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The Involvement of the T1R3 Receptor Protein in the Control of Glucose Metabolism in Mice at Different Levels of Glycemia

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Abstract

The heterodimeric protein T1R2/T1R3 is a chemoreceptor mediating taste perception of sugars, several amino acids, and non-caloric sweeteners in humans and many other vertebrate species. The T1R2 and T1R3 proteins are expressed not only in the oral cavity, but also in the intestine, pancreas, liver, adipose tissue, and in structures of the central nervous system, which suggests their involvement in functions other than gustatory perception. In this study, we analyzed the role of the T1R3 protein in regulation of glucose metabolism in experiments with the gene-knockout mouse strain C57BL/6J-*Tas1r3*^{tm1Rfm} (*Tas1r3*^{-/-}), with a deletion of the *Tas1r3* gene encoding T1R3, and the control strain C57BL/6ByJ with the intact gene. Glucose tolerance was measured in euglycemic or food-deprived mice after intraperitoneal or intragastric glucose administration. We have shown that in the *Tas1r3*^{-/-} strain, in addition to the disappearance of taste preference for sucrose, glucose tolerance is also substantially reduced, and insulin resistance is observed. The effect of the *Tas1r3* gene knockout on glucose utilization was more pronounced in the euglycemic state than after food deprivation. The baseline glucose level after food deprivation was lower in the *Tas1r3*^{-/-} strain than in the control strain, which suggests that T1R3 is involved in regulation of endogenous glucose production. These data suggest that the T1R3-mediated glucoreception interacts with the K_{ATP}-dependent mechanisms of regulation of the glucose metabolism, and that the main role is likely played by T1R3 expressed in the pancreas and possibly in the central nervous system, but not in the intestinal mucosa, as it was suggested earlier.

Keywords

gustatory receptors; *Tas1r3*; glucose tolerance; insulin resistance; glycemia; incretin effect

Introduction

Glucose homeostasis in vertebrates is regulated by central and peripheral feedback mechanisms that maintain concentration of this metabolite at a level currently required by the organism. The primary universal mechanism of the so-called metabolic detection of

glucose, which is well studied in β -cells of the islet tissue of mammals, is presumably also found in other classes of vertebrates [1]. On the whole, the metabolic detection of glucose can be thought of as a sequence of processes that include the transfer of glucose into the cell by a low-affinity transporter GLUT2, glucose phosphorylation by the specialized glucokinase (GK), and finally the glycolysis which results in an increase of the intracellular ratio of ATP/ADP. The higher concentration of ATP stimulates the closing of the ATP-sensitive potassium channels (K_{ATP}) which causes depolarization of the membrane and the inflow of Ca^{2+} ions to the cytoplasm, followed by the cell's response [2, 3]. This GK- K_{ATP} -dependent mechanism controls to a considerable extent the secretion of insulin in the pancreas β -cells [4], glucagon-like-peptide 1 (GLP-1), and glucose-dependent insulinotropic peptide (GIP) in the enteroendocrine cells of mucosa of the gastrointestinal tract [5, 6]. Besides, it mediates the response of glucosensory neurons in hypothalamus [7]. Nevertheless, in the beginning of the study it was apparent that the metabolic detection of glucose was not the only mechanism of glucoreception in the pancreas, intestine, and the central nervous system [5, 8–10].

The studies carried out in the recent decade revealed several alternative principles of tissue sensitivity to extracellular glucose, including a mechanism that is similar to sugar reception in gustatory cells, rather well-studied in mammals. The receptor involved in the perception of sweet taste of carbohydrates, certain amino acids, and non-caloric sugar substitutes represents a heterodimer that is composed of two transmembrane protein subunits, T1R2 and T1R3, encoded by the *Tas1r2* and *Tas1r3* genes [11, 12]. Polymorphism of the *Tas1r3* has been shown to underlie the among-strain differences in the extent of taste preference for sweet substances, amino acids, and ethanol in mice [13, 14] and among-population differences in that for sucrose and sodium glutamate in humans [12, 15]. Study on the *Tas-1* genes in different taxonomic groups of vertebrates allowed explaining many species-specific dietary preferences from the standpoint of physiological genetics. For example, the absence of behavioral and neurophysiological responses to sweet compounds in gallinaceous birds and in several groups of predatory mammals and amphibians correlates either with the loss of these genes or with pseudogene formation [12, 16].

