Predictive role of vital parameters for the outcomes of systemic inflammation and the role of TRP channels in pharmacological modulation of body temperature

Doctoral (PhD) thesis

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I. Introduction

I/1. Impact of body temperature and blood pH on outcomes of systemic inflammation

I/1.1. Sepsis and acute pancreatitis: two distinct manifestations of systemic inflammation

Systemic inflammation is a complex, generalized pathophysiological process, which can be clinically manifested in numerous forms [1]. This response describes an inflammatory process independent of its cause, and can result from infections, as well as from noninfectious causes. When the underlying origin of the systemic inflammatory response is infection, it is termed sepsis. Based on the latest consensus [2], it is defined as evidence of infection plus life-threatening organ dysfunction. Acute pancreatitis (AP) is initially a sterile inflammation of the pancreas most commonly triggered by noninfectious causes such as bile stones or excessive use of alcohol.

I/1.2. Association between body temperature and mortality in sepsis

Sepsis is often associated with changes of deep body temperature (T_b). In fact, many diagnostic and prognostic scoring systems (e.g., APACHE II, PIRO, SAPS II, SIRS) of sepsis, include an abnormal deviation of T_b from the normal range [1-5]. Based on experimental data from animal studies, Romanovksy and colleagues [6, 7] proposed that hypothermia and fever can both develop as two distinct adaptive mechanisms in sickness syndrome. The latter characteristically occurs at the beginning of an infection, while the

former is usually associated with progressed stage or severity of the disease [6, 7]. The two adaptive strategies can develop sequentially as the severity of the disease progresses [7], but hypothermia can be also one of the initial developing events in animal models of endotoxin shock [8], moreover, septic patients admitted to intensive care unit develop hypothermia more frequently in the early than in the late stages of their stay [8]. Despite the distinct pathological background, both fever and hypothermia are evaluated commonly as equally severe signs in the clinical praxis [9]. The definite association of T_b and mortality rate in a large study population has remained unknown.

I/1.3. Blood pH and the outcomes of acute pancreatitis

The inflammatory response is accompanied by changes in vital parameters, including T_b, heart rate, and respiratory rate. The changes in blood pH are also common in systemic inflammation [10]. Changes in acid-base balance significantly alter the release of inflammatory mediators which can affect the outcome of systemic inflammation. Multiple mechanisms have been implicated in AP which can lead to metabolic acidosis (MA), including direct mechanisms [11], as well as indirect ones [12]. Acidosis is often considered as a marker of disease severity, viz., a by-product of systemic dysregulation, and as such it is a proven prognostic factor in the assessment of critically ill patients [13]. Despite the fact that scoring systems, which are used to help the diagnosis and the assessment of the progression of AP, include the changes in systemic pH balance of the patients (e.g., Acute Physiology and Chronic Health Evaluation, APACHE II and Ranson scores), clinical trials aiming to reveal a correlation between the acid-base status and the outcome of AP are scarce.

I/2. Transient receptor potential (TRP) channels in pharmacological modulation of body temperature

I/2.1. The thermal effects of TRP vanilloid-1 (V1) antagonists

In the 1990s, during the in-vivo testing of TRPV1 antagonists, an unexpected adverse effect on T_b, hyperthermia, were repeatedly observed in animal studies and human clinical trials alike. Intriguingly, some TRPV1 antagonists (e.g., A-1165901, A-425619, AMG7905, and AMG8562) cause hypothermia instead of hyperthermia [14-16]. And yet other compounds appear to affect Tb regulation in a species-specific fashion.

I/2.2. Ammonium chloride-induced hypothermia

Ammonium chloride (NH_4Cl) is a systemic and urinary acidifying agent that can be used in the treatment of metabolic alkalosis [17-19]. The oral and/or parenteral administration of NH_4Cl is often used to induce systemic (extracellular) acidosis in animal models [20-24] and in human experiments [25-28]. In 1988, Gordon showed that the systemic administration of NH_4Cl leads to hypothermia in mice [29]. However, the molecular mediators of the NH_4Cl-induced hypothermia have remained largely unknown.

