REVIEW

Role of extracellular proton-sensing OGR1 in regulation of insulin secretion and pancreatic β -cell functions

Chihiro Mogi, Takashi Nakakura and Fumikazu Okajima

Laboratory of Signal Transduction, Institute for Molecular and Cellular Regulation, Gunma University, Maebashi 371-8512, Japan

Abstract. Insulin secretion with respect to pH environments has been investigated for a long time but its mechanism remains largely unknown. Extracellular pH is usually maintained at around 7.4 and, its change has been thought to occur in non-physiological situations. Acidification takes place under ischemic and inflammatory microenvironments, where stimulation of anaerobic glycolysis results in the production of lactic acid. In addition to ionotropic ion channels, such as transient receptor potential V1 (TRPV1) and acid-sensing ion channels (ASICs), metabotropic proton-sensing G protein-coupled receptors (GPCRs) have also been identified recently as proton-sensing machineries. While ionotropic ion channels usually sense strong acidic pH, proton-sensing GPCRs sense pH of 7.6 to 6.0 and have been shown to mediate a variety of biological actions in neutral and mildly acidic pH environments. Studies with receptor knockout mice have revealed that proton-sensing receptors, including ovarian cancer G protein-coupled receptor 1 (OGR1), a proton-sensing GPCRs, play a role in the regulation of insulin secretion and glucose metabolism under physiological conditions. Small molecule 3,5-disubstituted isoxazoles have recently been identified as OGR1 agonists working at neutral pH and have been shown to stimulate pancreatic β -cell differentiation and insulin synthesis. Thus, proton-sensing OGR1 may be an important player for insulin secretion and a potential target for improving β -cell function.

Key words: Proton-sensing GPCRs, OGR1, Insulin secretion, Pancreatic β -cell

Backgrounds: Glucose Metabolism and Insulin Secretion with Respect to pH Environments

CIRCULATING BLOOD pH is strictly maintained at around 7.4 by respiratory and renal compensation mechanisms. Disorders that lower the arterial pH <7.35 are defined as acidosis, whereas disorders with a pH >7.45 are termed alkalosis [1]. In 1975, Yajima and Ui reported that acidosis modifies basal and glucose-stimulated insulin secretion *in vivo* [2, 3]. Thus, when ammonium chloride was administered in rats, the procedure that is used for the induction of acidosis, basal and glucose-stimulated insulin levels in plasma were significantly higher than in control rats. On the contrary, alkalosis inhibited the glucose-stimulated insulin secretion activities *in vivo*. In spite of low insulin

response, however, alkalosis improved glucose tolerance or accelerated glucose utilization. The authors suggested that the change in plasma pH modifies reciprocally the adrenergic regulation of insulin secretion activities: acidosis enhances β -adrenergic receptor functions, whereas alkalosis enhances α -adrenergic receptor function [2]. However, it is also possible that a change in extracellular pH modulates insulin secretion activity in pancreatic β -cells directly rather than indirectly through adrenergic regulation.

There are many reports concerning the effects of extracellular and intracellular pH on insulin secretion *in vitro*, but the results are confusing. Insulin secretion caused by high glucose showed a bell-shaped dependence on extracellular pH with a peak at pH 7.4: the activity at pH 7.7 and 6.5 was about 50% of that of pH 7.4 [4]. A similar bell-shaped response to extracellular pH was confirmed [5]. Change in the extracellular pH is accompanied by a change in the intracellular pH, which has been thought to be a critical factor for insulin secretion. Although it is recognized that high glucose induces an increase in the intracellular pH [6, 7], intracellular acidification has been shown to enhance

Submitted Sep. 15, 2013; Accepted Sep. 17, 2013 as EJ13-0380
Released online in J-STAGE as advance publication Oct. 1, 2013

Correspondence to: Fumikazu Okajima, Laboratory of Signal Transduction, Institute for Molecular and Cellular Regulation, Gunma University, 3-39-15 Showa-machi, Maebashi 371-8512, Japan. E-mail: okajima@gunma-u.ac.jp

glucose-stimulated insulin secretion [8-11]. The inhibition of K_{ATP} channels is a possible mechanism of the intracellular acidic pH effect on insulin secretion [12]; K_{ATP} channels have histidine residues involved in either the inhibition or the activation of the activity in response to a change in the intracellular pH [13, 14]. The beneficial role of acidic pH on insulin secretion is supposed to be mainly based on the action of the Na^+/H^+ exchange blocker: inhibitors of Na^+/H^+ exchange caused the enhancement of glucose-stimulated insulin secretion in association with a decrease in the intracellular pH. However, the specificity of the Na^+/H^+ exchange blocker has recently been questioned [7]. Stiernet *et al.* showed that dimethyl amiloride (DMA), a classic blocker of Na^+/H^+ exchange, did not affect intracellular pH but increased insulin secretion in NHE1 mutant islets, indicating a nonspecific effect. They further showed that, even though intracellular pH was oppositely changed by a different buffer, *i.e.*, HCO_3^- -containing buffer or HEPES buffer without HCO_3^- , in normal and NHE1 mutant islets, no practical change in glucose-stimulated insulin secretion was observed [7]. These results suggest that intracellular pH is not causally related to changes in insulin secretion. Thus, the physiological relevance of a change in intracellular pH to insulin secretion is not established. In any event, previous studies on pH effects were performed based on the assumption that extracellular pH changes may affect insulin secretion activity through the changes in intracellular pH. However, recent studies have revealed that there are extracellular proton-sensing mechanisms in cell membranes in many biological systems.

