Project closing report of OTKA PD 105532: "Age-related development of obesity. Role of transient receptor potential vanilloid-1 channels in regulation of energy homeostasis."

1. Summary

During the three and a half years of the project, we published 9 scientific articles in pee-reviewed journals and one book chapter. I gave 11 oral presentations as an invited speaker and participated with a number (14) of poster presentations at national and international conferences. Further manuscripts from the findings of the research project are currently submitted or in preparation for submission. The total cumulative impact factor of the published articles is 42,085 (including abstracts) and 20,715 (without abstracts). The total number of citations of the articles published from the project is 32 based on Google Scholar (06/04/2016). Student researchers involved in project, presented the results of the supported research on 7 occasions at local, national, and international conferences. Six diploma theses were written in association with the project. Part of my habilitation degree was also based on the funded research.

According to the workplan and schedule of the funded research project, we investigated the parameters of energy balance regulation in mice genetically lacking the *Trpv1* gene (KO) and in rats after desensitization of the transient receptor potential vanilloid-1 (TRPV1) channels with high-dose capsaicin (CAP) in different age groups. Then, we measured indicators of thermoregulatory mechanisms in freely-moving and restrained TRPV1 KO mice and CAP desensitized rats of different age. Last, we studied the effects of central anabolic [ghrelin, neuropeptide Y (NPY)] and catabolic substances [leptin, alpha-melanocyte-stimulating hormone (α-MSH)] and of additional molecular mediators on the parameters of energy balance regulation in CAP desensitized rats of different age.

In harmony with our previous results, which also served as the basis of the research project, we found that TRPV1 KO mice gained more body mass than WT mice as a function of age. Thus, we confirmed that our animal model is appropriate for the study of the function of TRPV1 in the development of age-related obesity. Next, we investigated the involved mechanisms and as a novel finding, we showed that higher food intake and lower basal metabolic rate of the aged TRPV1 KO mice could also contribute to the development of their increased gain of body mass.

When we studied the age-related changes of energy balance regulation in CAP desensitized rats, to our surprise we found that their body mass was lower than that of controls as a function of age. We identified higher locomotor activity in the elder CAP desensitized rats as a mechanism of the observed change in their body mass.

By studying the effects of the key neuropeptides on the regulation of energy balance, we revealed that alterations in the energetic effects of mainly catabolic (leptin, α-MSH) and to a smaller extent anabolic neuropeptides (ghrelin, NPY) play a role in the development of the lower age-related gain of body mass in the CAP desensitized rats.

As the study progressed, we also implemented additional experiments to further clarify the molecular and neural mechanisms involved in the age-related changes in the absence of the TRPV1 channel. Therefore, we characterized the thermoregulatory effects of molecular mediators [e.g., pituitary adenylate cyclase-activating enzyme (PACAP), substance P (SP), bilirubin), which can be associated with TRPV1-expressing neural endings. Further, we studied the thermoregulatory role of TRP ankyrin-1 (A1), which is often co-expressed with TRPV1. Also, we investigated the physiological function of TRPV1 channels expressed in non-neural tissues such as the vascular wall and the endometrium.

In conclusion, our findings in relation with the funded project present significant advancements in understanding the physiological and pathological functions of the TRPV1 channel in the development of age-related obesity. In addition to the discovery of the physiological mechanisms, our results regarding the involved neuroendocrinological mediators also pave the way towards the prevention and, as a perspective, the targeted therapy of age-related obesity through the modulation of the TRPV1 channel.

2. Results

2.1 Characterization of the parameters of energy balance in TRPV1 KO mice of different age groups

As expected based on previous studies, the body mass of the elder TRPV1 KO mice was significantly higher than that of their wild type littermates. As a novel finding, we also observed an increased food intake in the elder TRPV1 KO mice which could have contributed to their higher body mass.

In all age groups of the mice, the recordings of day- and night-time thermoregulatory parameters have been also conducted in accordance with the plans. Day- and night-time deep body temperature and oxygen consumption of the TRPV1 KO mice were lower than those of controls, the difference between genotypes increased in the course of aging. We found no significant difference between the tail skin temperatures of TRPV1 KO and wild-type mice. In the biotelemetry study, we found that the abdominal temperature of freely-moving TRPV1 KO mice was higher than that of the controls, which could be explained – at least in part – by the higher locomotor activity of the KO mice. In case of freely-moving mice the difference in abdominal temperature and locomotor activity was less pronounced between genotypes over the course of aging, which can explain the age-related overweight of the TRPV1 KO mice. The excess weight gain of the TRPV1 KO mice was brought about by their higher food intake, lower resting metabolic rate (and core temperature, T_c), and decreasing general locomotor activity as a function of age. Assessment of body composition in the mice of different age groups revealed higher relative amount of body fat in the TRPV1 KO mice, which was in harmony with their increased body mass.