TRPV1 and TRP ankyrin-1 (A1) channels are temperature-sensitive members of the TRP channel family [30, 31]. In addition to thermal signals, they can be both activated by ligand agonists and by changes in pH [32, 33], especially on primary afferent neurons where

they are often co-expressed [34, 35]. 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 T_b [14, 36]. Interestingly, both channels can be activated by NH_4Cl [37, 38] and also by low pH [39-42]. 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.

II. Aims

The goal of the current work is to investigate the predictive role of vital parameters for the outcomes of systemic inflammation in human patients as well as to identify a potential pharmacological target for the modulation of T_b in animal experiments and human patients. The following main topics will be discussed in this thesis:

1. We studied the role of changes in Tb and blood pH as vital signs in the prediction of outcomes in two different manifestations of systemic inflammation (in sepsis and in AP) with meta-analysis. Regarding fever, different clinical trials came to controversial results in sepsis, while clinical trials aiming to reveal a correlation between the acid-base status and the outcomes of AP are scarce. We hypothesized that the increase and the decrease of T_b or blood pH from the normal range predicts the clinical outcomes in systemic inflammation differently.
2. Based on animal studies, nonthermal activation by protons is involved in thermoregulatory responses to TRPV1 antagonists. Therefore, we also aimed to

examine the thermal effect of TRPV1 antagonists in humans using meta-analysis of human trials.

3. Previously, it has been shown that NH_4Cl causes hypothermia in mice. In order to exclude the possibility that the NH_4Cl-induced hypothermic response is specific only to mice, we aimed to detect NH_4Cl-induced hypothermia in rats as well. Although TRPV1 and TRPA1 channels can be activated by NH_4Cl, the contribution of these channels in the thermal response to NH_4Cl has not been investigated yet. Thus, we studied the role of TRPV1 and TRPA1 channels in NH_4Cl-induced hypothermia using genetic deletion and pharmacological blockade of either channel in rodents.

III. Materials and Methods

III/1. Predictive role of body temperature and blood pH for the outcomes of systemic inflammation in humans

III/1.1. Body temperature and mortality in sepsis

III/1.1.1. Search strategy, study selection and data extraction

A search of the PubMed, EMBASE, and Cochrane Controlled Trials Registry databases was performed. The analysis was based on the Participants, Intervention (prognostic factor), Comparison, Outcome (PICO) model: in septic population, we aimed to assess the predictive role of T_b deviations on the mortality ratio. We included studies in which both the T_b values and the mortality ratios were reported for the same group(s) of

patients with systemic inflammation accompanied by suspected or confirmed blood infection. From all included articles we extracted the sample size, the reported mean T_b value of the patients with its standard error (SE), and the mortality ratio within the group during 28–30 days in most cases.

III/1.1.2. Statistical analysis

We have used event rates (mortality rates) as effect size data. Between-study heterogeneity was tested with Q homogeneity test and with I^2 statistical test. We applied the random effect model in our forest plot and meta-regression analyses. Publication bias was tested by inspecting the funnel plot. Meta-regression was performed to assess the overall effect of T_b to mortality.

III/1.2. Blood pH and outcomes of acute pancreatitis

III/1.2.1. Search strategy, study selection and data extraction

Our meta-analysis was based on the PICO model: in patients with AP, we aimed to assess the predictive role of the change in pH on disease severity, length of hospital stay (LOS), and mortality ratio. A search in the PubMed, EMBASE, and Cochrane Controlled Trials Registry databases was. We restricted our search to original human studies published in English without time period limitations. We included studies in which blood pH or a related parameter (e.g., base excess, base deficit, or bicarbonate) and severity scores or LOS or mortality ratios were reported for the same group(s) of patients with AP. From all included

articles we extracted the sample size, the reported mean pH or its related parameter for the studied patient groups with the corresponding SE or deviation, as well as the severity score, LOS, and mortality ratio within the group. To analyze the influence of the change in acid-base status on the severity and the outcome of AP, in each study we assigned the patient groups as a lower pH group and as a higher pH group, irrespective from the original basis for grouping used by the authors of the study. We used mortality ratio of the AP patients groups as the primary outcome. Regarding secondary outcomes, we used two commonly applied severity indices (i.e., APACHE II and Ranson scores) and the LOS.

