg/kg)	
Rat (Wistar)	N/A			220; 280	1
Mouse (*Trpv1*$^{-/-}$ and $^{+/+}$)	N/A			321	2
Mouse (C57BL/6)	AMG 517	0.21 s.c.	−120	321	3
Rat (Wistar)	AMG 517	0.21 i.v.	−20	280	4
Mouse (*Trpa1*$^{-/-}$ and $^{+/+}$)	N/A			321	5
Rat (Wistar)	A967079	5 i.v.	−20	280	6

N/A, not applicable.

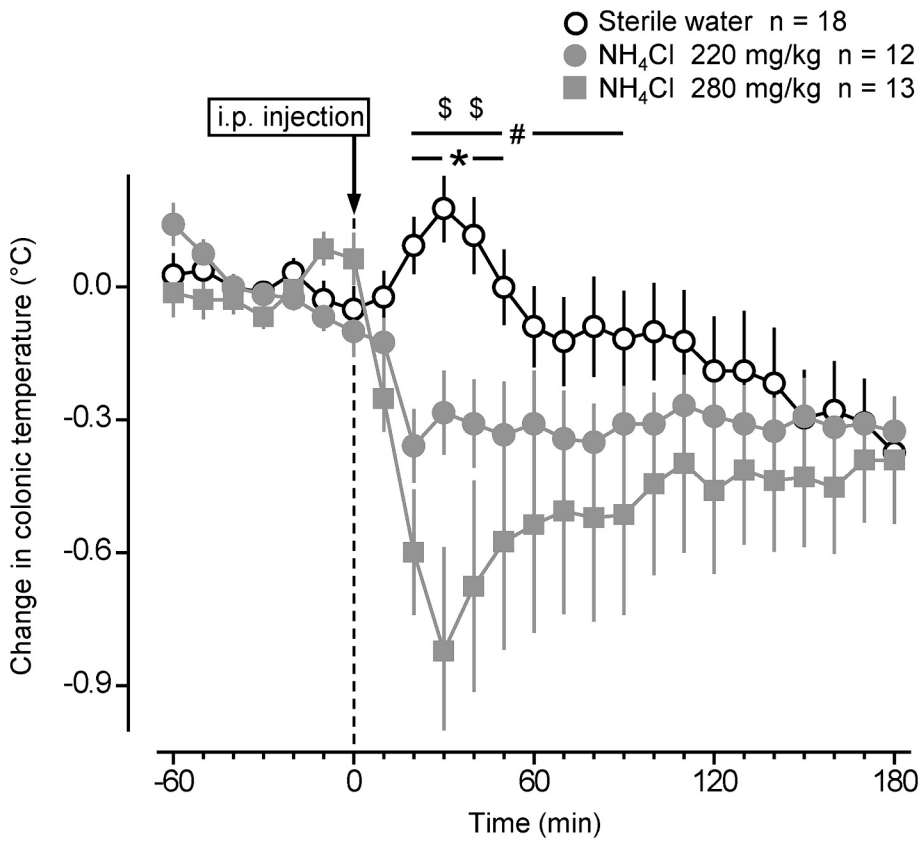

Fig. 1. Changes in deep (colonic) T_b in response to i.p. administration of NH_4Cl (doses indicated) or sterile water in rats. Here and in Figs. 2–6, n is the number of animals in each experimental group. $^{*}p < 0.05$, 220 mg/kg NH_4Cl vs. sterile water difference; $^{\#}p < 0.05$, 280 mg/kg NH_4Cl vs. sterile water difference; $^{\$}p < 0.05$, 220 vs. 280 mg/kg NH_4Cl difference.

3.2. NH_4Cl-induced hypothermia is augmented in mice genetically lacking the TRPV1 channel

After we showed that the hypothermic response to NH_4Cl is not specific to mice only, we wanted to know if two of the most studied thermo-TRP channels, TRPV1 or TRPA1, are involved in this thermoregulatory response. In our first approach, we compared the hypothermic response to NH_4Cl between *Trpv1*$^{-/-}$ and *Trpv1*$^{+/+}$ mice. Since the threshold i.p. dose of NH_4Cl to trigger hypothermia in mice was above 300 mg/kg according to Gordon [26], in our experiments we infused the mice i.p. with 321 mg/kg of NH_4Cl. As expected, NH_4Cl at this dose caused a sudden drop in the colonic temperature of *Trpv1*$^{+/+}$ mice, which reached the biggest mean decrease of -2.1 ± 0.5°C at 30 min post-injection (Fig. 2). Compared to the vehicle (sterile water), the deep T_b of NH_4Cl-treated *Trpv1*$^{+/+}$ mice was markedly lower between 20 and 50 min post-injection ($p < 0.05$). In *Trpv1*$^{-/-}$ mice, NH_4Cl also caused hypothermia compared to sterile water, which was significant between 20 and 70 min ($p < 0.05$) with a maximal mean decrease of -4.0 ± 0.4°C ($p < 0.001$). Interestingly, however, the hypothermic response to NH_4Cl was much more pronounced in the *Trpv1*$^{-/-}$ mice than in their *Trpv1*$^{+/+}$ littermates (Fig. 2). The intergenotype difference was significant ($p < 0.05$) between 20 and 60 min post-NH_4Cl administration with a maximum of ~2.0°C difference between the groups at 40 min ($p < 0.001$).

Nevertheless, it should be noted that the NH_4Cl-induced hypothermia developed in both genotypes of the mice (though to a different extent), which suggests that it also involves TRPV1-independent mechanisms.

3.3. Pharmacological blockade of the TRPV1 channel exaggerates NH_4Cl-induced hypothermia in mice and rats

Our findings in the genetically modified mouse model clearly indicated a bigger hypothermic response in *Trpv1*$^{-/-}$ mice. However, it could not be excluded that the *Trpv1*$^{-/-}$ mice had developed chronic compensatory mechanisms for the absence of TRPV1, which could have influenced the results. To avoid the potential development of chronic compensation, we decided to use genetically unaltered animals (*viz.*, C57BL/6 mice and Wistar rats) and block their TRPV1 channels acutely by a pharmacological antagonist, AMG 517.

AMG 517 is a highly potent and selective, in itself hyperthermia-inducing TRPV1 antagonist [52], which has been tested in hypothermic conditions associated with severe systemic inflammation and with general anesthesia [46,56]. Similarly as in our earlier study [56], in the present experiments the efficacy of AMG 517 could be confirmed by the higher deep T_b of the AMG 517-treated mice compared to vehicle-treated controls before NH_4Cl administration (Fig. 3a). As it could be expected based on our results obtained in *Trpv1*$^{+/+}$ mice, in the vehicle-pretreated C57BL/6 mice the hypothermic response to NH_4Cl developed rapidly and it reached the biggest mean decrease of -2.4 ± 0.4°C at 30 min ($p < 0.001$ compared to sterile water) (Fig. 3b). In the vehicle-pretreated mice, the NH_4Cl-induced decrease in deep T_b was significant ($p < 0.05$) compared to the sterile water-treated group between 20 and 70 min post-injection. Pretreatment with AMG 517 exaggerated the hypothermic effect of NH_4Cl to a maximal mean T_b decrease of -3.5 ± 0.6°C at 40 min ($p < 0.001$ compared to vehicle pretreatment) (Fig. 3b). The biggest difference between the mean T_b of the pretreatment groups was 1.6°C at 50 min post-NH_4Cl injection ($p < 0.001$). In response to NH_4Cl, the deep T_b of the AMG 517-pretreated mice was significantly