Proton-sensing Receptors

There are at least two types of proton-sensing mechanisms of cell membranes, which sense extracellular protons and transduce their signal inside the cells. Ionotropic ion channels, such as transient receptor potential V1 (TRPV1) and acid-sensing ion channels (ASICs), have been considered to be proton sensors for pain and nociception because these channels are predominantly expressed on sensory neurons [15, 16]. Recent studies, however, show that these ion channels are expressed on neurons in the central nervous system and non-neuronal cells and play a role in a wide range of biological systems [15-18]. Another type of proton-sensing receptors is the ovarian cancer G protein-coupled receptor 1 (OGR1)-family proton-sensing GPCR. While the ion

channel type of receptors sense strong acidic pH of less than 7.0 to pH 4.0, proton-sensing GPCRs sense neutral and mildly acidic pH of 7.6 to 6.0 through the histidine residues [19-21]. Thus, proton-sensing GPCRs are expected to regulate the physiological events at neutral pH as well. In the present review, we discuss the role of proton-sensing receptors, especially focusing on OGR1, a proton-sensing GPCR, in insulin secretion and glucose metabolism in relation to pH environments. As to the ion channels, we briefly summarize recent observations related to insulin secretion and energy metabolism obtained by using receptor knockout mice.

Proton-sensing ion channels

TRPV1 is one of 28 isotypes of the TRP superfamily and is activated by a diverse range of chemical and noxious stimuli, including protons [22-24]. Thus, proton is not the sole ligand for TRPV1. TRPV1 senses relatively strong acidic pH of below 6 through glutamic acid in the extracellular domain of the channel [22], is predominantly expressed on sensory neurons, and mediates pain and nociceptive signals [15]. In pancreatic β -cells, several types of thermosensitive TRP are expressed and contribute to β -cell function, including insulin secretion, in response to environmental temperature changes [25, 26]. TRPV1 is expressed in pancreatic β -cell lines as well, and capsaicin, a TRPV1 agonist, induces Ca^{2+} influx and insulin secretion [27, 28]. Capsaicin treatment also increases plasma insulin levels *in vivo* [27]. However, it is not generally accepted that islets express TRPV1 [28, 29]. Motter and Ahern have reported that, on a high-fat diet, TRPV1 knockout mice gained significantly less mass and adiposity as compared with wild-type mice [30]. Thus, TRPV1 deficiency protects from diet-induced obesity. Razavi *et al.* reported that knockout of TRPV1 brought improved glucose tolerance due to enhanced insulin sensitivity [29]. These observations are contrary to the finding that the TRPV1 agonist capsaicin stimulates insulin secretion and beneficial actions on adipose tissues [18]. Thus, capsaicin improves glucose tolerance and insulin sensitivity. The improvement of glucose tolerance is, at least partly, explained by glucagon-like peptide 1 (GLP-1) production, increased adiponectin levels, and decreased plasma levels of free fatty acid, triglycerides, and leptin [18, 31]. Thus, although loss-of-function and gain-of-function outcomes on TRPV1 are controversial, the ion channel seems to function under normal conditions. It should be remembered, however,

Fig. 1 Signaling mechanisms of proton-sensing GPCRs. Although overexpression of any proton-sensing receptors causes coupling with the Rho signaling pathways through $G_{12/13}$ proteins, OGR1 is usually coupled with $G_{q/11}$ proteins and phospholipase C (PLC)/ Ca^{2+} signaling pathways, and TDAG8 and GPR4 are coupled with the G_s proteins and adenylyl cyclase (AC)/cAMP pathways in native cells. The proton-sensing role of G2A is in question. See the text for more details.

that TRPV1 is a thermosensitive channel, and its thermosensitivity, rather than its proton sensitivity, may contribute to TRPV1's involvement in insulin secretion and its related metabolism [25].

ASICs are another family of proton-sensing channels. Six ASIC subunit proteins, encoded by four genes, have been identified: ASIC1a, ASIC1b, ASIC2a, ASIC2b, ASIC3, and ASIC4. ASICs are voltage-independent channels that mainly conduct Na^+ [32]. Protons are thought to be the sole physiological ligand for ASICs. Homomeric or heteromeric ASICs exhibit a variety of extracellular pH sensitivities, ranging from the near physiological pH of ~ 7.0 for ASIC3 to the severely acidic pH of ~ 5.0 for ASIC2a [16, 33]. Huang *et al.* showed that ASIC3 is expressed in adipocytes and that ASIC3 knockout mice were protected against age-dependent glucose tolerance with enhanced insulin sensitivity [34]. Thus, ASIC3 deficiency caused a smaller adipocyte size, improved glucose tolerance and insulin sensitivity, and less mitochondrial loss in white adipose tissue in 27-wk-old mice. However, these changes by ASIC3 deficiency were not observed in 9-wk-old mice. Supporting the phenotypes of ASIC3 knockout mice, an ASIC3-selective blocker, APETx2, improved insulin sensitivity in older mice. Although this report has not proved the phenotype change in ASIC3-deficient mice to be due to the lack of extracellular proton signals, mitochondrial loss in aged mice may cause more acidic microenvironments than in young mice. Indeed, mitochondrial dysfunction is shown to increase lactic acid production through the stimulation of anaerobic glucose metabolism [35] and is associated with insulin resistance in the elderly [36]. As to the pancreatic β -cell function,

expression of ASICs on islets and their involvement in insulin secretion have not been reported.

General information regarding proton-sensing GPCRs

OGR1 and its related GPCRs were first identified as receptors for sphingosylphosphorylcholine (SPC) and lysophosphatidylcholine (LPC) and have now been demonstrated to sense extracellular pH or protons, resulting in the stimulation of several types of G proteins and intracellular signaling pathways [see review articles 37-39] (Fig. 1). This family of GPCRs is composed of OGR1, G protein-coupled receptor 4 (GPR4), T cell death-associated gene 8 (TDAG8), and G2 accumulation (G2A). Several histidine residues on the extracellular domain of receptors are identified as sensors of protons [19-21]. Since the pK_a of histidine is around 7, certain histidine residues can be protonated in acidic environments, which, in turn, may change the conformation of the receptors to be coupled with G proteins. Although G2A stimulates Ca^{2+} and Rho signaling pathways when the cells are overexpressed with exogenous G2A [40], whether G2A is functioning as a proton-sensing receptor in the cells natively expressing G2A remains established [41]. Overexpression of any proton-sensing receptors causes coupling with the Rho signaling pathways through the $G_{12/13}$ proteins. In native cells, however, OGR1 is coupled with $G_{q/11}$ proteins and phospholipase C/ Ca^{2+} signaling pathways, and TDAG8 and GPR4 are coupled with the G_s proteins and adenylyl cyclase/cAMP pathways [37-39] (Fig. 1).