III/1.2.2. Statistical analysis

We used logit transformation of event rates for mortality ratios and standardized mean difference (SMD) for LOS and severity scores as the effect size data. The secondary outcomes were compared between the lower and higher pH groups (see above) within each study, and then the estimated pooled mean values were calculated. The relevant studies were compared with standard meta-analysis tools (e.g., forest plot) in case of each outcome. Between-study heterogeneity was assessed similarly to our earlier meta-analysis [43]. Publication bias was assessed by funnel-plot analysis, Egger's test and Duval and Tweedie trim and fill method. We performed meta-regression analysis of those studies in which both blood pH and mortality rate were reported within the same patient group.

III/2. The role of TRP channels in pharmacological modulation of body temperature

III/2.1. Relationship between body temperature and pH: TRPV1 antagonists induced thermal changes

III/2.1.1. Search strategy, study selection and data extraction

We used standard meta-analysis tools, in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis Protocols [44]. We included the studies that reported deep T_b values in both TRPV1 antagonist-treated and placebo groups at least at two time points: (i) at shortly before the drug (or placebo) administration and (ii) at 3 h after the time of administration. For each included study, we calculated the change in deep T_b as a difference between the T_b values at 3 h after the drug (placebo) administration vs. at the time of administration (0 h). We then calculated the difference between the T_b changes induced by a drug and those induced by placebo (difference in means); we considered the latter difference to represent the thermal effect of the drug in our meta-analysis.

III/2.1.2. Statistical analysis

For all doses, the differences in means were standardized (based on variances) to obtain standardized differences in means (SDMs). The SDMs with 95% confidence intervals

(CIs) were used as primary measures of effect size and are presented as a “forest plot”. We considered each TRPV1 antagonist to be either mode-nonselective or mode-selective.

III/2.2. The roles of TRPV1 and TRPA1 in NH_4Cl-induced hypothermia in rodents

III/2.2.1. Animals

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 WT) a homozygous targeted mutation in the *Trpv1* gene (KO: n = 21; WT: n = 14) or in the *Trpa1* gene (KO: n = 24; WT: n = 16) were also obtained from the Laboratory Animal Centre of the University of Pecs. Animals were extensively habituated to the experimental setup, as described elsewhere [45, 46].

III/2.2.2. Surgeries

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.

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. 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 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.

III/2.2.3. Thermocouple thermometry

The mice and the rats were placed in cylindrical confiners and equipped with copper-constantan thermocouples 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 connected to a computer. Animals in their confiners were then placed into a biochemistry incubator. 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.

III/2.2.4. Drugs and drug administration

For the i.p. administration of NH_4Cl to mice, the working solution (32.1 mg/ml) was infused (10 ml/kg) over 16 minutes 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 minutes to deliver NH_4Cl at 220 and 280 mg/kg (ca. 4 and 5 mmol/kg), respectively. Control animals were infused with sterile water. 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 minutes 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 minutes before the i.p. infusion of NH_4Cl. Control rats were infused with the vehicle of the antagonist of interest. In mice, the working solution (or its vehicle) was injected subcutaneous (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 minutes before the administration of NH_4Cl). Then, the mice were allowed to accommodate to the experimental conditions for ~2 hours before they received the i.p. infusion of NH_4Cl (321 mg/kg).