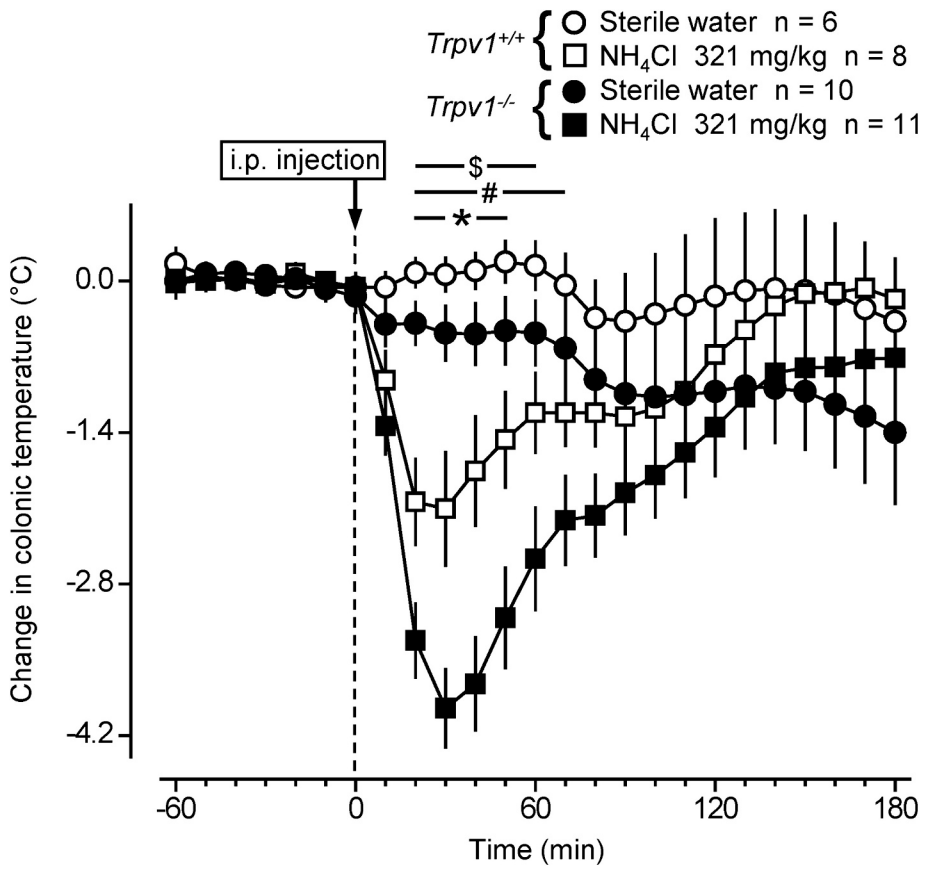

Fig. 2. Deep T_b responses of *Trpv1*$^{+/+}$ and *Trpv1*$^{-/-}$ mice to i.p. administration of NH_4Cl (dose indicated) or sterile water. $^{*}p < 0.05$, NH_4Cl vs. sterile water difference in *Trpv1*$^{+/+}$ mice; $^{\#}p < 0.05$, NH_4Cl vs. sterile water difference in *Trpv1*$^{-/-}$ mice; $^{\$}p < 0.05$, intergenotype difference in response to NH_4Cl.

lower than that of the vehicle-pretreated mice between 30 and 70 min ($p < 0.05$).

We also wanted to confirm that the blockade of TRPV1 leads to the augmentation of NH_4Cl-induced hypothermia not only in mice, but also in rats. We administered the same dose (210 μg/kg) of AMG 517 to rats i.v. 20 min before the i.p. injection of NH_4Cl. As expected, AMG 517 caused prompt hyperthermia, which was present also at the time of the i.p NH_4Cl injection (Fig. 4a). After the injection of NH_4Cl, the deep T_b of vehicle-pretreated rats started to decrease immediately and reached a mean maximal decrease of -1.2 ± 0.2°C at 30 min ($p < 0.001$ compared to sterile water injection); it was significantly ($p < 0.05$) different between the two i.p. treatment groups (NH_4Cl vs. sterile water) between 20 and 100 min (Fig. 4b). Importantly, in AMG 517-pretreated rats both the magnitude and the duration of the NH_4Cl-induced hypothermia was markedly exaggerated. The maximal mean T_b fall of -1.7 ± 0.2°C developed at 40 min, and the T_b of the NH_4Cl-treated rats remained lower than the T_b of the sterile water-treated rats between 20 and 130 min ($p < 0.05$). Accordingly, the effect of NH_4Cl on T_b was significantly different between the i.v. pretreatment groups (AMG 517 vs. vehicle) from 40 to 70 and at 110 min post-NH_4Cl injection (Fig. 4b).

Similarly to our results with the genetic blockade of TRPV1, the NH_4Cl-induced hypothermia developed both with and without the pharmacological blockade of the TRPV1 channel (though to a different extent), which suggests the involvement of TRPV1-independent mechanisms.

3.4. The hypothermic response to NH_4Cl is attenuated in the absence of the TRPA1 channel in mice

Because TRPV1 and TRPA1 channels are often co-expressed [31,32,58] and crosstalk between them was also reported [59,60], after discovering the exaggeration of NH_4Cl-induced hypothermia in different models of TRPV1 blockade, we studied whether the TRPA1 channel also plays a role in this thermal response. Similarly as in the case of TRPV1, in our first approach we used mice genetically lacking the channel (*Trpa1*$^{-/-}$) and their WT littermates (*Trpa1*$^{+/+}$). As expected, the injection of NH_4Cl (321 mg/kg, i.p.) caused marked hypothermia in *Trpa1*$^{+/+}$ mice as compared to sterile water (Fig. 5). In *Trpa1*$^{+/+}$ mice, the biggest NH_4Cl-induced drop in T_b (-2.8 ± 0.3°C) developed 30 min after the injection ($p < 0.001$), and the T_b of NH_4Cl-tretaed mice was significantly lower than the T_b of the sterile water-treated group from 20 to 110 min post-injection ($p < 0.05$). In the *Trpa1*$^{-/-}$ mice, however, the hypothermic response to NH_4Cl was greatly attenuated: the biggest mean T_b decrease was -2.5 ± 0.2°C at 20 min, and it was significantly ($p < 0.05$) lower than that of sterile water-treated mice only between 20 and 50 min (Fig. 5). With regards to intergenotype difference, *Trpa1*$^{+/+}$ mice had significantly lower deep T_b than *Trpa1*$^{-/-}$ mice between 40 and 100 min after the administration of NH_4Cl with a maximal mean T_b difference of 1.0°C at 70 min ($p = 0.008$).

It should be noted that although the NH_4Cl-induced hypothermia was markedly attenuated in the genetic absence of the TRPA1 channel, it was still present in the KO mice suggesting the contribution of TRPA1-independent mechanisms to the response.