While proton-sensing ion channels are expressed mainly on sensory neurons, proton-sensing GPCRs are expressed not only in neurons but also in a vari-

ety of cell types. In fact, OGR1 has been reported to be involved in a variety of physiologically important responses to extracellular acidification using a knock-down strategy of the receptor in several types of cells *in vitro*, e.g., receptor activator of nuclear factor- κ B ligand (RANKL)-induced differentiation in osteoclasts [42], Ca^{2+} mobilization, cyclooxygenase (COX)-2 induction, and prostaglandin synthesis in osteoblasts [43, 44] and vascular smooth muscle cells [45, 46], and IL-6 and connective tissue growth factor production in airway smooth muscle cells [47-49]. Similarly, TDAG8 has been shown to mediate acidic pH-induced inhibition of apoptosis in eosinophils [50], inflammatory cytokine production in macrophages [51], and O_2^- production in neutrophils [52]. As for GPR4, this receptor subtype has been reported to mediate acidic-pH-induced stimulation of monocyte adhesion in association with the expression of VCAM-1 [53] and a number of inflammatory genes [54]. In these previous studies, proton-sensing GPCRs mediate responses to acidic environments of less than pH 7.0, which may be attained under inflammatory lesions [39]. Studies using knockout mice have suggested the involvement of host cell OGR1 in tumorigenesis [55] and GPR4 in reduced angiogenesis and tumor growth *in vivo* as well [56]. In tumors, acidification of less than pH 6.0 has been reported to occur [57]. However, recent studies suggested that the OGR1 family of GPCRs is also involved in the physiological events as well. For example, GPR4 deficiency causes vascular abnormality during development [58] and decreased net acid secretion by the kidney [59], and OGR1 deficiency enhances pH-dependent proton extrusion in proximal tubules from the kidney [60] and changes insulin secretion and insulin sensitivity [61]. In the following sections, we will focus on the role of OGR1 in insulin secretion and β -cell differentiation. For further information about proton-sensing GPCRs in expression profile, signal transduction mechanism, and their functions other than insulin secretion and β -cell functions, refer to our review articles [37, 39].

Role of OGR1 in Insulin Secretion and Its Related Metabolism

In vivo analysis

In the fasting state, there were no significant differences in the basal plasma insulin and glucose levels before glucose administration between wild-type and *OGR1*^{-/-} mice [61]. In order to evaluate the role of

OGR1 on insulin secretion activity and insulin sensitivity, a glucose tolerance test was performed. Insulin levels after glucose administration were significantly lower in OGR1-deficient mice than in WT mice. In spite of the lower insulin level, blood glucose was not appreciably changed or slightly decreased rather than increased by OGR1 deficiency. To further elucidate the role of OGR1 on glucose metabolism, the effects of OGR1 deficiency on the ability of insulin to decrease blood glucose (insulin sensitivity) and of pyruvate to increase blood glucose (gluconeogenesis activity) were evaluated. OGR1 deficiency enhanced insulin sensitivity but did not change gluconeogenesis activity. The basal plasma glucagon level before glucose injection was significantly lower in OGR1-deficient mice than in WT mice. These results suggest that increased insulin sensitivity to glucose utilization and the lower level of plasma glucagon may explain the masking of the expected hyperglycemia due to impaired insulin secretion in OGR1-deficient mice.

The finding that OGR1 deficiency increases insulin sensitivity means that proton/OGR1 is inhibitory for glucose utilization. Among the tissues and organs involved in glucose metabolism, OGR1 is expressed in adipose tissues but not in liver and skeletal muscle [61]. This reminds us the similarity of the phenotypes of knockout mice of ASIC3, which improve glucose tolerance and insulin sensitivity probably through the change in adipocyte functions [34]. In OGR1-deficient mice, adipose tissue weight is lower than that in WT mice (unpublished results). Thus, proton environments of adipose tissues may regulate adipocyte functions and, finally, whole body glucose utilization activity through OGR1, although the mechanism underlying it remains unknown. With respect to glucose utilization, the inhibitory role of proton/OGR1 in glucose utilization might be related to the finding of Yajima and Ui that alkalosis is stimulatory, whereas acidosis is inhibitory, for glucose utilization [2, 3]. According to Nakakura *et al.* [61], OGR1 seems to be inhibitory for glucose utilization at normal or physiological pH values. If so, alkalosis may reduce OGR1 activity by decreasing proton or ligand concentrations and thereby de-inhibit or stimulate glucose utilization activity or insulin sensitivity.

In vitro analysis of insulin secretion

Ohta *et al.* previously reported that glucose-induced insulin secretion exhibited a bell-shaped dependence on extracellular pH with a peak value at pH 7.4 in rat islets

[4]. Nakakura *et al.* also observed a similar bell-shaped dose response curve of protons in mouse islets, although the peak was attained at pH 7.0 to 6.8 [61]. Thus, glucose-stimulated insulin secretion activity was increased from pH 8.0 to 7.0. The role of OGR1 was examined using islets from *OGR1*^{-/-} mice. Insulin secretion activity was inhibited by OGR1 deficiency depending on the extracellular pH; insulin secretion at pH 7.4 to 7.0, but not at 8.0, was significantly suppressed by OGR1 deficiency. Insulin secretion induced by KCl and tolbutamide was also significantly inhibited even in the absence of high glucose, whereas that induced by several insulin secretagogues, including vasopressin, a GLP-1 receptor agonist, and forskolin, was not suppressed by OGR1 deficiency. Moreover, glucose-induced ATP production was not affected by extracellular pH and OGR1 deficiency. Thus, glucose metabolism and insulin secretion machinery in islets seem to be unchanged by OGR1 deficiency. The inhibition of insulin secretion was associated with the reduction of a glucose-induced increase in the intracellular Ca^{2+} concentration mostly depending on Ca^{2+} influx. The acidic pH-induced insulin secretion was inhibited by YM-254590 and 2-APB, suggesting mediation by $\text{G}_{\text{q/11}}$ proteins and inositol trisphosphate (InsP_3) channels. These results suggest that the OGR1/ $\text{G}_{\text{q/11}}$ protein pathway is activated by extracellular protons existing in the physiological extracellular pH 7.4 and further stimulated by acidification, resulting in the enhancement of insulin secretion in response to high glucose concentrations and KCl. (Fig. 2, the upper side). Thus, the OGR1/ $\text{G}_{\text{q/11}}$ protein signaling pathway may affect the process(es) of K_{ATP} channels and/or voltage-dependent Ca^{2+} channels; however, the precise mechanism remains unknown [61].