The described receptor proteins and some others associated with them (G-protein α -gustducin and the enzymes of the intracellular signaling cascade) are expressed not only in the mucosa of the oral cavity, but also outside of the gustatory system: in β -cells of the pancreatic islet tissue [17–19], enteroendocrine L-cells of the intestinal epithelium [20–23], hypothalamic centers of hunger and satiety [24]. Both *in vivo* and *in vitro* studies showed that T1R2 and T1R3 proteins expressed in various tissues, the same as enzymes of their intracellular cascades, are potentially able to affect the glucose metabolism. To a certain extent, they participate in the control of the production of incretins, i.e., hormones secreted in the epithelium of the small intestine, which stimulate hypoglycemia, in particular glucagon-like-peptide 1 (GLP-1) [25–27]. Besides, they have an effect on expression of sodium-dependent glucose co-transporter 1 (SGLT1) [28] and GLUT 2 [29] in the gastrointestinal tract. The role of the T1R3 protein in enhancement of insulin production was shown *in vitro* in β -cells of the pancreatic islet tissue [18, 30]. At the same time, it cannot be considered established that a shifts in expression of glucose transporters and in production of

incretins and insulin, which occur *in vivo* during the T1R2/T1R3 activation in intestine and pancreas, are sufficient to change the glucose metabolism at the level of the whole organism. On the one hand, in mice *in vivo*, deletion of the genes encoding the T1R3 protein or α -gustducin reduces production of incretins and insulin and decreases glucose tolerance after intragastric administration of glucose or artificial sweeteners [20, 25, 27]. In humans, lactisole blockade of sweet taste receptors inhibits production of GLP-1 provoked by intraduodenal administration of glucose [26]. *In vitro* experiments on isolated fragments of the small intestine from non-starved mice with the deletion of the above-mentioned genes also showed reduction in the secretion of incretins in response to caloric and non-caloric sweeteners [25, 27]. On the other hand, it is noted in a number of articles that, in contrast to sucrose, non-caloric sweeteners alone do not affect secretion of incretins and glucose tolerance both in humans and in rodents [21, 31, 32]. It has been also shown that, in preliminarily starved mice with the deletion of *Tas1r3*, intraperitoneal administration of glucose does not affect glucose tolerance [27]. Further studies are expected to resolve these controversies. Yee with co-authors [33] put forward a suggested that GLUT2 and K_{ATP} that they revealed in taste receptor cells actively regulate transmission of signals from T1R, as these are the elements of the metabolic glucose detection. It has also been established that the T1R2/T1R3-mediated stimulating effect of fructose and non-caloric sweeteners on insulin secretion by β -cells *in vitro* depends on glucose concentration in the medium [17, 19]. Apparently, the metabolic detection of glucose creates the required depolarization of the cell, and it is on this background that nutrients and non-metabolized sweeteners can enhance insulin production via the T1R-mediated signaling pathway.

Thus, it may be presumed that in many vertebrates, including humans and rodents, there are two molecular mechanisms of reception coexisting in all specialized cells sensitive to sweet substances, one of which (the T1R2/T1R3-mediated one) determines the presence of a ligand, while the other one assesses its metabolic value. Both mechanisms of metabolic and specific reception act synergistically [9]. The absence of either of them may be compensated by the other only to a certain degree.

The goal of the present study was to evaluate the physiological effect of the T1R3 receptor protein in control of glucose homeostasis *in vivo*, including that in the so-called incretin effect. This was performed on a *Tas1r*-knockout mouse strain and the parental strain used as control. The glucose tolerance test was performed under conditions of intragastric and intraperitoneal glucose load in euglycemic and starved animals. A separate experimental series was used for posing the classical behavioral tasks with brief and prolonged access to study effect of the T1R3 on the dietary preference of mice for carbohydrates.