2.2 Characterization of the parameters of energy balance in CAP desensitized rats of different age groups

We also investigated the resting thermoregulatory parameters in restrained, as well as the circadian changes of energy balance in freely-moving CAP desensitized and vehicle-treated rats in different age groups. As a surprise, we found that contradictory to our findings in TRPV1 KO mice, the body mass of CAP desensitized rats became less than that of controls as a function of age. When we investigated the possible mechanisms, we found that the smaller body mass of the aging desensitized rats was, at least in part, the result of their higher general locomotor activity (and T_c) as compared to age-matched controls. At the age of 6 months, that is when the body mass curves between CAP desensitized and control rats started to diverge, the night-time resting metabolic rate of the desensitized rats was higher than that of controls. We did not observe such difference in the metabolic rate between desensitized and control rats when the animals became older (at 18 and 24 months), at which age the difference in their body mass was also less pronounced.

The contradiction between the age-related changes of body mass of TRPV1 KO mice and of the CAP desensitized rats is presumably due to the different nature of TRPV1 channels, which are absent in the desensitized rats compared to the KO mice. While in CAP

desensitized rats the impairment of the function of TRPV1 channels is limited mainly to neural elements, due to the genetic ablation of TRPV1 in KO mice, TRPV1 is absent from all cells and tissues, including both neural and non-neural structures. To support the hypothesis that neural and non-neural TRPV1 channels can have different physiological functions, we investigated the role of non-neural TRPV1 channels in isolated vessels and in endometrium. In line with our hypothesis, we found that neural and non-neural TRPV1 channels differently mediate the vasomotor responses to chemical stimuli. Furthermore, we showed for the first time that functional non-neuronal TRPA1 and TRPV1 receptor proteins are expressed in the rat endometrium. Our findings also supported the close interactions between the TRPV1 and TRPA1 channels. It can be suggested that TRPV1 channels on non-neural structures can also play an important role in the development of age-related changes of body mass.

2.3 Investigation of the TRPV1-associated neuroendocrinological mechanisms in the development of age-related changes of body mass

In order to better understand the molecular mechanisms involved in TRPV1-mediated changes in the regulation of energy balance, we studied the thermoregulatory effects of pituitary adenylate cyclase-activating polypeptide (PACAP), which is one of the most important among the neuropeptides (e.g., substance P, PACAP, somatostatin, etc.) released from the capsaicin-sensitive neural endings. We found that acute administration of PACAP increases deep body temperature, while chronic absence of the peptide leads to higher locomotor activity and body temperature similarly to the results found in TRPV1 KO mice. These results suggested that the lack of PACAP might contribute to the thermoregulatory changes observed in the TRPV1 KO mice. Thus, we continued to study the causative role of PACAP in the energetic changes observed in TRPV1 KO mice and found that the thermoregulatory phenotype of mice lacking PACAP is similar to that of TRPV1 KO mice, moreover the absence of PACAP results in the alteration of the activity of those hypothalamic nuclei, which play a crucial role in the maintenance of energy balance and also express TRPV1 channels. These results suggest a direct causative interaction between the thermoregulatory effects of TRPV1 and PACAP.

As we planned, we also investigated the roles of different neuropeptides in energy balance regulation in the absence of the TRPV1 channel. We found that the effects of the studied catabolic neuropeptides (leptin and α-MSH) were more pronounced in CAP desensitized rats than in controls at the age of 6 months, when the body mass of the desensitized rats started to decline significantly compared to that of age-matched controls. In old age, however, no such difference could be observed between desensitized and control rats in the case of either neuropeptide, which is in accordance with the observed smaller difference in the body mass of the elder groups of rats (for details, see section 2.2). Among the anabolic substances, the effect of NPY tended to be attenuated in younger CAP desensitized rats than in controls, which tendency was not present in elder rats. While the effect of ghrelin was not meaningfully altered in the CAP desensitized rats, when administered to TRPV1 KO mice its stimulatory effect on food intake was enhanced as compared to controls, which could contribute to the increased food intake and body mass of the elder KO mice (see section 2.1 for details). The revealed changes in the energetic effect of the neuropeptides can explain the lower body mass gain of CAP desensitized rats (increased effects of catabolic substances) as well as the higher body mass gain of TRPV1 KO mice (enhanced effect of ghrelin) as a function of age. These results are also in accordance with a difference in the role between neural and non-neural TRPV1 channels in the regulation of complex energy balance described in section 2.2.