3.5. The hypothermic response to NH_4Cl is attenuated by the pharmacological blockade of the TRPA1 channel in rats

Similarly as in the case of TRPV1, it was important to exclude the potential presence of chronic compensatory mechanisms that may have developed in the absence of the TRPA1 channel in the *Trpa1*$^{-/-}$ mice. For that, we used pharmacological blockade of the channel with a highly potent and selective TRPA1 antagonist, A967079 [53]. However, we

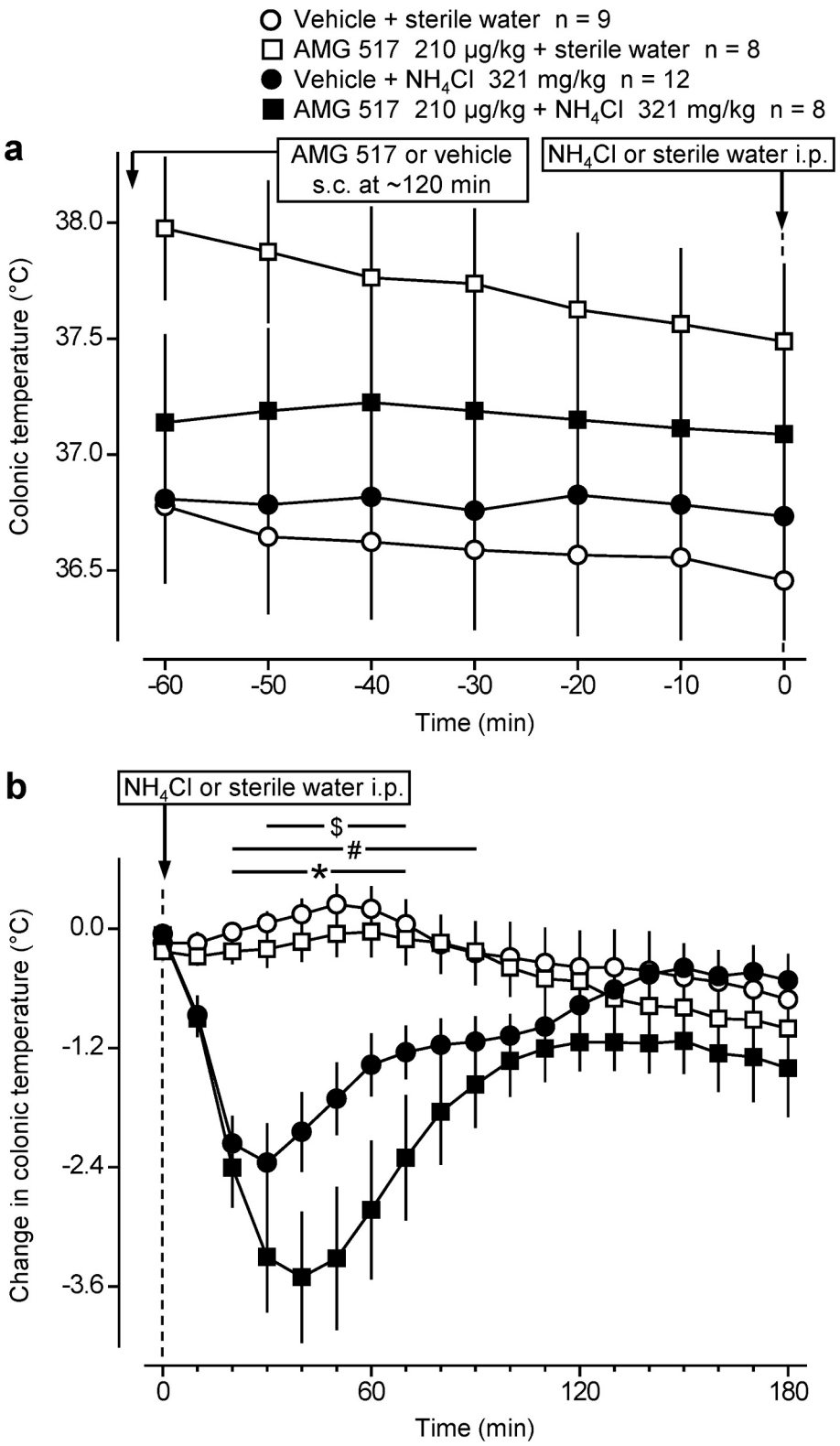

Fig. 3. (a) Colonic temperature responses to s.c. administration of the TRPV1 antagonist AMG 517 (dose indicated) or vehicle in C57BL/6 mice. (b) Changes in deep T_b of the mice in response to i.p. administration of NH_4Cl (dose indicated) or sterile water after s.c. pretreatment at −120 min with AMG 517 (dose indicated) or vehicle. $^{*}p < 0.05$, NH_4Cl vs. sterile water difference in vehicle-pretreated mice; $^{\#}p < 0.05$, NH_4Cl vs. sterile water difference in AMG 517-pretreated mice; $^{\$}p < 0.05$, AMG 517 vs. vehicle pretreatment difference in response to NH_4Cl.

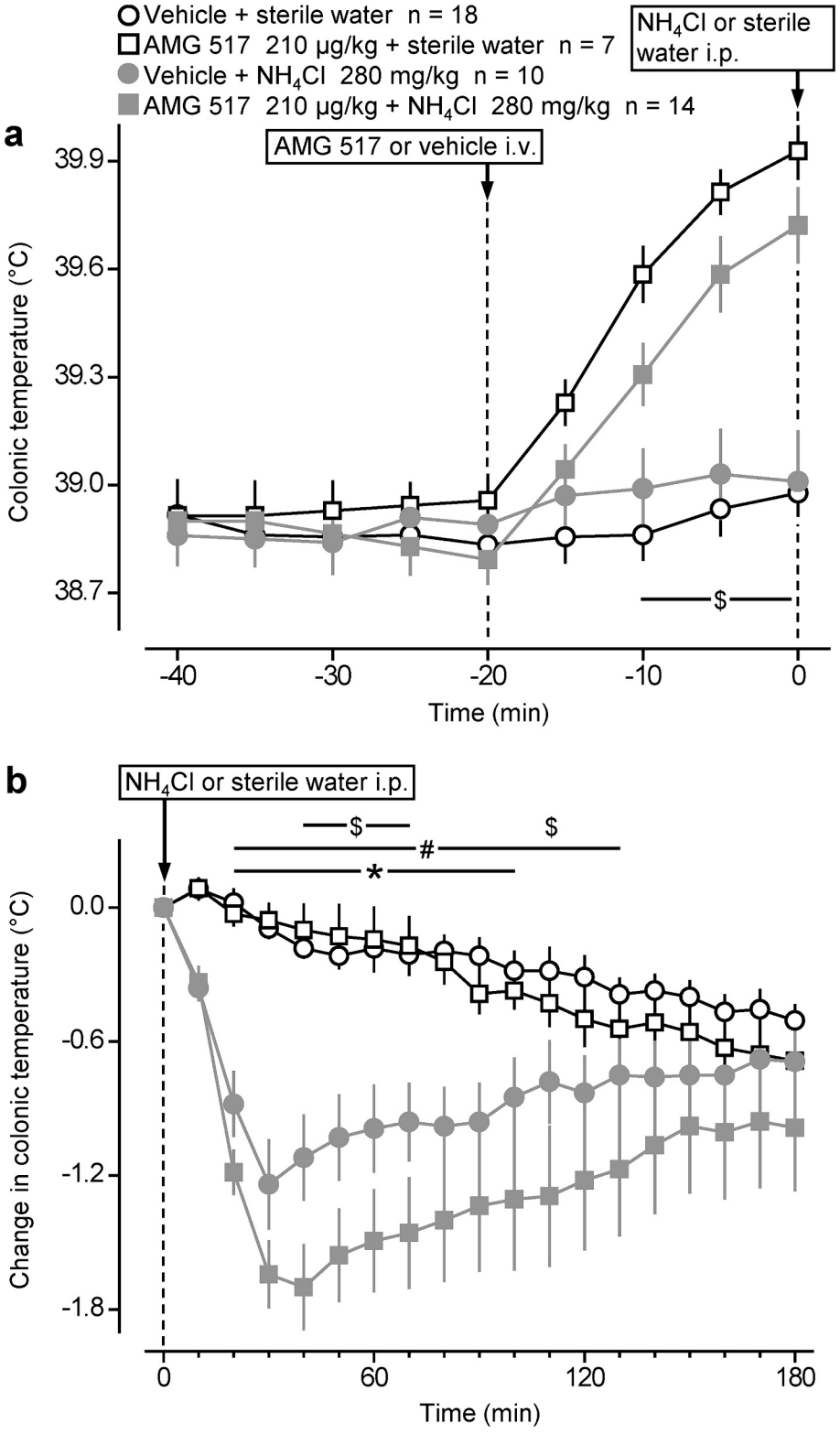

Fig. 4. (a) Colonic temperature responses to i.v. administration of the TRPV1 antagonist AMG517 (dose indicated) or vehicle in rats. $^{\$}p < 0.05$, AMG 517 vs. vehicle pretreatment difference. (b) Changes in deep T_b of the mice in response to i.p. administration of NH_4Cl (dose indicated) or sterile water after i.v. pretreatment at −20 min with AMG 517 (dose indicated) or vehicle. $^{*}p < 0.05$, NH_4Cl vs. sterile water difference in vehicle-pretreated rats; $^{\#}p < 0.05$, NH_4Cl vs. sterile water difference in AMG 517-pretreated rats; $^{\$}p < 0.05$, AMG 517 vs. vehicle pretreatment difference in response to NH_4Cl.