III/2.2.5. Blood pH measurements

One hour after the i.p. administration of NH_4Cl, the animals were anesthetized with 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 within 1 minute after collection.

III/2.2.6. 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 [47]. Sigmaplot 11.0 software was used for statistical analyses. Differences were considered significant when $p < 0.05$. Data are presented as mean ± SE.

IV. Results

IV/1. Predictive role of body temperature and blood pH for the outcomes of systemic inflammation in humans

IV/1.1. Body temperature and mortality in sepsis

Until February 29, 2016, 42 full-text publications were found eligible for statistical analysis which included data from a total of 10,834 septic patients. First, we performed a common (Pearson) correlation analysis between T_b and mortality rate of all septic patients. A weak negative linear correlation was found. This method, however, did not allow us to weight the collected data according to the size of the studied populations, thus a detailed meta-analysis was needed.

We investigated the incidence of mortality in fever associated with sepsis. 40 groups of septic patients could be separated and included in the analysis with the random effect model. The meta-analysis of the mortality rates in the septic patients with fever revealed an

average event rate of 22.2%. This percentage was significantly lower than the 50% chance of mortality, which could be regarded as a random outcome.

Next, we analyzed the mortality ratios of normothermic patients. We found that the average mortality ratio was 31.2%, which was higher than in the fever group. The mortality rate was significantly lower than 50% in this study population.

Then, we examined the incidence of mortality in hypothermic septic patients. The random effect model revealed that the average mortality rate was the highest, 47.3% in the hypothermic patients, which did not significantly differ from the 50% random chance.

As a further statistical approach, we also performed a meta-regression analysis. We found a significant negative linear correlation between T_b and mortality rate.

Last, we divided the patients into quartiles (Q1-Q4) of mortality and calculated the average T_b for each mortality quartile. The weighted average T_bs indicate that in sepsis a higher T_b is associated with better outcome, while a lower T_b is related with higher risk of mortality.

IV/1.2. Blood pH and outcomes in acute pancreatitis

Until January 2017 13 full-text publications were found eligible for statistical analysis which included data from a total of 2,311 patients [12, 48-59]. First, we investigated the association between systemic (blood) pH status and mortality. Our meta-analysis revealed a logit event rate of -0.09, corresponding to an average mortality rate of 51.0% in the more acidotic patient groups, while in the patient groups with higher pH or bicarbonate level the logit event rate was -3.68, which corresponds to an average mortality rate of 3.0%.

The mortality ratios were significantly different between the two groups. We also performed a meta-regression analysis on the collected data. We found a significant correlation between pH and mortality rate.

Next, we analyzed the LOS in patients with AP by using the same grouping of acid-base status as we did for mortality and severity scores. We found that this difference was significant between the more acidotic patient groups and the groups with higher pH or bicarbonate concentrations, which difference corresponds to 15.05 days longer LOS in the more acidotic AP patient group.

We also studied the association between blood pH and clinical severity scores. Meta-analysis revealed that the estimated standardized mean differences of the Ranson score and the APACHE II score were significant between the patient groups with lower pH or bicarbonate levels compared with less acidotic groups of patients. These standardized values correspond to 1.60 higher Ranson score and 7.40 higher APACHE II score in the more acidotic patients with AP.

IV/2. The role of TRP channels in pharmacological modulation of body temperature

IV/2.1. Relationship between body temperature and pH: TRPV1 antagonists induced thermal changes. Meta-analysis of human clinical trials

All three antagonists in the mode-nonselective group caused hyperthermia, which was dose-dependent for those compounds that were administered at multiple doses.

NEO6860, the only mode-selective TRPV1 antagonist, did not cause hyperthermia at the dose used (1.2 mmol) but, instead, decreased the deep T_b.