Although extracellular acidic pH-induced insulin secretion is dependent on OGR1, extracellular acidification significantly enhanced the insulin secretion even in OGR1-deficient islets, suggesting the additional mechanism of OGR1-independent pH action. TDAG8 and GPR4 are expressed in islets; however, the pH-induced enhancement of insulin secretion was not affected by the knockout of these proton-sensing GPCRs (unpublished observation). In islets, neither TRPV1 nor ASIC expression has been reported, although β -cell lines express TRPV1 and its stimulation causes insulin secretion [27, 28]. The proton-sensing receptor-independent insulin secretion may be related to intracellular acidification as described in the introduction. Intracellular acidification has a potential

ability to inhibit K_{ATP} channels and, thereby, to induce depolarization of the cells [12, 14].

OGR1 deficiency inhibited insulin secretion at neutral pH 7.4, suggesting that OGR1 is functioning at a physiological pH. One main concern is that OGR1 is practically active at physiological pH 7.4. Most of previous studies on the role of OGR1 were observed at pathophysiological acidic pH of less than pH 7.0 [39]. For example, COX-2 expression and PGE_2 production in osteoclasts [43] and IL-6 and connective tissue growth factor (CTGF) production in airway smooth muscle cells [47, 48] in response to acidic pH are observed at pH 6.8 to 6.3. The activation of OGR1 at neutral pH 7.4, however, is not unusual because a remarkable activation of OGR1 at pH 7.4 is observed in CHO cells and in HEK293 cells, in which OGR1 expression was forced [19, 62]. These differences in the pH sensitivity of OGR1 between cell types may simply reflect differences in the expression levels of OGR1 and its coupling efficiency to G proteins and subsequent signaling pathways.

It should be noted, however, that the pH of insulin secretory granules is maintained at 5.5 to 5.0 with the assistance of vacuolar ATPase (V-ATPase) [63]. Acidic granules are necessary for the processing of insulin and the exocytosis of insulin secretion [64, 65]. The activity of V-ATPase is regulated by intracellular pH; interestingly, an increase in the intracellular pH stimulates V-ATP activity, resulting in acidification of the granules [66]. Upon insulin secretion, protons may be released simultaneously into the extracellular space [67]. Thus, we can speculate that a quite high level of protons may stimulate OGR1 in the vicinity of the insulin secretion site (Fig. 2), although this speculation has not been proved.

Role of OGR1 in β -cell Differentiation and Insulin Synthesis

Schneider and his colleagues have identified 3,5-disubstituted isoxazoles (Isx) by screening a chemical library based on activation of the gene encoding the homeodomain transcription factor, NK2 transcription factor-related, locus 5 (NKx2.5), in mouse pluripotent stem cells [68]. They also found that Isx increased $[\text{Ca}^{2+}]_i$ in Notch-activated epicardium-derived cells (NECs) in a manner sensitive to inhibitors for phospholipase C and InsP_3 channels, suggesting a $\text{G}_{\text{q/11}}$ protein mediation [69]. They tested the hypothesis that Isx activate GPCRs by screening a library of recom-

Fig. 2 Postulated roles of OGR1 in insulin secretion and synthesis in pancreatic β -cells. Glucose-stimulated insulin secretion is thought to be as follows. Glucose is metabolized and finally ATP is generated in β -cells. This results in closure of the K_{ATP} channel and membrane depolarization, which causes the opening of voltage-dependent Ca^{2+} channel (VDCC) and stimulation of the Ca^{2+} influx leading to insulin secretion. OGR1 activated by neutral or mildly acidic pH (pH 7.4–7.0) stimulates the process(es) of the K_{ATP} channel and/or VDCC and, thereby, enhances glucose-stimulated insulin secretion through the phospholipase C (PLC)/ Ca^{2+} signaling pathways (the upper side of the figure). Even at neutral pH, OGR1 can be activated by small molecular compounds (3,5-disubstituted isoxazoles, or Isx) and stimulate insulin synthesis through a mechanism involving extracellular signal-regulated kinase (ERK) and p300 histone acetyltransferase (HAT) (the lower side of the figure). Since the pH in insulin granules is acidified by vacuolar ATPase (V-ATPase), protons can be released simultaneously with insulin. Thus, even though circulating blood pH is maintained at 7.4, OGR1 may be activated by high levels of protons in the vicinity of the secretory site. See the text for more details.

binant GPCR-overexpressing cell lines in a functional assay based on $[Ca^{2+}]_i$ increase and found that Isx activate only the OGR1-expressing cell line among 158 different GPCR-expressing cell lines [69]. Moreover, knockdown of OGR1 resulted in the attenuation of Isx-induced $[Ca^{2+}]_i$ increase, NKx2.5 activity, and biosynthesis and assembly of α -actinin pre-sarcomeres [69].