could not use the mouse experimental model that was applied in case of AMG 517, because the half-life of A967079 is relatively short, ca. 49 min [53], thus the efficacy of the drug could have been questionable 2 h after its s.c. administration, when NH_4Cl could be injected to mice (for details, see Table 1). In addition, it was of crucial importance to test the contribution of the TRPA1 channel in another species than mice, because interspecies differences were demonstrated in the temperature-sensitivity of the channel as well as in its response to pharmacological

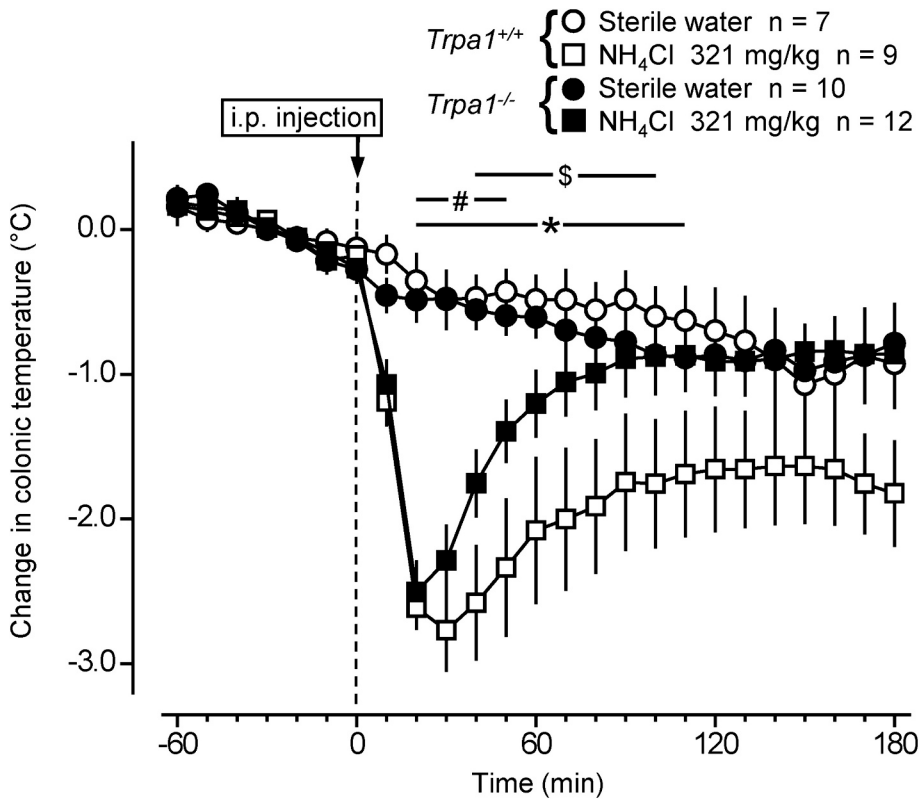

Fig. 5. Deep T_b responses of *Trpa1*$^{+/+}$ and *Trpa1*$^{-/-}$ mice to i.p. administration of NH_4Cl (dose indicated) or sterile water. $^{*}p < 0.05$, NH_4Cl vs. sterile water difference in *Trpa1*$^{+/+}$ mice; $^{\#}p < 0.05$, NH_4Cl vs. sterile water difference in *Trpa1*$^{-/-}$ mice; $^{\$}p < 0.05$, intergenotype difference in response to NH_4Cl.

agents (for review, see [61]). Therefore, we studied the effects of i.v. administered A967079 on NH_4Cl-induced hypothermia in rats with a similar experimental design as in the case of AMG 517 (for details, Table 1). In accordance with previous studies [35,53], A967079 did not have any meaningful effect on the colonic temperature of the rats before the i.p injection of NH_4Cl or sterile water (Fig. 6). On the contrary, when the same dose (5 mg/kg) of A967079 was infused 20 min before the i.p. injection of NH_4Cl, it markedly attenuated the hypothermic response. While the T_b of NH_4Cl-treated rats was significantly ($p < 0.05$) lower at 20–150 min compared to sterile water injection after pretreatment with vehicle, in the A967079-pretreated rats the NH_4Cl-induced hypothermia was significant only between 10 and 90 min (Fig. 6). In accordance, a statistically significant difference was also present between the two pretreatments in the NH_4Cl-treated rats between 40 and 120 min with a maximal difference of 0.7°C at 50 min ($p = 0.003$).

In line with our results with the genetic blockade of TRPA1, the NH_4Cl-induced hypothermia was substantially attenuated, but still present after the pharmacological inhibition of the TRPA1 channel suggesting the contribution of TRPA1-independent mediators to the response.

3.6. I.p. administration of NH_4Cl decreases the blood pH in rats and mice

Last, we wanted to know how the applied doses of NH_4Cl affected the blood pH of the rats and the mice. In rats, the blood pH after i.p. administration of NH_4Cl was decreased at both doses (to 7.47 and 7.24) compared to sterile water treatment (pH = 7.51), however, the difference was significant only at the higher (280 mg/kg) dose ($p < 0.001$) (Fig. 7). The blood pH was also statistically different between two doses of NH_4Cl ($p < 0.001$). In mice, the i.p. injection of NH_4Cl (321 mg/kg) resulted in substantial drop in blood pH in all genotypes compared to sterile water injection (Fig. 7). The fall in the blood pH of the mice reached a similar extent in all genotypes ranging between 7.23 and 7.30 after the i.p. injection of NH_4Cl.

4. Discussion

In the present study, we show that the i.p. administration of NH_4Cl decreases deep T_b in rats and mice. The genetic and pharmacological blockade of the TRPV1 channel exaggerates the hypothermic effect of NH_4Cl. On the contrary, the hypothermic response to NH_4Cl is attenuated by the genetic ablation and pharmacological inhibition of the TRPA1 channel. Nevertheless, the hypothermic response occurred (to a different extent) in all of our experimental models, indicating that *per se* neither TRPA1 nor TRPV1 is essential for NH_4Cl-induced hypothermia. These findings suggest that TRPV1 channels are limiting regulators, whereas TRPA1 channels are potentiating signaling molecules of NH_4Cl-induced hypothermia.