IV/2.2. NH_4Cl-induced hypothermia is attenuated by transient receptor potential channel vanilloid-1, but augmented by ankyrin-1 in rodents

IV/2.2.1. 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. Statistically, both doses of NH_4Cl had a significant effect as compared to controls, and a statistical difference was also present between the lower and the higher dose groups. At 220 mg/kg, the NH_4Cl-induced decrease in deep T_b was significant compared to the control group between 20-50 min, while at 280 mg/kg, the T_b was significantly lower than in controls between 20-90 min. The development of hypothermia in response to NH_4Cl in rats is a novel finding of our study.

IV/2.2.2. NH_4Cl-induced hypothermia is augmented in mice genetically lacking the TRPV1 channel

We also 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. In our experiments

we infused the mice i.p. with 321 mg/kg of NH_4Cl. As expected, at this dose NH_4Cl caused a sudden drop in the colonic temperature of *Trpv1*$^{+/+}$ mice. In Trpv1$^{-/-}$ mice, NH_4Cl also caused hypothermia compared to sterile water, which was significant between 20-70 min. Interestingly, however, the hypothermic response to NH_4Cl was much more pronounced in the *Trpv1*$^{-/-}$ mice than in their *Trpv1*$^{+/+}$ littermates. The intergenotype difference was significant between 20-60 min post-NH_4Cl administration with a maximum of ~2.0°C difference between the groups at 40 min.

IV/2.2.3. Pharmacological blockade of the TRPV1 channel exaggerates NH_4Cl-induced hypothermia in mice and rats

Next, to avoid the potential development of chronic compensation, we decided to use genetically unaltered animals and block their TRPV1 channels by AMG 517. Pretreatment with AMG 517 exaggerated the hypothermic effect of NH_4Cl. The biggest difference between the mean T_b of the pretreatment groups was 1.6°C at 50 min post-NH_4Cl injection. 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. Importantly, in AMG 517-pretreated rats both the magnitude and the duration of the NH_4Cl-induced hypothermia was markedly exaggerated. 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.

IV/2.2.4. 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, we studied whether the TRPA1 channel also plays a role in this thermal response. 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.

IV/2.2.5. The hypothermic response to NH_4Cl is attenuated by the pharmacological blockade of the TRPA1 channel in rats

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 a highly potent and selective TRPA1 antagonist, A967079 [60]. When the 5 mg/kg of A967079 was infused 20 min before the i.p. injection of NH_4Cl, it markedly attenuated the hypothermic response, and a statistically significant difference was present between the two pretreatments groups in the NH_4Cl-treated rats between 40-120 min with a maximal difference of 0.7°C at 50 min.

IV/2.2.6. 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 compared to sterile water treatment, however, the difference was significant only at the higher (280 mg/kg) dose. 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. The fall in the blood pH of the mice reached a similar extent in all genotypes.

V. Discussion

In the present thesis, we investigated the predictive role of vital parameters (T_b and blood pH) for the outcomes of two different manifestations of systemic inflammation and the role of TRP channels in pharmacological modulation of T_b.

In the first part of our work, we found that fever reduces, while hypothermia promotes mortality in septic patients as compared to normothermic subjects. In addition, we demonstrated a strong negative correlation between T_b and mortality. Furthermore, when we calculated the mean T_bs of septic patients in the mortality quartiles, we found that it was significantly higher in the lowest than in the highest quartile of mortality. Taken together the results from all of our statistical approaches, our data strongly suggest a predictive role of T_b for the outcome of sepsis.

With regard to the adaptive biological value of T_b alterations in mammals, fever itself is assumed to have a direct, advantageous effect on the mortality ratio in systemic inflammation, when it is affordable for the host. Although the results of our analysis showed that hypothermia is associated with higher mortality, it should be noted that we can not be sure how mortality ratio of the patients would have changed if hypothermia had not developed or if the patients were rewarmed. Therefore, hypothermia in itself should not be

regarded harmful for the body as the associated higher mortality rate of the septic patients is presumably due to their more severe clinical condition.