Materials and Methods

All the described experimental procedures complied with the Committee for Bioethics at the Institute of Physiology of the Russian Academy of Sciences. The experiments were carried out on male mice of a *Tas1r3*-knockout strain C57BL/6J-*Tas1r3*^{tm1Rfm} (*Tas1r3*^{-/-}), which were bred from the C57BL/6J strain by means of homologous recombination in the embryonic stem cells [34] and was obtained by courtesy of Dr. R.F. Margolskee (Monell Chemical Senses Center, Philadelphia, PA, USA). As the control, the wild-type (*Tas1r3*^{+/+})

mice of the C57BL/6ByJ strain were used; they were descendants from the stock colony maintained at the Jackson Laboratory (Bar Harbor, ME, USA). The mice were kept by 4–5 individuals per cage, with sawdust bedding, under artificial light (12L:12D) turned on at 7.30 am and a constant temperature of $23 \pm 1^\circ\text{C}$. The animals used in the feeding preference tests were placed into individual cages 1–2 weeks before the experiment. A standard granulated food PK-120 (carbohydrates: 67%, proteins: 19%, fats: 5%, energy value: 13000 kJ; OOO Mest, Moscow) and purified tap water were available *ad lib*, unless stated otherwise.

In the brief access test (BAT) [35], 1–32% solutions of sucrose (pure for analysis, ZAO Vekton, St. Petersburg, Russia) were given to an animal in the box of a Davis MS-160 automated gustometer (DiLog Instruments, Tallahassee, FL, USA) in a series of increasing concentrations, alternated with distilled water. The drinking pan was available for 5 s during each of the total of 24 trials. The gustometer recorded the number of lapping movements of the tongue during a 5-s trial. The “preference” was calculated from these data as a percentage of the mean number of lapping movements for a given concentration of sucrose relative to the mean number of lapping movements calculated across all trials with water. The BAT was preceded with water deprivation which lasted 22–24 h and was total in the case of preliminary training and moderate (1.5 ml of water left) before the experiments with sucrose. After the experiments, animals were provided with a free access to water for 1 h.

In the two-bottle preference test (2BT) [36], animals in their cages had a free 48-h access to two graduated tubes containing 1–32% solutions of sucrose or distilled water. The volume consumed was measured 24 h later, after which the solutions were refreshed and the tubes were transposed with each other. After another 24 h, the second measurement was made. The preference for sucrose was calculated as a ratio of its consumption to total consumption of liquids, averaged across 48 h and expressed as percentage. A 50% level of preference was considered as indifference to a solution, higher values indicated the preference for the consumed substance and the lower values were a sign of avoidance.

The glucose tolerance test (GTT) [37] was performed by means of intraperitoneal or intragastric administration of glucose (2 g/kg). Solutions of glucose (pure for analysis, ZAO Vekton, St. Petersburg, Russia) were prepared *de novo*. Glucose for intraperitoneal injections was dissolved in saline and that for intragastric injections was dissolved in distilled water. Both non-starved (euglycemic) and starved (hypoglycemic) animals were used in this test. The GTT without food deprivation was started at a fixed time from 3 to 3.30 pm. Food deprivation lasted 18–19 h and began on the previous day at 7–9 pm when the food was taken away and the bedding in the cage was renewed. In the insulin tolerance test (ITT) [37], non-starved animals were intraperitoneally administered with 2 MU/kg of insulin (Insulin aspart, Novo Nordisk A/S, Bagsværd, Denmark). The volume of glucose and insulin injections was 0.1 ml per 10 g of animal's body mass. Blood samples were drawn from tail vessels immediately prior to and 2–120 min after glucose administration or 15–120 min after the insulin administration. The blood glucose level was measured with aid of OneTouch® Ultra™ glucometer (LifeScan, Inc., USA).

Statistical analyses were done with Statistica 7.0 software (StatSoft, Inc., Tulsa, USA). One-way and two-way ANOVA was used with genotype (strain), administration method, concentration and time as categorical predictors. *Post-hoc* tests were performed by using the criteria of Fisher and Tukey (for the results of the 2BT). The data in text are expressed as the mean $\pm$ SE. Differences were considered significant at $p < 0.05$.