The hypothermic effect of i.p. administered NH_4Cl has been shown in mice long time ago [26]. However, it has remained unknown whether it also develops in other species and which receptors are involved in the NH_4Cl-induced hypothermic response. NH_4Cl administration is often used to induce metabolic acidosis in rodents [12–15]. The mechanism of NH_4Cl-induced metabolic acidosis is consumption of bicarbonate during conversion of ammonia to urea nitrogen via the urea cycle [62]. In severe cases of the NH_4Cl-induced metabolic acidosis, a fall in intracellular pH also developed [19], although the intra- and extracellular pH levels did not always correlate in milder cases of acidosis (extracellular pH drop of 0.11–0.19) [14,19,63]. The different effects on pH can originate from the used species (rat vs. mouse), experimental model (*in vitro* vs. *in vivo*), and acid loading protocol (acute vs. chronic). In our experiments, NH_4Cl decreased the blood pH to ~7.25 in rats and mice, which was markedly lower than in the control animals, however, in this *in vivo* experimental design the intracellular pH could not be measured. The decreased blood pH could potentially serve as a direct mechanism for the development of the hypothermia, for example, through the

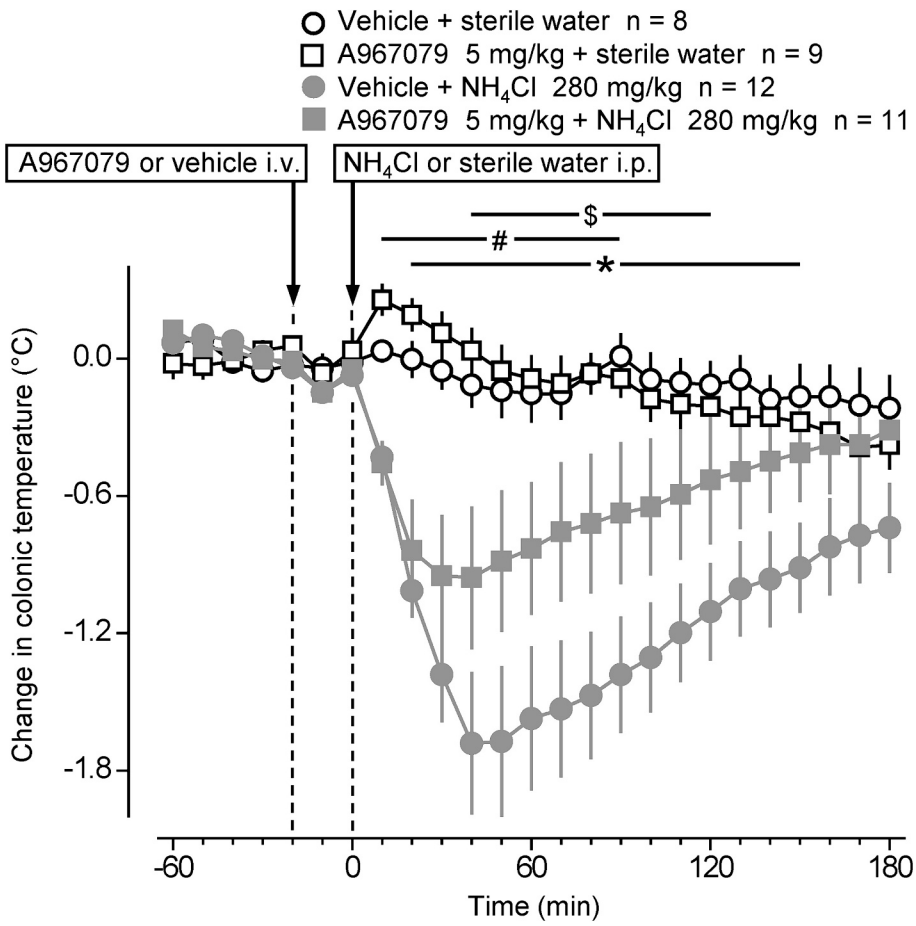

Fig. 6. Changes in deep T_b of rats in response to i.p. administration of NH_4Cl (dose indicated) or sterile water after i.v. pretreatment at −20 min with A967079 (dose indicated) or vehicle. $^{*}p < 0.05$, NH_4Cl vs. sterile water difference in vehicle-pretreated rats; $^{\#}p < 0.05$, NH_4Cl vs. sterile water difference in A967079-pretreated rats; $^{\$}p < 0.05$, A967079 vs. vehicle pretreatment difference in response to NH_4Cl.

stimulation of the TRPV1-mediated acido-antithermogenic and acido-antivasoconstrictor reflexes that originate from trunk muscles and limit the increase in deep T_b during physical exercise (for review, see [64]). However, since the NH_4Cl-induced hypothermia was still detectable after genetic and pharmacological blockade of both the TRPV1 and the TRPA1 channels, it cannot be excluded that the hypothermic response to the decreased blood pH involved TRPV1- and TRPA1-independent mechanisms. Several acid-sensitive ion channels, other than TRPV1 and TRPA1, are expressed on primary sensory neurons, which are involved in a number of physiological and pathophysiological reactions to acidosis (for review, see [65]). Whether any of these acid sensors contribute to the development of NH_4Cl-induced hypothermia remains subject for future studies.

It is also possible, however, that the hypothermic effect of NH_4Cl was triggered by mechanisms that are not related solely to the decreased blood pH, because the lower dose of NH_4Cl caused hypothermia in rats, but it did not influence the blood pH significantly, which argues against a direct acid-induced effect. Unfortunately, the pH of the mice was not reported in the study by Gordon [26]. It should be also noted that although the acidosis clearly developed in the rats (at the higher dose) as well as in the mice of all genotypes in our experiments, the extent of decrease in blood pH was not very severe. The drop in blood pH was probably not sufficient to reach the proton activation threshold of the TRPV1 channel, because the half-maximal response of the rat TRPV1 channel to protons occurs at a pH of ~5.78 *in vitro* [66]. However, the elevated proton concentrations could potentiate responses evoked by other agonists, resulting in increased activity of the channel [43].

With regards to the TRPA1 channel, the pH activation is complex. It was demonstrated that weak acids activate rodent TRPA1, but this was due to intracellular acidosis and direct proton activation of the channel from the cytosolic side [44,67]. Although protons can rapidly permeate through the membrane to induce an intracellular acidosis when the extracellular H^+ concentration is increased [68], rodent TRPA1 failed to respond to extracellular acidosis, and protons even inhibited the channel in a later study [41]. Nevertheless, exclusively in humans, TRPA1 is an important (nociceptive) proton sensor [41], which complicates the translation of results from animal experiments to humans. Moreover, in case of NH_4Cl administration the intracellular pH level does not always correlate with the extracellular pH and an increased pH within the cell was also described instead of acidosis (for review, see [69]). Ammonia and intracellular alkalization were shown to activate both the TRPV1 and the TRPA1 channels [39,40]. Hence, the rapid inward diffusion of gaseous ammonia and the resulting intracellular alkalization could have also contributed to our results. It is known that systemic (i.v.) administration of NH_4Cl leads to formation of ammonia, which can readily cross the blood-brain barrier [70]. This raises the possibility that activation of TRPA1 channels in thermoregulatory neurons in the brain triggered the hypothermic response, as shown earlier in case of another gasotransmitter, hydrogen sulfide [37], but the investigation of the exact molecular interaction between NH_4Cl and TRP channels, as well as finding the site of the thermoregulatory action of NH_4Cl were beyond the scope of the present study and remain subjects for future research.