Next, we investigated the effect of blood pH on outcomes in AP. Our analyses showed that lower blood pH predicts higher mortality rate, longer LOS, and worsens the severity of AP. There were huge differences between the protocols of the individual studies, but it is remarkable that no matter how the patients were grouped by the authors originally, the patient group with lower pH had always worse outcomes than the group with higher pH in AP, which suggests that in the early stages of AP acidosis is an important influencing factor of the outcome regardless from the actual progression of the disease.

Then, we studied the role of TRP channels in the pharmacological modulation of T_b by conducting meta-analysis of clinical trials and animal experiments [61, 62]. Our meta-analysis of the human-trial data has confirmed that the first-generation TRPV1 antagonists cause hyperthermia in humans, whereas the second-generation compounds may lack this effect. The location of TRPV1 channels that sense T_bs to drive thermoeffector responses in humans is unknown and can be different from the location of the channels that are tonically activated by protons. Knowing that the skin plays a prominent thermosensory role in all species, at least some TRPV1 channels that mediate thermal signals to drive thermoeffectors in humans can be speculated to be located in the skin.

Finally, in animal experiments, we revealed that 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. These findings suggest that TRPV1

channels are limiting regulators, whereas TRPA1 channels are potentiating signaling molecules of NH_4Cl-induced hypothermia.

The decreased blood pH could potentially serve as a direct mechanism for the development of the hypothermia through the stimulation of the TRPV1-mediated acido-antithermogenic and acido-antivasoconstrictor reflexes [for review, see 61]. 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. 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. We can only speculate that NH_4+ ions could inhibit TRPV1 channels located in the abdominal wall, which were shown to tonically suppress skin vasoconstriction and thermogenesis [63]. 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 [64], thus it can be assumed that the blockade of these TRPV1 channels could result in higher heat loss and exaggerated hypothermic response to NH_4Cl. 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 [39, 65]. Although protons can rapidly permeate through the membrane [66], rodent TRPA1 failed to respond to extracellular acidosis, and protons even inhibited the channel in a later study [40]. Ammonia and intracellular alkalization were shown to activate both the

TRPV1 and the TRPA1 channels [37, 38]. 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 [67]. 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 [36].

VI. Conclusions

In the present work, we investigated the predictive role of T_b and the blood pH in an infectious (sepsis) and mainly noninfectious (AP) manifestation of systemic inflammation. The deviations of deep T_b are strongly associated with mortality in sepsis. Similar to the T_b, the correlation between blood pH and mortality showed significant negative correlation in AP. We also found that lower systemic pH predicts longer LOS, and worsens the severity of AP.

In the second part of my work, we highlighted the importance of the nonthermal activation of TRPV1 and TRPA1 channels in thermoregulation with different methodological designs in humans and rodents. Our meta-analysis of human trials showed that the first-generation TRPV1 antagonists cause hyperthermia in humans, whereas the second-generation compounds may lack this effect. In our animal experiments we found that (i) i.p. administration of NH_4Cl induces hypothermia in rats as well as in mice; (ii) TRPA1 channels contribute to the development of NH_4Cl-induced hypothermia in both mice and

rats; (iii) TRPV1 channels play a limiting function in this process. In systemic inflammation, the pharmacological modulation of T_b may be beneficial in humans and animals, and TRPV1 and TRPA1 channels are probably good targets to modify T_b.

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VIII. Appendix

Publications related to the subject of the thesis

- Number of publications related to the subject of the thesis: 5
- Number of publications not related to the subject of the thesis: 36
- Number of book chapters: 1
- Sum of all impact factors: 157.789
- Sum of impact factors from publications related to the topic of PhD thesis: 23.477
- All citations: 866
- Independent citations: 779