Results

The *Tas1r3* deletion and taste preference for sucrose

The consumption of sucrose in the BAT differed significantly in wild-type mice and the *Tas1r3*-knockout strain (Fig. 1a; effect of genotype: $F_{1,36} = 14.39$, $p < 0.0006$; effect of concentration: $F_{5,180} = 4.79$, $p < 0.0004$; interaction of the two factors: $F_{5,180} = 3.69$, $p < 0.004$). *Post-hoc* comparisons showed that the greatest differences from the control were observed during the consumption of 8% and 16% sucrose solutions (Fig. 1a; Fisher's test, $p < 0.05$). While mice of the *Tas1r3*^{-/-} strain did not prefer sucrose to water and exhibited equally moderate sucrose consumption in the whole range of concentrations, the response of *Tas1r3*^{+/+} mice was concentration-dependent. In the latter, a considerable increase in sucrose preference was detected starting from the lowest concentrations (the differences between 2–32% and 1% were significant, $p < 0.05$) and the maximum consumption of sugar was already observed during the exposure to 4% solution.

The *Tas1r3* genotype also had a significant effect on sucrose consumption during long-term exposure in the 2BT (Fig. 1b; effect of genotype: $F_{1,22} = 443.31$, $p < 0.0001$; effect of concentration: $F_{5,110} = 63.88$, $p < 0.0001$; interaction of the two factors: $F_{5,110} = 35.97$, $p < 0.0001$). The *post-hoc* comparisons revealed lower preference for sucrose in the *Tas1r3*^{-/-} mice as compared with the *Tas1r3*^{+/+} in the range of concentrations 1–16% (Tukey's test, $p < 0.001 \sim 0.05$), except for the maximum concentration of 32%. The wild type was characterized by high preference to sucrose over water, starting from the minimum concentration of 1%. The gene-knockout animals did not respond to the increase of sucrose concentration up to 4%, but, with its further increase, they did exhibit preference for sucrose (the values at 16% and 32% were significantly different from that at 8%; the Tukey's test, $p < 0.001$).

The effect of the *Tas1r3* gene on glucose tolerance

In the euglycemic state, i.e., without preliminary starvation, the *Tas1r3*^{-/-} and *Tas1r3*^{+/+} mice did not differ in their basal blood sugar levels (8.52 ± 0.17 and 8.34 ± 0.14 mmol/l, respectively). The prolonged 18-h starvation decreased the basal level in both lines, as expected (the Fisher's test, $p < 0.00001$). However, in the *Tas1r3*^{-/-} strain, blood glucose concentration dropped lower than that in the *Tas1r3*^{+/+} mice (down to 3.80 ± 0.13 and 4.46 ± 0.15 mmol/l, respectively; the Fisher's test, $p < 0.0007$). At the same time, there were no differences in body mass between the *Tas1r3*^{-/-} and the wild type either before the food deprivation (27.53 ± 0.61 vs. 26.68 ± 0.35 g) or after it (23.78 ± 0.28 vs. 24.04 ± 0.28 g).

After the intraperitoneal administration of glucose (Figs. 2a, 2b), the *Tas1r3*^{-/-} mice had blood sugar levels higher than those in the *Tas1r3*^{+/+} strain both in the euglycemic and in

the hypoglycemic states (effects of the genotype: $F_{1,30} = 7.60$, $p < 0.001$ and $F_{1,27} = 8.30$, $p < 0.01$; time: $F_{7,210} = 114.37$, $p < 0.0001$ and $F_{7,189} = 155.22$, $p < 0.0001$; their interaction: $F_{7,210} = 6.79$, $p < 0.0001$ and $F_{7,189} = 5.87$, $p < 0.0001$, respectively). The maximum difference in both cases was reached 30–45 min after the glucose administration (the Fisher's test, $p < 0.05$).