The exaggeration of NH_4Cl-induced hypothermia by the blockade of TRPV1 channels was an unexpected novel finding. However, it should be mentioned that it was shown earlier that inhibition of TRPV1 channels potentiated the neuronal responses to NH_4^+ [71]. We can only speculate

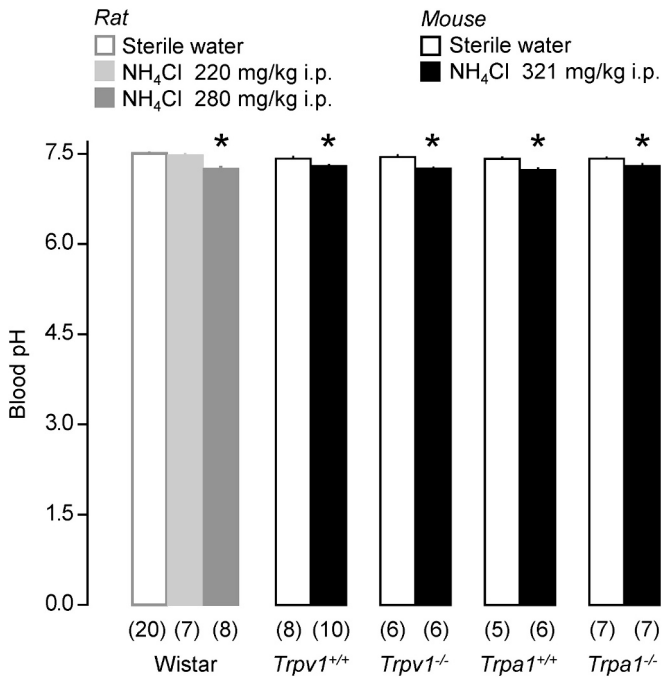

Fig. 7. Blood pH of rats and mice of different genotypes (*Trpv1*$^{+/+}$, *Trpv1*$^{-/-}$, *Trpa1*$^{+/+}$, and *Trpa1*$^{-/-}$) one hour after the i.p. administration of NH_4Cl (doses indicated) or sterile water. Numbers in parentheses indicate the number of animals in the corresponding groups. *$p < 0.05$, NH_4Cl vs. sterile water difference within the same genotype.

that NH_4^+ ions could inhibit TRPV1 channels located in the abdominal wall, which were shown to tonically suppress skin vasoconstriction and thermogenesis [72]. When these TRPV1 channels are blocked with genetic or pharmacological tools the hypothermia-counteracting effect is absent, hence the thermal response to NH_4Cl becomes augmented. In support of this assumption, it was found that different quaternary ammonium ions blocked the TRPV1 channel from the intracellular surface with the bigger molecules becoming slower blockers [73]. As an alternative theory, we showed earlier that acidosis-induced vasodilation of rat and mouse tail arteries are limited by non-neuronal TRPV1 channels in the vascular wall [74], thus it can be assumed that the blockade of these vasodilation-limiting TRPV1 channels could result in higher heat loss and exaggerated hypothermic response to NH_4Cl. It has to be noted, however, that the abovementioned action mechanisms of NH_4Cl on TRPV1 channels remain hypothetical until directly tested in future experiments. As another limitation of our study, it should be mentioned that we used only male animals in our experiments, hence sex differences in the thermal response to NH_4Cl could not be investigated. Nevertheless, our results can also serve as an encouraging basis for designing future studies, which are required to determine the potential presence of sex differences in the development of NH_4Cl-induced hypothermia and its relation to TRPA1 and TRPV1 channels.

In conclusion, TRPA1 channels contribute to the development of NH_4Cl-induced hypothermia in mice and rats, whereas TRPV1 channels play a limiting function, although in itself neither of them is essential for the occurrence of the response. It can be hypothesized that activation of TRPA1 channels in thermoregulation-related brain nuclei are responsible for the induction of NH_4Cl-induced hypothermia, while TRPV1 channels on the periphery, possibly in abdominal muscles or vascular smooth muscle exert a limiting effect on the response. Our findings highlight the importance of the nonthermal activation of TRPV1 and TRPA1 channels in thermoregulation. Since NH_4Cl is still used in clinical practice (*e.g.*, as a diuretic, expectorant, and perhaps COVID-19 treatment), the present results warrant for regular T_b control and careful consideration of the treatment, especially in patients with TRPA1 and TRPV1 channel function disorders.

Funding

This work was supported by the National Research, Development and Innovation Office (FK 138722 to A.G.) and the New National Excellence Program of the Hungarian Ministry for Innovation and Technology (UNKP-23-2-I-PTE-1938 to B.G. and UNKP-23-3-I-PTE-2000 to K.F.). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CRediT authorship contribution statement

Zoltan Rumbus: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kata Fekete:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Leonardo Kelava:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Bibor Gardos:** Writing – review & editing, Investigation, Formal analysis. **Krisztian Klonfar:** Formal analysis, Investigation, Writing – review & editing. **Patrik Keringer:** Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. **Erika Pinter:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Resources, Writing – review & editing. **Eszter Pakai:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation. **Andras Garami:** Investigation, Funding acquisition, Formal analysis, Conceptualization, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analyzed during this study are included in this published article.

Acknowledgements

The authors thank Aniko Varnagyne Rozsafi, Terez Bagoly, and Victor Nabseth for their excellent technical assistance.

References

[1] A.L. Sellers, E.C. Kast, The treatment of alkalosis with intravenous ammonium chloride solution, Perm. Found. Med. Bull. 3 (1945) 171–174.
[2] W.J. Martin, G.R. Matzke, Treating severe metabolic alkalosis, Clin. Pharm. 1 (1982) 42–48.
[3] S.A. Doxiadis, M.K. Goldfinch, K.S. Holt, Alkalosis in infants; treatment by intravenous infusion of ammonium chloride, Lancet 265 (1953) 801–804.
[4] K.M. Wong, W.L. Chak, C.Y. Cheung, Y.H. Chan, K.S. Choi, K.F. Chau, et al., Hypokalemic metabolic acidosis attributed to cough mixture abuse, Am. J. Kidney Dis. 38 (2001) 390–394.
[5] S. MacRury, R. Neilson, K. Goodwin, Benylin dependence, metabolic acidosis and hyperglycaemia, Postgrad. Med. J. 63 (1987) 587–588.
[6] F.J. Wood, Ammonium chloride acidosis, Clin. Sci. 14 (1955) 81–89.
[7] A.S. Relman, P.F. Shelburne, A. Talman, Profound acidosis resulting from excessive ammonium chloride in previously healthy subjects. A study of two cases, N. Engl. J. Med. 264 (1961) 848–852.
[8] M.H. Sleisenger, A.S. Freedberg, Ammonium chloride acidosis; a report of six cases, Circulation 3 (1951) 837–845.
[9] Z. Siami, S. Aghajanian, S. Mansouri, Z. Mokhames, R. Pakzad, K. Kabir, et al., Effect of ammonium chloride in addition to standard of care in outpatients and hospitalized COVID-19 patients: a randomized clinical trial, Int. J. Infect. Dis. 108 (2021) 306–308.