It has already been found that Isx have strong neurogenic activity with the expression of neurogenic transcription factors, including neurogenic differentiation 1 (NeuroD1) [70], which is critical for the development of the pancreas and for insulin production in pancreatic β -cells. Based on these findings, Dioum *et al.* characterized Isx actions and their mechanisms in the differentiation of β -cells and insulin production in human cadaveric islets and MIN6 pancreatic β -cells [71]. They found that Isx stimulate the expression of insulin and its secretion in response to high glucose, which was associated with several transcription factors important for β -cell differentiation and insulin synthesis, in primary cultured human islets. Transcription factors included NeuroD1, MafA, PDX-1, paired box gene (Pax) 6, Pax4, Nkx6.1, Nkx2.2, forkhead box A2 (Foxa2), and Ngn3. Isx also stimulate the expression of insulin, glut2, and

glucokinase. Similar effects of Isx were observed in MIN6 β -cells. Thus, Isx stimulated the expression of transcription factors, *e.g.*, NeuroD1 and MafA, and insulin secretion in response to glucose, amino acids, and GLP-1 agonist. Isx caused biphasic activation of ERK1/2 and increased bulk histone acetylation. Although there was little effect on histone deacetylase activity, Isx increased histone acetyl transferase activity in nuclear extracts. Reconstitution assays indicated that Isx increased the activity of the histone acetyl transferase p300 through an ERK1/2-dependent mechanism. The authors concluded that Isx stimulates ERK and histone acetyl transferase activity and, thereby, stimulates several transcription factors critical for β -cell differentiation through phosphorylation and acetylation [71]. Since a target of Isx is now identified to be OGR1 [69], the results suggest that OGR1 may be involved in the stimulation of intracellular signaling pathways leading to the activation of transcription factors for insulin synthesis and β -cell differentiation (Fig. 2, the lower side).

They had also found that Isx drive cardiomyogenic and pro-survival transcription programs in myocardial infarction models [69, 72] and promote neurogenesis in young mice *in vivo* [73]. The small molecules also

inhibit proliferation and induce neuronal gene expression in malignant astrocytes [74]. These results suggest that OGR1 may be involved in cardiomyogenesis and neurogenesis in acidic microenvironments as well. However, the possibility still cannot be ruled out that Isx affect receptors and intracellular signaling molecules other than OGR1. Isx stimulate ERK activity [71] in addition to Ca^{2+} mobilization [69]. OGR1 is coupled with the $\text{G}_{q/11}$ protein and Ca^{2+} signaling pathway and has been shown to activate ERK at least in airway smooth muscle cells [47, 49] and Ca^{2+} mobilization in several cell types [39]. OGR1 deficiency in mice resulted in no apparent change in the morphology of islets, number of islets, insulin content, or mRNA expression of insulin, glucagon, *glut2*, *Kir6.2*, and *SUR1* [61], whereas Isx stimulated insulin production and mRNA expression of insulin and *glut2* [71]. However, it is not always necessary to obtain opposite results in gain-of-function and loss-of-function: $\text{G}_{q/11}$ protein-coupled receptors, such as M3 muscarinic receptors, may compensate for the loss of OGR1. As for insulin secretion, the experiment for glucose-stimulated insulin secretion was always carried out at least 24 h after treatment with Isx [71], whereas acidification effects were observed with simultaneous treatment with glucose for 30 to 60 min [61]. It would be interesting to examine whether Isx stimulate insulin secretion in a manner sensitive to OGR1, as was case for extracellular acidification. The following two observations are not simply explained by the hypothesis that OGR1 is the sole target of Isx for diverse actions. Isx-induced increase in $[\text{Ca}^{2+}]_i$ in neural stem cells (NSCs) was completely blocked by nifedipine, an L-type Ca^{2+} channel blocker and MK801, a specific N-methyl-D-aspartic acid (NMDA) receptor antagonist, suggesting Ca^{2+} influx through Ca^{2+} channels but not intracellular Ca^{2+} mobilization through InsP_3 [70]. Moreover, acidic pH failed to stimulate NKx2.5 activity, whereas Isx stimulated it depending on the extracellular pH in the NECs [69]. Thus, although there is uncertainty in the specificity of Isx, the small molecules with antidiabetic activity provide a tool not only for exploring islet functions but also for further characterizing the role of OGR1 in biological actions just like capsaicin for TRPV1 functions.

Conclusion and Perspective

Since extracellular pH is maintained at around 7.4 under physiological conditions, proton-sensing receptors have been speculated to play a role in pathophys-

iological situations, such as ischemia and inflammation, where extracellular pH reaches below 6.0. Indeed, recent studies have revealed that severe acidosis-induced pain and nociception are mediated by TRPV1 and ASICs, reflecting their predominant expression in sensory neurons; and a wide range of inflammatory responses and related responses are mediated by proton-sensing GPCRs, reflecting their coupling with a variety of intracellular signaling pathways. In addition, proton-sensing receptors, especially proton-sensing OGR1 and ASIC3, sense near-physiological pH and have been proved to function in physiological events, such as insulin secretion and/or normal glucose metabolism, as described in the present review. Although a TRPV1 agonist capsaicin exerts diverse responses, including glucose metabolism and insulin secretion, whether capsaicin-induced actions are related to and reproduced by extracellular protons remains unknown. TRPV1 is thermosensitive, and its thermosensitivity, rather than its proton sensitivity, may be involved in the actions observed in the knock-out mice *in vivo*.

OGR1 deficiency influenced insulin secretion and glucose metabolism under near-physiological conditions such as glucose tolerance tests. This suggests that proton-sensing OGR1 is activated at physiological pH 7.4. However, an alternative possibility was presented: OGR1 activation may be accelerated by protons released simultaneously with insulin from secretory granules in which pH is maintained at 5.5 to 5.0. It would be interesting to extend the hypothesis to neurons, where protons may be released with neurotransmitters into synapses and act on proton-sensing receptors in post-synapses in the nervous system. Proton-sensing GPCRs are expressed in a wide range of cell types, including neurons.

Acidic pH still significantly enhances glucose-stimulated insulin secretion, even in OGR1-deficient islets. This suggests that there is another mechanism, other than OGR1, to regulate insulin secretion with respect to proton environments. It is unlikely, however, that TRPV1, ASICs, and proton-sensing GPCRs other than OGR1 are involved in the secretion on insulin based on their expression profiles and knockout phenotypes. Intracellular pH change is potentially involved in insulin secretion. As to the change in insulin sensitivity, the roles of proton-sensing receptors are mostly left unclear except for the possible involvement of adipose tissues.