After the intragastric glucose infusion (Figs. 2c, 2d), the non-starved *Tas1r3*^{−/−} mice had blood sugar levels higher than those in the wild type (effect of genotype: $F_{1,22} = 11.11$, $p < 0.0031$; time: $F_{7,154} = 88.86$, $p < 0.0001$; their interaction: $F_{7,154} = 3.34$, $p < 0.0025$). The maximum difference was observed at the 15th–30th minute after the administration, i.e., somewhat earlier than in the case of intraperitoneal administration (the Fisher's test, $p < 0.05$; Fig. 2c). However, after starvation (in the hypoglycemic state), the intragastric glucose load (Fig. 2d) led to an equal increase in the blood glucose levels in both strains (the effect of genotype: $F_{1,28} = 0.05$, $p > 0.82$; time: $F_{7,196} = 212.67$, $p < 0.0001$; their interaction: $F_{7,196} = 1.83$, $p < 0.083$). In other words, in the GTT, the effect of the gene on the blood glucose concentration depended on the metabolic state of the organism only during the intragastric infusion, but not during the intraperitoneal administration.

To evaluate effect of incretins, the areas delimited by the concentration–time curves were compared for intragastric and intraperitoneal methods of glucose administration (data not shown). The incretin effect, i.e., more active blood glucose utilization rates after the intragastric administration as compared with the intraperitoneal one, was well-defined in both strains in the euglycemic state ($p < 0.001$). In the hypoglycemic state, the incretin effect turned out to become non-significant for both genotypes.

The effect of the *Tas1r3* gene on insulin tolerance

The ITT (Fig. 3) revealed the interstrain differences only in the dynamics of response (effect of genotype is nonsignificant: $F_{1,36} = 0.93$, $p > 0.33$; effect of time: $F_{3,108} = 136.62$, $p < 0.000$ and their interaction: $F_{3,108} = 7.34$, $p < 0.0001$). The *a posteriori* comparisons showed that the gene-knockout animals had higher blood sugar levels 60 min after insulin administration (Fig. 3).

Discussion

On the whole, the differences in the consumption of sucrose solutions between the *Tas1r3*-knockout mice and the wild-type strain, which are revealed in behavioral tests (Fig. 1), confirm the leading role of the T1R3 protein subunit of sweet taste receptor in development of preference for sweet taste. In the brief access test, where post-absorption effects are considered to be minimal, it is the presence of the working *Tas1r3* gene that determines lower thresholds for taste recognition and the higher preference for sucrose in the wild-type mice. Meanwhile, it is known from earlier studies that even after the deletion of either T1R3 or T1R2, but not both of them together, there remains some residual gustatory sensitivity to high concentrations of caloric sweet substances, which is confirmed in the BAT as well as in neurophysiological experiments [34, 38]. Such residual sensitivity is believed to be associated with homodimerization of either T1R2 or T1R3 subunits that form functional low-affinity receptors able to respond to high concentrations of sugars [38, 39]. However,

the temperature-dependence of responses recorded in gustatory fibers of the facial and the glossopharyngeal nerves in *Tas1r2*- or *Tas1r3*-knockouts suggests the existence of alternative mechanisms of sensitivity to sweet metabolized substances, other than the mechanism associated with T1R proteins and their signaling system [39]. This is confirmed by recent findings of the key elements of the metabolic detector of glucose, K_{ATP} and GLUT2, in gustatory sensory cells expressing the T1R3 protein [33]. However, in our present study, no residual response to high concentration of glucose was observed in the *Tas1r3*^{−/−} strain (Fig. 1a), which might have been a consequence of the thirst experienced before the tests. Thirst can lead to a certain caloric deprivation that was absent in experiments by Zhao et al. [38] who revealed the residual sensitivity to metabolized sugars in the BAT. Nonetheless, the sensitivity to high concentrations of glucose was quite clearly observed in the gene-knockout mice in the arbitrary choice test (2BT; Fig. 1b). This is accounted for by the conditions of the test, where preference is affected not only by the taste recognition, but also by the post-absorption effect, i.e., by metabolism of sucrose after its absorption [40].