Publications related to the topic of the PhD thesis

1. **Rumbus, Z**., Fekete, K., Kelava, L., Gardos, B., Klonfar, K., Keringer, P., Pinter, E., Pakai, E., & Garami, A. Ammonium chloride-induced hypothermia is attenuated by transient receptor potential channel vanilloid-1, but augmented by ankyrin-1 in rodents. Life sciences, 2024;346, 122633. Advance online publication. **IF: 5.2 Q1/D1**
2. Garami, A., Shimansky, Y. P., **Rumbus, Z**., Vizin, R. C. L., Farkas, N., Hegyi, J., Szakacs, Z., Solymar, M., Csenkey, A., Chiche, D. A., Kapil, R., Kyle, D. J., Van Horn, W. D., Hegyi, P., & Romanovsky, A. A. Hyperthermia induced by transient receptor potential vanilloid-1 (TRPV1) antagonists in human clinical trials: Insights from mathematical modeling and meta-analysis. Pharmacology & therapeutics, 2020, 208, 107474 **IF: 12.31 Q1/D1**
3. **Rumbus, Z**., & Garami, A. Fever, hypothermia, and mortality in sepsis: Comment on: Rumbus Z, Matics R, Hegyi P, Zsiboras C, Szabo I, Illes A, Petervari E, Balasko M, Marta K, Miko A, Parniczky A, Tenk J, Rostas I, Solymar M, Garami A. Fever is associated with reduced, hypothermia with increased mortality in septic patients: a meta-analysis of clinical trials. PLoS One. 2017;12(1):e0170152. DOI: 10.1371/journal.pone.0170152. Temperature (Austin, Tex.), 2018. 6(2), 101–103.
4. **Rumbus, Z.,** Toth, E., Poto, L., Vincze, A., Veres, G., Czako, L., Olah, E., Marta, K., Miko, A., Rakonczay, Z., Jr, Balla, Z., Kaszaki, J., Foldesi, I., Maleth, J., Hegyi, P., & Garami, A. Bidirectional relationship between reduced blood pH and acute pancreatitis: A translational study of their noxious combination. Frontiers in physiology, 2018; 9, 1360. **IF: 3.201 Q2**

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Other publications, not related to the topic of the PhD thesis

1. Bálint, A., Hanák, L., Hegyi, P., Szakács, Z., Eitmann, S., Garami, A., Solymár, M., Márta, K., **Rumbus, Z.,** & Komócsi, A. Increased risk of adverse events in patients with low-on clopidogrel platelet reactivity after percutaneous coronary intervention: A systematic review and meta-analysis. Cardiology journal, 2023;30(3), 391–400. **IF: 2.9 Q2**
2. Garai, J., Radnai, B., Vámos, E., Kovács, D., Vántus, V. B., **Rumbus, Z.,** Pákai, E., Garami, A., Gulyás-Fekete, G., Agócs, A., Krekó, M., Zaman, K., Prókai, L., Őrfi, L., Jakus, P. B., & Lóránd, T. Synthesis and evaluation of a new class of MIF-inhibitors in activated macrophage cells and in experimental septic shock in mice. European journal of medicinal chemistry, 2023; 247, 115050. **IF: 6.0 Q1**
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11. Olah, E., **Rumbus, Z**., Kormos, V., Tekus, V., Pakai, E., Wilson, H. V., Fekete, K., Solymar, M., Kelava, L., Keringer, P., Gaszner, B., Whiteman, M., Keeble, J., Pinter, E., & Garami, A. The hypothermic effect of hydrogen sulfide is mediated by the transient receptor potential ankyrin-1 channel in mice. Pharmaceuticals (Basel, Switzerland), 2021; 14(10), 992. **IF: 5.215 Q1**
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20. Bui, T. Q., Bui, Q. V. P., Németh, D., Hegyi, P., Szakács, Z., **Rumbus, Z.**, Tóth, B., Emri, G., Párniczky, A., Sarlós, P., & Varga, O. Epidermal growth factor is effective in the treatment of diabetic foot ulcers: meta-analysis and systematic review. International journal of environmental research and public health, 2019; 16(14), 2584. **IF: 2.849 Q2**
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