[10] N. Baker, A.J. Williams, A. Tropsha, S. Ekins, Repurposing quaternary ammonium compounds as potential treatments for COVID-19, Pharm. Res. 37 (2020) 104.
[11] A.C. Celotto, L.G. Ferreira, V.K. Capellini, A.A. Albuquerque, A.J. Rodrigues, P. R. Evora, Acute but not chronic metabolic acidosis potentiates the acetylcholine-induced reduction in blood pressure: an endothelium-dependent effect, Braz. J. Med. Biol. Res. 49 (2016) e5007.
[12] J. Galicek, F. Seow, J.M. Lingard, The effect of chronic acid/base disturbances on renal amino acid clearances in the rat, Aust. J. Exp. Biol. Med. Sci. 59 (1981) 383–391.
[13] M. Nowik, N.B. Kampik, M. Mihailova, D. Eladari, C.A. Wagner, Induction of metabolic acidosis with ammonium chloride (NH4Cl) in mice and rats—species differences and technical considerations, Cell. Physiol. Biochem. 26 (2010) 1059–1072.
[14] K.F. Rothe, F. Schimek, New aspects of acid-base balance influences of NH4Cl on intra- and extracellular acid-base equilibrium studies in the rat, J. Med. 15 (1984) 135–148.
[15] Z. Rumbus, E. Toth, L. Poto, A. Vincze, G. Veres, L. Czako, et al., Bidirectional relationship between reduced blood pH and acute pancreatitis: a translational study of their noxious combination, Front. Physiol. 9 (2018) 1360.
[16] J. Edge, T. Mundel, H. Pilegaard, E. Hawke, M. Leikis, N. Lopez-Villalobos, et al., Ammonium chloride ingestion attenuates exercise-induced mRNA levels in human muscle, PloS One 10 (2015) e0141317.
[17] G.R. Kleger, M. Turgay, R. Imoberdorf, M.A. McNurlan, P.J. Garlick, P.E. Ballmer, Acute metabolic acidosis decreases muscle protein synthesis but not albumin synthesis in humans, Am. J. Kidney Dis. 38 (2001) 1199–1207.
[18] D. Reaich, S.M. Channon, C.M. Scrimgeour, T.H. Goodship, Ammonium chloride-induced acidosis increases protein breakdown and amino acid oxidation in humans, Am. J. Physiol. 263 (1992) E735–E739.
[19] A. Tizianello, G. De Ferrari, G. Gurreri, N. Acquarone, Effects of metabolic alkalosis, metabolic acidosis and uraemia on whole-body intracellular pH in man, Clin. Sci. Mol. Med. 52 (1977) 125–135.
[20] A. Stauber, T. Radanovic, G. Stange, H. Murer, C.A. Wagner, J. Biber, Regulation of intestinal phosphate transport. II. Metabolic acidosis stimulates Na(+)-dependent phosphate absorption and expression of the Na(+)-P(i) cotransporter NaPi-IIb in small intestine, Am. J. Physiol. Gastrointest. Liver Physiol. 288 (2005) G501–G506.
[21] R. Lane, L. Adams, Metabolic acidosis and breathlessness during exercise and hypercapnia in man, J. Physiol. 461 (1993) 47–61.
[22] L.M. Bento, M.M. Fagian, A.E. Vercesi, J.A. Gontijo, Effects of NH4Cl-induced systemic metabolic acidosis on kidney mitochondrial coupling and calcium transport in rats, Nephrol. Dial. Transplant. 22 (2007) 2817–2823.
[23] A. Jara, A.J. Felsenfeld, J. Bover, C.R. Kleeman, Chronic metabolic acidosis in azotemic rats on a high-phosphate diet halts the progression of renal disease, Kidney Int. 58 (2000) 1023–1032.
[24] R. Daher, N. Ducrot, T. Lefebvre, S. Zineeddine, J. Ausseil, H. Puy, et al., Crosstalk between acidosis and iron metabolism: data from in vivo studies, Metabolites 12 (2022) 89.
[25] F.L. Coe, J.J. Firpo Jr., D.L. Hollandsworth, L. Segil, J.M. Canterbury, E. Reiss, Effect of acute and chronic metabolic acidosis on serum immunoreactive parathyroid hormone in man, Kidney Int. 8 (1975) 263–273.
[26] C.J. Gordon, Thermoregulatory responses in mice following acute administration of principal nitrogenous excretory substances, Pharmacol. Biochem. Behav. 31 (1988) 699–703.
[27] A.A. Romanovsky, M.C. Almeida, A. Garami, A.A. Steiner, M.H. Norman, S. F. Morrison, et al., The transient receptor potential vanilloid-1 channel in thermoregulation: a thermosensor it is not, Pharmacol. Rev. 61 (2009) 228–261.
[28] H. Wang, J. Siemens, TRP ion channels in thermosensation, thermoregulation and metabolism, Temperature (Austin) 2 (2015) 178–187.
[29] D. Bamps, J. Vriens, J. de Hoon, T. Voets, TRP channel cooperation for nociception: therapeutic opportunities, Annu. Rev. Pharmacol. Toxicol. 61 (2021) 655–677.
[30] P. Holzer, Transient receptor potential (TRP) channels as drug targets for diseases of the digestive system, Pharmacol. Ther. 131 (2011) 142–170.
[31] D. Huang, S. Li, A. Dhaka, G.M. Story, Y.Q. Cao, Expression of the transient receptor potential channels TRPV1, TRPA1 and TRPM8 in mouse trigeminal primary afferent neurons innervating the dura, Mol. Pain 8 (2012) 66.
[32] K. Kobayashi, T. Fukuoka, K. Obata, H. Yamanaka, Y. Dai, A. Tokunaga, et al., Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors, J. Comp. Neurol. 493 (2005) 596–606.
[33] D. Julius, TRP channels and pain, Annu. Rev. Cell Dev. Biol. 29 (2013) 355–384.
[34] M. Kashio, M. Tominaga, TRP channels in thermosensation, Curr. Opin. Neurobiol. 75 (2022) 102591.
[35] C. de Oliveira, A. Garami, S.G. Lehto, E. Pakai, V. Tekus, K. Pohoczky, et al., Transient receptor potential channel ankyrin-1 is not a cold sensor for autonomic thermoregulation in rodents, J. Neurosci. 34 (2014) 4445–4452.
[36] A.A. Steiner, V.F. Turek, M.C. Almeida, J.J. Burmeister, D.L. Oliveira, J.L. Roberts, et al., Nonthermal activation of transient receptor potential vanilloid-1 channels in abdominal viscera tonically inhibits autonomic cold-defense effectors, J. Neurosci. 27 (2007) 7459–7468.
[37] E. Olah, Z. Rumbus, V. Kormos, V. Tekus, E. Pakai, H.V. Wilson, et al., The hypothermic effect of hydrogen sulfide is mediated by the transient receptor potential ankyrin-1 channel in mice, Pharmaceuticals (Basel) 14 (2021) 992.
[38] A. Garami, E. Pakai, H.A. McDonald, R.M. Reilly, A. Gomtsyan, J.J. Corrigan, et al., TRPV1 antagonists that cause hypothermia, instead of hyperthermia, in rodents: compounds' pharmacological profiles, in vivo targets, thermoeffectors recruited and implications for drug development, Acta Physiol. (Oxf.) 223 (2018) e13038.
[39] A. Dhaka, V. Uzzell, A.E. Dubin, J. Mathur, M. Petrus, M. Bandell, et al., TRPV1 is activated by both acidic and basic pH, J. Neurosci. 29 (2009) 153–158.
[40] F. Fujita, K. Uchida, T. Moriyama, A. Shima, K. Shibasaki, H. Inada, et al., Intracellular alkalization causes pain sensation through activation of TRPA1 in mice, J. Clin. Invest. 118 (2008) 4049–4057.
[41] J. de la Roche, M.J. Eberhardt, A.B. Klinger, N. Stanslowsky, F. Wegner, W. Koppert, et al., The molecular basis for species-specific activation of human TRPA1 protein by protons involves poorly conserved residues within transmembrane domains 5 and 6, J. Biol. Chem. 288 (2013) 20280–20292.
[42] S.E. Jordt, M. Tominaga, D. Julius, Acid potentiation of the capsaicin receptor determined by a key extracellular site, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8134–8139.
[43] M. Tominaga, M.J. Caterina, A.B. Malmberg, T.A. Rosen, H. Gilbert, K. Skinner, et al., The cloned capsaicin receptor integrates multiple pain-producing stimuli, Neuron 21 (1998) 531–543.
[44] Y.Y. Wang, R.B. Chang, S.D. Allgood, W.L. Silver, E.R. Liman, A TRPA1-dependent mechanism for the pungent sensation of weak acids, J. Gen. Physiol. 137 (2011) 493–505.
[45] K. Bolcskei, Z. Helyes, A. Szabo, K. Sandor, K. Elekes, J. Nemeth, et al., Investigation of the role of TRPV1 receptors in acute and chronic nociceptive processes using gene-deficient mice, Pain 117 (2005) 368–376.
[46] A. Garami, M. Ibrahim, K. Gilbraith, R. Khanna, E. Pakai, A. Miko, et al., Transient receptor potential vanilloid 1 antagonists prevent anesthesia-induced hypothermia and decrease postincisional opioid dose requirements in rodents, Anesthesiology 127 (2017) 813–823.
[47] A. Garami, E. Pakai, D.L. Oliveira, A.A. Steiner, S.P. Wanner, M.C. Almeida, et al., Thermoregulatory phenotype of the Trpv1 knockout mouse: thermoeffector dysbalance with hyperkinesis, J. Neurosci. 31 (2011) 1721–1733.
[48] A.A. Romanovsky, A.I. Ivanov, Y.P. Shimansky, Selected contribution: ambient temperature for experiments in rats: a new method for determining the zone of thermal neutrality, J. Appl. Physiol. 2002 (92) (1985) 2667–2679.
[49] P. Keringer, N. Furedi, B. Gaszner, A. Miko, E. Pakai, K. Fekete, et al., The hyperthermic effect of central cholecystokinin is mediated by the cyclooxygenase-2 pathway, Am. J. Physiol. Endocrinol. Metab. 322 (2022) E10–E23.
[50] A.A. Romanovsky, M.C. Almeida, D.M. Aronoff, A.I. Ivanov, J.P. Konsman, A. A. Steiner, et al., Fever and hypothermia in systemic inflammation: recent discoveries and revisions, Front. Biosci. 10 (2005) 2193–2216.
[51] E. Pakai, V. Tekus, C. Zsiboras, Z. Rumbus, E. Olah, P. Keringer, et al., The neurokinin-1 receptor contributes to the early phase of lipopolysaccharide-induced fever via stimulation of peripheral cyclooxygenase-2 protein expression in mice, Front. Immunol. 9 (2018) 166.
[52] N.R. Gavva, J.J. Treanor, A. Garami, L. Fang, S. Surapaneni, A. Akrami, et al., Pharmacological blockade of the vanilloid receptor TRPV1 elicits marked hyperthermia in humans, Pain 136 (2008) 202–210.
[53] J. Chen, S.K. Joshi, S. DiDomenico, R.J. Perner, J.P. Mikusa, D.M. Gauvin, et al., Selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or body temperature regulation, Pain 152 (2011) 1165–1172.
[54] A. Garami, Y.P. Shimansky, E. Pakai, D.L. Oliveira, N.R. Gavva, A.A. Romanovsky, Contributions of different modes of TRPV1 activation to TRPV1 antagonist-induced hyperthermia, J. Neurosci. 30 (2010) 1435–1440.
[55] S. McGaraughty, K.L. Chu, R.J. Perner, S. Didomenico, M.E. Kort, P.R. Kym, TRPA1 modulation of spontaneous and mechanically evoked firing of spinal neurons in uninjured, osteoarthritic, and inflamed rats, Mol. Pain 6 (2010) 14.
[56] S.P. Wanner, A. Garami, E. Pakai, D.L. Oliveira, N.R. Gavva, C.C. Coimbra, et al., Aging reverses the role of the transient receptor potential vanilloid-1 channel in systemic inflammation from anti-inflammatory to proinflammatory, Cell Cycle 11 (2012) 343–349.
[57] E.M. Doherty, C. Fotsch, A.W. Bannon, Y. Bo, N. Chen, C. Dominguez, et al., Novel vanilloid receptor-1 antagonists: 2. Structure-activity relationships of 4-oxopyrimidines leading to the selection of a clinical candidate, J. Med. Chem. 50 (2007) 3515–3527.
[58] E.S. Fernandes, M.A. Fernandes, J.E. Keeble, The functions of TRPA1 and TRPV1: moving away from sensory nerves, Br. J. Pharmacol. 166 (2012) 510–521.
[59] M.J. Patil, N.A. Jeske, A.N. Akopian, Transient receptor potential V1 regulates activation and modulation of transient receptor potential A1 by Ca2+, Neuroscience 171 (2010) 1109–1119.
[60] A. Staruschenko, N.A. Jeske, A.N. Akopian, Contribution of TRPV1-TRPA1 interaction to the single channel properties of the TRPA1 channel, J. Biol. Chem. 285 (2010) 15167–15177.
[61] J. Chen, D.H. Hackos, TRPA1 as a drug target—promise and challenges, Naunyn Schmiedebergs Arch. Pharmacol. 388 (2015) 451–463.
[62] S. Matsumoto, J. Haberle, J. Kido, H. Mitsubuchi, F. Endo, K. Nakamura, Urea cycle disorders-update, J. Hum. Genet. 64 (2019) 833–847.
[63] J.I. Shapiro, M. Whalen, R. Kucera, N. Kindig, G. Filley, L. Chan, Brain pH responses to sodium bicarbonate and Carbicarb during systemic acidosis, Am. J. Physiol. 256 (1989) H1316–H1321.
[64] A. Garami, Y.P. Shimansky, Z. Rumbus, R.C.L. Vizin, N. Farkas, J. Hegyi, et al., Hyperthermia induced by transient receptor potential vanilloid-1 (TRPV1) antagonists in human clinical trials: insights from mathematical modeling and meta-analysis, Pharmacol. Ther. 208 (2020) 107474.
[65] P. Holzer, Acid-sensitive ion channels and receptors, Handb. Exp. Pharmacol. (2009) 283–332.
[66] P. McIntyre, L.M. McLatchie, A. Chambers, E. Phillips, M. Clarke, J. Savidge, et al., Pharmacological differences between the human and rat vanilloid receptor 1 (VR1), Br. J. Pharmacol. 132 (2001) 1084–1094.