Finally, it is quite exciting that OGR1 agonists Isx stimulate differentiation not only of pancreatic β -cells but also of progenitor or stem cells of neurons and cardiomyocytes. At present, however, the possibility cannot be ruled out that Isx may have targets other than OGR1. It is a subject urgently needing to be clarified. Apart from the specificity of the compounds, the finding that Isx stimulate the differentiation of β -cells and insulin secretion suggests that Isx are lead compounds for improving β -cell function in diabetics.

Acknowledgments

This work was supported by grants from Grant-in-Aid for Scientific Research (B)(21390016), Grant-in-Aid for Challenging Exploratory Research (22659014, 23659029, 25670617), and Grant-in-Aid for Scientific Research on Innovative Areas (23112503) from the Japan Society for the Promotion of Science (JSPS).

Disclosure

The authors have no conflict of interest.

References

1. Ayers P, Dixon C (2012) Simple acid-base tutorial. *JPEN J Parenter Enteral Nutr* 36: 18-23.
2. Yajima M, Ui M (1975) Carbohydrate metabolism and its response to catecholamines as modified in alkalotic rat. *Am J Physiol* 228: 1046-1052.
3. Yajima M, Ui M (1975) Hydrocortisone restoration of the pH-dependent metabolic responses to catecholamines. *Am J Physiol* 228: 1053-1059.
4. Ohta M, Nelson D, Nelson J, Meglasson MD, Erecinska M (1991) Relationships between energy level and insulin secretion in isolated rat islets of Langerhans. A study at various pH values. *Biochem Pharmacol* 42: 593-598.
5. Hyder A, Laue C, Schrezenmeir J (2001) Effect of extracellular pH on insulin secretion and glucose metabolism in neonatal and adult rat pancreatic islets. *Acta Diabetol* 38: 171-178.
6. Juntti-Berggren L, Arkhammar P, Nilsson T, Rorsman P, Berggren PO (1991) Glucose-induced increase in cytoplasmic pH in pancreatic beta-cells is mediated by Na^+/H^+ exchange, an effect not dependent on protein kinase C. *J Biol Chem* 266: 23537-23541.
7. Stiernet P, Nenquin M, Moulin P, Jonas JC, Henquin JC (2007) Glucose-induced cytosolic pH changes in beta-cells and insulin secretion are not causally related: studies in islets lacking the Na^+/H^+ exchanger NHE1. *J Biol Chem* 282: 24538-24546.
8. Best L, Bone EA, Meats JE, Tomlinson S (1988) Is intracellular pH a coupling factor in nutrient-stimulated pancreatic islets? *J Mol Endocrinol* 1: 33-38.
9. Gunawardana SC, Sharp GW (2002) Intracellular pH plays a critical role in glucose-induced time-dependent potentiation of insulin release in rat islets. *Diabetes* 51: 105-113.
10. Gunawardana SC, Rocheleau JV, Head WS, Piston DW (2004) Nutrient-stimulated insulin secretion in mouse islets is critically dependent on intracellular pH. *BMC Endocr Disord* 4: 1.
11. Nabe K, Fujimoto S, Shimodahira M, Kominato R, Nishi Y et al. (2006) Diphenylhydantoin suppresses glucose-induced insulin release by decreasing cytoplasmic H^+ concentration in pancreatic islets. *Endocrinology* 147: 2717-2727.
12. Misler S, Gillis K, Tabcharani J (1989) Modulation of gating of a metabolically regulated, ATP-dependent K^+ channel by intracellular pH in B cells of the pancreatic islet. *J Membr Biol* 109: 135-143.
13. Xu H, Cui N, Yang Z, Wu J, Giwa LR et al. (2001) Direct activation of cloned $\text{K}(\text{atp})$ channels by intracellular acidosis. *J Biol Chem* 276: 12898-12902.
14. Xu H, Wu J, Cui N, Abdulkadir L, Wang R et al. (2001) Distinct histidine residues control the acid-induced activation and inhibition of the cloned $\text{K}(\text{ATP})$ channel. *J Biol Chem* 276: 38690-38696.
15. Morales-Lazaro SL, Simon SA, Rosenbaum T (2013) The role of endogenous molecules in modulating pain through transient receptor potential vanilloid 1 (TRPV1). *J Physiol* 591: 3109-3121.
16. Wemmie JA, Taugher RJ, Kreple CJ (2013) Acid-sensing ion channels in pain and disease. *Nat Rev Neurosci* 14: 461-471.
17. Ho KW, Ward NJ, Calkins DJ (2012) TRPV1: a stress response protein in the central nervous system. *Am J Neurodegener Dis* 1: 1-14.
18. Zsombok A (2013) Vanilloid receptors--do they have a role in whole body metabolism? Evidence from TRPV1. *J Diabetes Complications* 27: 287-292.
19. Ludwig MG, Vanek M, Guerini D, Gasser JA, Jones CE et al. (2003) Proton-sensing G-protein-coupled receptors. *Nature* 425: 93-98.
20. Wang JQ, Kon J, Mogi C, Tobo M, Damirin A et al. (2004) TDAG8 is a proton-sensing and psychosine-sensitive G-protein-coupled receptor. *J Biol Chem* 279: 45626-45633.
21. Liu JP, Nakakura T, Tomura H, Tobo M, Mogi C et