The obtained results show that the T1R3-mediated visceral reception of sugars and most probably of sugar substitutes produces a significant effect on the basal blood glucose level during starvation. After 18 h of food deprivation, mice with deletion of the *Tas1r3* gene had the lower basal levels of blood glucose, although this difference from the parental strain was absent in the euglycemic state. Thus, the T1R3 receptor protein can be regarded as a possible participant in control of the endogenous production of glucose in liver, intestines or kidneys [41]. Meanwhile, our results certainly contradict those obtained by Geraedts et al. [27] who recorded similar concentrations of blood glucose (133–134 mg/dl, or 7.4 mmol/l) after 18 h of starvation in the *Tas1r3*-knockout strain and in the control. It is to be noted that the values obtained by these authors differ considerably from our data or from the values recorded by other experimenters and are closer to blood glucose levels in the euglycemic state or after moderate starvation for 0–6 h [42].

Although studies *in vitro* and most studies on gene-knockout animals *in vivo* have confirmed the involvement of intestinal T1R-type taste receptors and proteins of their signaling cascade in enhancement of expression of glucose transporters (GLUT2, SGLT1) and in production of incretins [20, 25, 27–29], it has remained unknown until recently to what extent these mechanisms are involved in the physiological control of insulin secretion and in maintenance of blood glucose level, as well as how much their efficiency depends on the simultaneously occurring metabolic detection of glucose. Currently, the possible role of the T1R3-mediated reception in pancreas in the glucose homeostasis has only been shown *in vitro* [17–19]. It is also known that the deletion of T1R3 results in decreased glucose tolerance *in vivo* during intragastric load with large doses of glucose (5 g/kg) after long-term starvation [25, 27]. However, there has been only one study analyzing the role of T1R3 during intragastric load, and its results are negative [27]. Therefore, it is believed that the primary mechanism of action of T1R3, besides gustation, is the enhancement of expression of glucose transporters and stimulation of incretin production in intestine. Our comparison of the *Tas1r3*^{−/−} and *Tas1r3*^{+/+} mice showed that, in the euglycemic state (without starvation), the T1R-mediated reception accelerates glucose utilization after both intragastric

and intraperitoneal administration of glucose (Fig. 2). This observation proves the physiological role of the T1R3 receptor protein expressed in β -cells of the pancreatic islet tissue [17–19] and, probably, also in hypothalamic nuclei [24]. On the hypoglycemic background (after 18 h of starvation), T1R3 only affects glucose tolerance after systemic administration, but not after intragastric glucose infusion. The causes of this are rather understandable as the nighttime starvation, which is an unnatural state to rodents, transforms regulation of carbohydrate homeostasis and, in particular, inhibits the basal production of incretins and insulin [37]. In contrast to humans, the insulin-dependent utilization of glucose in mice increases during the long-term starvation [43], and this is why it is considered that glucose load on the starvation-induced hypoglycemic background mainly allows estimating glucose utilization rates in tissues, whereas the production of insulin is better characterized without preliminary starvation [37, 42]. Apparently, hypoglycemia attenuates K_{ATP} -independent mechanisms of sensitivity to glucose. There is evidence that initiation of these mechanisms requires a certain level of cell depolarization that occurs at the closing of K_{ATP} channels. For example, in the culture of the pancreatic islet tissue or MIN6 β -cell line, fructose and non-caloric sweeteners stimulate the production of insulin only if the concentration of glucose in the medium is optimal [17, 19]. Maximal depolarization of β -cells in the presence of a K_{ATP} blocker tolbutamide makes the K_{ATP} -independent mechanisms ineffective [19].