[67] Y.Y. Wang, R.B. Chang, E.R. Liman, TRPA1 is a component of the nociceptive response to CO2, J. Neurosci. 30 (2010) 12958–12963.
[68] D.A. Andersson, H.W. Chase, S. Bevan, TRPM8 activation by menthol, icilin, and cold is differentially modulated by intracellular pH, J. Neurosci. 24 (2004) 5364–5369.
[69] A. Roos, W.F. Boron, Intracellular pH, Physiol. Rev. 61 (1981) 296–434.
[70] S.I. Rapoport, H.K. Thompson, Effect of intravenous NH4Cl and NaHCO3 on the pH of the brain surface, as related to respiration and the blood-brain barrier, Exp. Neurol. 42 (1974) 320–333.
[71] S. Balakrishnan, S.L. Mironov, Instant activation of TRP channels by NH(4) (+) promotes neuronal bursting and glutamate spikes in CA1 neurons, Curr Res Physiol 3 (2020) 20–29.
[72] A. Garami, A.A. Steiner, E. Pakai, S.P. Wanner, M.C. Almeida, P. Keringer, et al., The neural pathway of the hyperthermic response to antagonists of the transient receptor potential vanilloid-1 channel, Temperature (Austin) 10 (2023) 136–154.
[73] A. Jara-Oseguera, I. Llorente, T. Rosenbaum, L.D. Islas, Properties of the inner pore region of TRPV1 channels revealed by block with quaternary ammoniums, J. Gen. Physiol. 132 (2008) 547–562.
[74] I. Ivic, M. Solymar, E. Pakai, Z. Rumbus, E. Pinter, A. Koller, et al., Transient receptor potential vanilloid-1 channels contribute to the regulation of acid- and base-induced vasomotor responses, J. Vasc. Res. 53 (2016) 279–290.