- al. (2010) Each one of certain histidine residues in G-protein-coupled receptor GPR4 is critical for extracellular proton-induced stimulation of multiple G-protein-signaling pathways. *Pharmacol Res* 61: 499-505.
22. Tominaga M, Tominaga T (2005) Structure and function of TRPV1. *Pflugers Arch* 451: 143-150.
23. Banner KH, Igney F, Poll C (2011) TRP channels: emerging targets for respiratory disease. *Pharmacol Ther* 130: 371-384.
24. Lee LY, Gu Q, Xu F, Hong JL (2013) Acid-sensing by airway afferent nerves. *Pulm Pharmacol Ther* 26: 491-497
25. Uchida K, Tominaga M (2011) The role of thermosensitive TRP (transient receptor potential) channels in insulin secretion. *Endocr J* 58: 1021-1028.
26. Zhu Z, Luo Z, Ma S, Liu D (2011) TRP channels and their implications in metabolic diseases. *Pflugers Arch* 461: 211-223.
27. Akiba Y, Kato S, Katsube K, Nakamura M, Takeuchi K et al. (2004) Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet beta cells modulates insulin secretion in rats. *Biochem Biophys Res Commun* 321: 219-225.
28. Jabin Fagelskiold A, Kannisto K, Bostrom A, Hadrovic B, Farre C et al. (2012) Insulin-secreting INS-1E cells express functional TRPV1 channels. *Islets* 4: 56-63.
29. Razavi R, Chan Y, Afifyan FN, Liu XJ, Wan X et al. (2006) TRPV1+ sensory neurons control beta cell stress and islet inflammation in autoimmune diabetes. *Cell* 127: 1123-1135.
30. Motter AL, Ahern GP (2008) TRPV1-null mice are protected from diet-induced obesity. *FEBS Lett* 582: 2257-2262.
31. Wang P, Yan Z, Zhong J, Chen J, Ni Y et al. (2012) Transient receptor potential vanilloid 1 activation enhances gut glucagon-like peptide-1 secretion and improves glucose homeostasis. *Diabetes* 61: 2155-2165.
32. Lingueglia E (2007) Acid-sensing ion channels in sensory perception. *J Biol Chem* 282: 17325-17329.
33. Wu WL, Cheng CF, Sun WH, Wong CW, Chen CC (2012) Targeting ASIC3 for pain, anxiety, and insulin resistance. *Pharmacol Ther* 134: 127-138.
34. Huang SJ, Yang WS, Lin YW, Wang HC, Chen CC (2008) Increase of insulin sensitivity and reversal of age-dependent glucose intolerance with inhibition of ASIC3. *Biochem Biophys Res Commun* 371: 729-734.
35. Noda M, Yamashita S, Takahashi N, Eto K, Shen LM et al. (2002) Switch to anaerobic glucose metabolism with NADH accumulation in the beta-cell model of mitochondrial diabetes. Characteristics of betaHC9 cells deficient in mitochondrial DNA transcription. *J Biol Chem* 277: 41817-41826.
36. Petersen KF, Befroy D, Dufour S, Dziura J, Ariyan C et al. (2003) Mitochondrial dysfunction in the elderly: possible role in insulin resistance. *Science* 300: 1140-1142.
37. Tomura H, Mogi C, Sato K, Okajima F (2005) Proton-sensing and lysolipid-sensitive G-protein-coupled receptors: a novel type of multi-functional receptors. *Cell Signal* 17: 1466-1476.
38. Meyer zu Heringdorf D, Jakobs KH (2007) Lysophospholipid receptors: signalling, pharmacology and regulation by lysophospholipid metabolism. *Biochim Biophys Acta* 1768: 923-940.
39. Okajima F (2013) Regulation of inflammation by extracellular acidification and proton-sensing GPCRs. *Cell Signal* 25: 2263-2271.
40. Murakami N, Yokomizo T, Okuno T, Shimizu T (2004) G2A is a proton-sensing G-protein-coupled receptor antagonized by lysophosphatidylcholine. *J Biol Chem* 279: 42484-42491.
41. Radu CG, Nijagal A, McLaughlin J, Wang L, Witte ON (2005) Differential proton sensitivity of related G protein-coupled receptors T cell death-associated gene 8 and G2A expressed in immune cells. *Proc Natl Acad Sci U S A* 102: 1632-1637.
42. Komarova SV, Pereverzev A, Shum JW, Sims SM, Dixon SJ (2005) Convergent signaling by acidosis and receptor activator of NF-kappaB ligand (RANKL) on the calcium/calciueurin/NFAT pathway in osteoclasts. *Proc Natl Acad Sci U S A* 102: 2643-2648.
43. Tomura H, Wang JQ, Liu JP, Komachi M, Damirin A et al. (2008) Cyclooxygenase-2 expression and prostaglandin E2 production in response to acidic pH through OGR1 in a human osteoblastic cell line. *J Bone Miner Res* 23: 1129-1139.
44. Wang J, Sun Y, Tomura H, Okajima F (2012) Ovarian cancer G-protein-coupled receptor 1 induces the expression of the pain mediator prostaglandin E2 in response to an acidic extracellular environment in human osteoblast-like cells. *Int J Biochem Cell Biol* 44: 1937-1941.
45. Tomura H, Wang JQ, Komachi M, Damirin A, Mogi C et al. (2005) Prostaglandin I(2) production and cAMP accumulation in response to acidic extracellular pH through OGR1 in human aortic smooth muscle cells. *J Biol Chem* 280: 34458-34464.
46. Liu JP, Komachi M, Tomura H, Mogi C, Damirin A et al. (2010) Ovarian cancer G protein-coupled receptor 1-dependent and -independent vascular actions to acidic pH in human aortic smooth muscle cells. *Am J Physiol Heart Circ Physiol* 299: H731-742.
47. Ichimonji I, Tomura H, Mogi C, Sato K, Aoki H et al. (2010) Extracellular acidification stimulates IL-6 production and Ca(2+) mobilization through proton-sensing OGR1 receptors in human airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 299: L567-577.
48. Matsuzaki S, Ishizuka T, Yamada H, Kamide Y, Hisada T et al. (2011) Extracellular acidification induces connective tissue growth factor production through proton-sensing receptor OGR1 in human airway smooth mus-