Sweet taste receptors in the intestinal mucosa attract researchers' attention as a possible way for various impacts on appetite, carbohydrate metabolism, and diabetes [44]. Egan and Margolskee [45] suggested that low-caloric sweeteners, via the intestinal mechanisms of sweet taste reception, may produce obesity and metabolic syndrome, as they create an imbalance between the activity of the hormonal system and the intake of nutrients. However, some researchers are quite skeptic about such a possibility, as their own data show that non-caloric substitutes of sugar, unlike sucrose, have no effect on blood glucose levels in humans and mice [21, 31, 32]. The enhancement of expression of glucose transporters GLUT2 and SGLT1 during activation of sweet taste receptors can also potentially be used in preventive care and therapy of malabsorption, diabetes, and obesity [20, 28, 29]. In accordance with classical views, oral administration of glucose triggers greater insulin production and faster glucose utilization than intravenous injection does [46]. This phenomenon, which has been termed the incretin effect, is associated with after-meal secretion of insulinotropic hormones (GLP-1 and GIP) by the intestine enteroendocrine cells. The activity of these hormones is responsible for up to 50% of insulin secretion [47]. The comparison of wild-type and *Tas1r3*^{−/−} mice by the rate of utilization of the same dose of intragastrically or intraperitoneally administered glucose showed that the incretin effect is almost equally pronounced in both genotypes in the euglycemic state, whereas after starvation the effect is observed in neither of them. Therefore, the small number of taste receptor proteins in the enteroendocrine cells of the mouse duodenum and jejunum [23, 31], albeit capable of changing the GLP-1 production in a cell culture or in an isolated gut segment [20, 25, 27, 28], are most probably unable to affect glucose metabolism in the whole organism to any considerable extent. Therefore, the comparison of *Tas1r3*^{−/−} and *Tas1r3*^{+/+} mice also indicates the physiological importance of the T1R3 protein expressed outside of the oral

cavity and intestine, e.g., in the pancreas, and the insignificant role of the T1R3-mediated intestinal reception of sugars *in vivo*.

The lower glucose tolerance is usually accompanied by the lower sensitivity to insulin [48, 49]. A similar tendency was noted in the reported experiments (Fig. 3). Excessive fat deposition cannot account for the lower sensitivity to insulin, as we did not observe any detectable increase in body mass in the *Tas1r3*-knockout strain. On the other hand, the higher postprandial glucose levels in *Tas1r3*^{−/−} mice may cause a decrease in the transcription of insulin substrates via enhanced biosynthesis of hexosamines [50].

Conclusion

This study has confirmed that the *Tas1r3* gene and the T1R3 taste receptor protein subunit encoded by this gene, besides modulation of taste preferences and carbohydrate consumption, also play a significant role in control of glucose metabolism. The T1R3 receptor protein expressed outside of the oral cavity and intestine, presumably in the pancreas as well as in the hypothalamus and possibly in other central nervous system structures, mediates the enhancement of glucose utilization *in vivo*, in addition to basal K_{ATP}-dependent mechanisms. The T1R3 receptor protein expressed in the intestinal mucosa is unlikely to play any prominent role in the control of glucose homeostasis *in vivo*. The physiological efficiency of T1R3-mediated visceral reception of sugars depends on the basal glucose level and is more pronounced in the euglycemic state than after starvation. T1R3 may also be involved in regulation of endogenous production of glucose, as the basal glucose levels after starvation decreased greater in *Tas1r3*^{−/−} mice than in the control group.

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Fig. 1.

The preference of sucrose dissolved in water in C57BL/6ByJ and C57BL/6J-*Tas1r3*^{tm1Rfm} mice (a) in test with the brief access ($n = 15\sim 29$) and (b) in test with prolonged access (the two-bottle test ($n = 12$)). *Abscissa*: glucose concentration (%); *ordinate*: (a) relative consumption (%), (b) preference (%). In Figs. 1–3: solid line, dark symbols and columns—the control strain C57BL/6ByJ; broken line, light symbols and columns—*Tas1r3*-gene-knockout strain C57BL/6J-*Tas1r3*^{tm1Rfm}. Statistical significance of differences between mouse strains: ***- $p < 0.001$, **- $p < 0.01$, *- $p < 0.05$.

Fig. 2. Blood glucose concentrations in the 13–20-week-old C57BL/6ByJ and C57BL/6J–*Tas1r3*^{tm1Rfm} mice after (a, b) intraperitoneal or (c, d) intragastric glucose load of 2 g/kg ($n = 11\sim16$) at testing without food deprivation (a, c) or after 18-h starvation (b, d). In Figs. 2–3: *abscissa*: time after the start of the experiment (min); *ordinate*: blood glucose level (mmol/l).

Fig. 3. Blood glucose concentration in the non-starved C57BL/6ByJ ($n = 19$) and C57BL/6J-*Tas1r3*^{tm1Rfm} ($n = 24$) mice after intraperitoneal administration of 2 MU/kg of insulin. Age of the animals—8–37 weeks. *Solid* and *broken lines*, see Fig. 1.