2.4 Investigation of the TRPV1-associated neurotransmission mechanisms in the regulation of complex energy balance

To clarify the mechanism of the age-related overweight in TRPV1 KO mice, we needed to exclude a potential compensatory mechanism in the TRPV1 KO mice, which could develop through the increased function of the TRPA1 channel, which is co-expressed with TRPV1. For this, we conducted a detailed thermoregulatory study to investigate the role of TRPA1 in the maintenance of energy balance and found that the absence of TRPA1 does not contribute to the maintenance of normal T_b under thermoneutral or cold conditions, therefore its potential compensatory role in the regulation of energy balance in the TRPV1 KO mice is presumably not meaningful. Next, we wanted to know whether the modulation of the activity of TRPA1 with ligand agonists can cause changes in T_b regulation. When we studied the thermoregulatory effects of dimethyl trisulfide, which is a hydrogen-sulfide donor, we found that its hypothermic and hypokinetic effects are evoked, at least in part, through the TRPA1 channel, therefore a role for TRPA1 in thermoregulation can not be fully excluded.

Another important TRPV1-associated neuronal mechanism is the SP signaling, which could also contribute to the development of the energetic phenotype of the TRPV1 KO mice. When we studied mice, in which the neurokinin-1 receptor (NK1R), the main receptor of SP, is genetically ablated, we found that similarly to the TRPV1 KO mice, the NK1R KO mice were also hyperactive and hyperthermic compared to controls. We revealed further similarities between the thermoregulatory phenotype of NK1R KO and TRPV1 KO mice by showing an attenuation of local and systemic inflammatory responses of the NK1R KO mice. Since similar reduction in the inflammatory reactions were also shown in TRPV1 KO mice, our findings suggest a close interaction between the SP signaling and the TRPV1 channel in inflammation and in the regulation of energy balance.

Because of the well-known role of the TRPV1 channel in inflammatory processes, we also wanted to know whether one of the most important endogenous antioxidant, bilirubin, has similar effects to TRPV1 in systemic inflammation, which we found in earlier studies. Such similarities could suggest an interaction between bilirubin (and the reactive oxygen species neutralized by it) and the TRPV1 channel. For that, we compared the responses of normobilirubinemic and hyperbilirubinemic rats to bacterial endotoxin. These two genotypes of rats correspond to undisturbed versus drastically suppressed (by bilirubin) tissue accumulation of reactive oxygen species, respectively. Low dose of endotoxin caused a typical triphasic fever in both genotypes, without any intergenotype differences. A high dose of endotoxin caused early hypothermia followed by late fever. The hypothermic response was markedly exaggerated, whereas the subsequent fever response was strongly attenuated in the hyperbilirubinemic rats compared to controls. The hyperbilirubinemic rats also responded with blunted increase in liver enzymes (damage) than controls, which could be due to either direct actions of bilirubin on thermoeffectors or the antioxidant action of bilirubin. Since the effects of bilirubin (current findings) and the TRPV1 channel (previous results) are vastly different on the processes of systemic inflammation, we rejected the hypothesis about the possible interaction between bilirubin and the TRPV1 channel.

3. Setbacks during the research project

As technical obstacles, we faced two unexpected difficulties during the second year of the project: 1) the environmental temperature control system in our experimental setup has lost its ability to maintain a steady temperature during the experiments; and 2) survival rate of the animals after intracerebroventricular cannula implantation was lower than the expected over 90% ratio, presumably due to a newly occurred inaccuracy of the stereotaxic manipulator. To

cope with these problems and to try to avoid substantial delays in the project, we contacted the OTKA Office to obtain permission to dedicate part of our budget to the purchase of the two new devices. The permission has been granted and we initiated the purchase of the needed equipment. Unexpectedly, the delivery of the new devices took longer time than usual due to reasons beyond our control, thus we also needed to ask for the extension of the project duration to finish all experiments proposed in the workplan. The extra time allowed to complete all proposed experiments and to publish one article in an international and one in a national journal. We also presented our newest findings at international scientific conferences. A further manuscript is currently under review, another one in submission and additional ones in preparation from the novel results of the funded project.

4. Importance and impact of the findings of the research project

Our results in association with the funded project help us to better understand the physiological and pathological mechanisms involved in the development of age-related obesity. We showed that the TRPV1 channel plays a crucial role in age-related body mass gain through modulation of the activity of different effectors (e.g., metabolic rate, locomotor activity), which are involved in the regulation of energy balance. We also revealed or rejected a number of neural and molecular processes, which are responsible for the alternation of energy balance regulation in the absence of the TRPV1 channels. By comparing two animal models (KO mice and desensitized rats), we concluded that the loss of solely the neural TRPV1 channels prevents, whilst the loss of all (neural and non-neural) TRPV1 promotes the development of age-related obesity. From a translational point of view our results can help to understand why people with a certain lifestyle and diet are prone to develop obesity while others are not. Our results can also open new perspectives for drug development for the treatment of body mass disorders, for example through the targeted, selective inhibition of neuronal TRPV1 channels.