- cle cells. *Biochem Biophys Res Commun* 413: 499-503.
49. Saxena H, Deshpande DA, Tiegs BC, Yan H, Battafarano RJ et al. (2012) The GPCR OGR1 (GPR68) mediates diverse signalling and contraction of airway smooth muscle in response to small reductions in extracellular pH. *Br J Pharmacol* 166: 981-990.
 50. Kottyan LC, Collier AR, Cao KH, Niese KA, Hedgebeth M et al. (2009) Eosinophil viability is increased by acidic pH in a cAMP- and GPR65-dependent manner. *Blood* 114: 2774-2782.
 51. Mogi C, Tobo M, Tomura H, Murata N, He XD et al. (2009) Involvement of proton-sensing TDAG8 in extracellular acidification-induced inhibition of proinflammatory cytokine production in peritoneal macrophages. *J Immunol* 182: 3243-3251.
 52. Murata N, Mogi C, Tobo M, Nakakura T, Sato K et al. (2009) Inhibition of superoxide anion production by extracellular acidification in neutrophils. *Cell Immunol* 259: 21-26.
 53. Chen A, Dong L, Leffler NR, Asch AS, Witte ON, Yang LV (2011) Activation of GPR4 by acidosis increases endothelial cell adhesion through the cAMP/Epac pathway. *PLoS One* 6: e27586.
 54. Dong L, Li Z, Leffler NR, Asch AS, Chi JT, Yang LV (2013) Acidosis activation of the proton-sensing GPR4 receptor stimulates vascular endothelial cell inflammatory responses revealed by transcriptome analysis. *PLoS One* 8: e61991.
 55. Yan L, Singh LS, Zhang L, Xu Y (2014) Role of OGR1 in myeloid-derived cells in prostate cancer. *Oncogene* 33: 157-164.
 56. Wyder L, Suply T, Ricoux B, Billy E, Schnell C et al. (2011) Reduced pathological angiogenesis and tumor growth in mice lacking GPR4, a proton sensing receptor. *Angiogenesis* 14: 533-544.
 57. Wike-Hooley JL, Haveman J, Reinhold HS (1984) The relevance of tumour pH to the treatment of malignant disease. *Radiother Oncol* 2: 343-366.
 58. Yang LV, Radu CG, Roy M, Lee S, McLaughlin J et al. (2007) Vascular abnormalities in mice deficient for the G protein-coupled receptor GPR4 that functions as a pH sensor. *Mol Cell Biol* 27: 1334-1347.
 59. Sun X, Yang LV, Tiegs BC, Arend LJ, McGraw DW et al. (2010) Deletion of the pH sensor GPR4 decreases renal acid excretion. *J Am Soc Nephrol* 21: 1745-1755.
 60. Mohebbi N, Benabbas C, Vidal S, Daryadel A, Bourgeois S et al. (2012) The proton-activated G protein coupled receptor OGR1 acutely regulates the activity of epithelial proton transport proteins. *Cell Physiol Biochem* 29: 313-324.
 61. Nakakura T, Mogi C, Tobo M, Tomura H, Sato K et al. (2012) Deficiency of proton-sensing ovarian cancer G protein-coupled receptor 1 attenuates glucose-stimulated insulin secretion. *Endocrinology* 153: 4171-4180.
 62. Mogi C, Tomura H, Tobo M, Wang JQ, Damirin A et al. (2005) Sphingosylphosphorylcholine antagonizes proton-sensing ovarian cancer G-protein-coupled receptor 1 (OGR1)-mediated inositol phosphate production and cAMP accumulation. *J Pharmacol Sci* 99: 160-167.
 63. Urbe S, Dittie AS, Tooze SA (1997) pH-dependent processing of secretogranin II by the endopeptidase PC2 in isolated immature secretory granules. *Biochem J* 321 (Pt 1): 65-74.
 64. Sun-Wada GH, Toyomura T, Murata Y, Yamamoto A, Futai M, Wada Y (2006) The $\alpha 3$ isoform of V-ATPase regulates insulin secretion from pancreatic beta-cells. *J Cell Sci* 119: 4531-4540.
 65. Louagie E, Taylor NA, Flamez D, Roebroek AJ, Bright NA et al. (2008) Role of furin in granular acidification in the endocrine pancreas: identification of the V-ATPase subunit Ac45 as a candidate substrate. *Proc Natl Acad Sci U S A* 105: 12319-12324.
 66. Dechant R, Binda M, Lee SS, Pelet S, Winderickx J, Peter M (2010) Cytosolic pH is a second messenger for glucose and regulates the PKA pathway through V-ATPase. *EMBO J* 29: 2515-2526.
 67. Luckie DB, Wilterding JH, Maleszewski JJ, Hobbs EC, Olson LK (2002) Extracellular acidification parallels insulin secretion in INS-1 and HIT-T15 beta-cell lines. *Biochem Biophys Res Commun* 293: 1168-1173.
 68. Sadek H, Hannack B, Choe E, Wang J, Latif S et al. (2008) Cardiogenic small molecules that enhance myocardial repair by stem cells. *Proc Natl Acad Sci U S A* 105: 6063-6068.
 69. Russell JL, Goetsch SC, Aguilar HR, Coe H, Luo X et al. (2012) Regulated expression of pH sensing G Protein-coupled receptor-68 identified through chemical biology defines a new drug target for ischemic heart disease. *ACS Chem Biol* 7: 1077-1083.
 70. Schneider JW, Gao Z, Li S, Farooqi M, Tang TS et al. (2008) Small-molecule activation of neuronal cell fate. *Nat Chem Biol* 4: 408-410.
 71. Dioum EM, Osborne JK, Goetsch S, Russell J, Schneider JW, Cobb MH (2011) A small molecule differentiation inducer increases insulin production by pancreatic beta cells. *Proc Natl Acad Sci U S A* 108: 20713-20718.
 72. Russell JL, Goetsch SC, Aguilar HR, Frantz DE, Schneider JW (2012) Targeting native adult heart progenitors with cardiogenic small molecules. *ACS Chem Biol* 7: 1067-1076.
 73. Schneider JW, Goetsch SC, Leng X, Ludwig SM, Russell JL et al. (2012) Coupling hippocampal neurogenesis to brain pH through proneurogenic small molecules that regulate proton sensing G protein-coupled receptors. *ACS Chem Neurosci* 3: 557-568.
 74. Zhang L, Li P, Hsu T, Aguilar HR, Frantz DE et al. (2011) Small-molecule blocks malignant astrocyte proliferation and induces neuronal gene expression. *Differentiation* 81: 233-242.