We published our results in peer-reviewed journals with impact factor, for instance in the Journal of Neuroscience (IF: 6.747), Cell Cycle (4.565), and the Journal of Molecular Neuroscience (IF: 2.757). We also published three articles of a revolutionary type in the recently established peer-reviewed journal “Temperature: Multidisciplinary Biomedical Journal”. For one of those (Szekely et al 2015), we were awarded “The Best Teaching Slide Publication in 2015” by the Editor-in-Chief. We wrote one book chapter in association with our findings from the research project. Our results were presented at national and international conferences as poster and oral presentations, from several of which the abstracts have been published in peer-reviewed scientific journal. Student researchers under my supervision, who have been involved in the project disseminated our results at local and international student conferences, several of them wrote their theses based on the findings. The list of selected publications can be found at the end of the current report (see section 5. Publications).

5. Selected publications

5.1 Articles in peer-reviewed scientific journals

Ivic I, Solymar M, Pakai E, Rumbus Z, Pinter E, Koller A, **Garami A**. Neural and non-neural transient receptor potential vanilloid-1 channels differently mediate the vasomotor responses to changes in pH. *Am J Physiol Regul Integr Comp Physiol*
IF: 3.106
(*in submission*)

Pozsgai G, Payrits M, Saghy E, Sebestyen-Batai R, Steen E, Szoke E, Sandor Z, Solymar M, **Garami A**, Orvos P, Talosi L, Helyes Z, Pinter E. Analgesic effect of dialkyl polysulfide compound dimethyl trisulfide in mice is mediated by TRPA1 and sst4 receptors. *Neuroscience*
IF: 3.357
(*under review*)

Garai J, Kanizsai P, Rumbus Z, Toldi J, **Garami A**. Az akut szisztémás gyulladás kórélettana az alapkutatásoktól a klinikai vonatkozásokig. *Aneszteziológia és Intenzív Terápia*
(*accepted*)

Pohoczky K, Kun J, Szalontai B, Szoke E, Saghy E, Payrits M, Kajtar B, Kovacs K, Kornyei JL, Garai J, **Garami A**, Perkecz A, Czegledi L, Helyes Z. Estrogen-dependent up-regulation of TRPA1 and TRPV1 receptor proteins in the rat endometrium. *J Mol Endocrinol* 56: 135-49, 2016.
IF: 3.081
doi: 10.1530/JME-15-0184

Pakai E, **Garami A**, Nucci TB, Ivanov AI, Romanovsky AA. Hyperbilirubinemia exaggerates endotoxin-induced hypothermia. *Cell Cycle* 14: 1260-1267, 2015.
IF: 4.565
doi:10.1080/15384101.2015.1014150

Garami A, Romanovsky AA. The transient receptor potential vanilloid-4 channel: detecting body temperatures that drive defences against mild warmth. *Acta Physiol (Oxford, England)* 214: 154-156, 2015.
IF: 4.382
doi: 10.1111/apha.12510

Szekely M, Carletto L, **Garami A**. The pathophysiology of heat exposure. *Temperature* 2: 452, 2015.
doi: 10.1080/23328940.2015.1051207

Banki E, Pakai E, Gaszner B, Zsiboras C, Czett A, Bhuddi PR, Hashimoto H, Toth G, Tamas A, Reglodi D, **Garami A**. Characterization of the Thermoregulatory Response to Pituitary Adenylate Cyclase-Activating Polypeptide in Rodents. *J Mol Neurosci* 54: 543-554, 2014.
IF: 2.343
doi: 10.1007/s12031-014-0361-0

De Oliveira C*, **Garami A***, Lehto SG, Pakai E, Tekus V, Pohoczky K, Youngblood BD, Wang W, Kort ME, Kym PR, Pinter E, Gavva NR, Romanovsky AA. Transient receptor potential channel ankyrin-1 is not a cold sensor for autonomic thermoregulation in rodents. *J Neurosci* 34: 4445-4452, 2014.
IF: 6.344
**Authors contributed equally to this work.*
doi: 10.1523/JNEUROSCI.5387-13.2014.

Armbruszt S, **Garami A**. The short- and long-term effects of food intake on thermogenesis. *Temperature* 1: 96-96, 2014.
doi: 10.4161/temp.29733

Garami A, Székely M. Body temperature: Its regulation in framework of energy balance. *Temperature* 1: 28-29, 2014.
doi: 10.4161/temp.29060

5.2 Book chapter

Garami A, Pakai E, Rumbus Z, Solymar M. The role of PACAP in the regulation of body temperature. In: *Pituitary Adenylate Cyclase Activating Polypeptide – PACAP*, ed. Reglodi D, Tamas A. New York: Springer Nature (*in press*)