Role of Na/H exchange in insulin secretion by islet cells

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Purpose of review
Sodium/hydrogen exchangers (NHEs) are a large family of transport proteins catalyzing the exchange of cations for protons across lipid bilayer membranes. Several isoforms are expressed in β cells of the endocrine pancreas, including the recently discovered and poorly characterized isoform NHA2. This review will summarize advances in our understanding of the roles of NHEs in the regulation of insulin secretion in β cells.

Recent findings
Plasmalemmal full-length NHE1 defends β cells from intracellular acidification, but has no role in stimulus-secretion coupling and is not causally involved in glucose-induced alkalinization of the β cell. The function of a shorter NHE1 splice variant, which localizes to insulin-containing large dense core vesicles, remains currently unknown. In contrast, in-vitro and in-vivo studies indicate that the NHA2 isoform is required for insulin secretion and clathrin-mediated endocytosis in β cells.

Summary
Recent data highlight the importance of NHEs in the regulation of cellular pH, clathrin-mediated endocytosis and insulin secretion in β cells. Based on these studies, a pathophysiological role of NHEs in human disorders of the endocrine pancreas seems likely and should be investigated.

Keywords
β cell, insulin, islet, NHE, sodium/hydrogen exchanger

INTRODUCTION

Sodium/hydrogen exchangers (NHEs) are membrane transport proteins catalyzing the exchange of cations with protons (antiporters) [1–2]. In mammals, 13 evolutionary conserved NHE isoforms, encoded by the *SLC9* (solute carrier classification of transporters) gene family, are currently known [3▪]. SLC9 transporters are divided into three subgroups [4]. The SLC9A subgroup encompasses plasmalemmal isoforms NHE1–5 (SLC9A1–5) and intracellular isoforms NHE6–9 (SLC9A6–9). The SLC9B subgroup consists of two recently cloned isoforms, NHA1 and NHA2 (SLC9B1 and SLC9B2, respectively). The latter are also known as NHEDC1 and NHEDC2. The SLC9C subgroup consists of a sperm-specific NHE (SLC9C1) and a putative NHE (SLC9C2). Each NHE isoform possesses unique structural features that dictate its functional role, mode of regulation and cellular as well as subcellular distribution. NHEs participate in a wide variety of physiological processes including cytosolic and organellar pH homeostasis, transepithelial salt transport and both systemic and single cell volume regulation.

As shown in Fig. 1, eight NHE isoforms can be found in murine islets of the endocrine pancreas on mRNA level. Of these, two are plasmalemmal (NHE1, NHE5) and six are intracellular (NHA1, NHA2, NHE6, 7, 8 and 9). To the best of our knowledge, only the ubiquitous isoform NHE1 and the recently cloned isoform NHA2 have been thoroughly studied in β cells [5▪▪,6,7].

MOLECULAR MECHANISMS OF INSULIN SECRETION BY β CELLS

Insulin secretion by β cells is tightly coupled to circulating glucose levels. Plasmalemmal glucose

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KEY POINTS

- NHA2 plays an important role in clathrin-mediated endocytosis and insulin secretion in β cells.
- NHE1 defends β cells from intracellular acidification but is not involved in glucose-induced alkalinization of the β cell or insulin secretion.
- Several additional NHE isoforms are expressed in β cells, but their physiological role remains currently unknown.

transporters of the SLC2 transporter family ensure efficient uptake of extracellular glucose into β cells and thereby initiation of the insulin secretion cascade [8]. After uptake, glucose metabolism results in an increase of the cellular ATP/ADP ratio leading to closure of ATP-sensitive potassium (K_{ATP}) channels, the main determinants of the β cell membrane potential. In addition to glucose, K_{ATP} channel closure can also be attained pharmacologically by sulfonylureas, which target the SUR1 K_{ATP} channel regulatory subunit [9]. Membrane depolarization evoked by K_{ATP} channel closure directly activates voltage-sensitive L-type Ca^{++} channels. The ensuing rise of intracellular Ca^{++} then drives the exocytosis of insulin-containing large dense core vesicles (LDCVs) [10]. Concurrently, glucose metabolism elicits signals that augment insulin secretion independent of K_{ATP} channels and intracellular Ca^{++} [11]. K_{ATP} channel-dependent and channel-independent pathways are referred to as the triggering and amplifying pathways, respectively [12]. Although there is general consensus on the basic molecular mechanisms underlying the K_{ATP} channel-dependent pathway, as outlined above, the K_{ATP} channel-independent pathways are still incompletely understood [13].

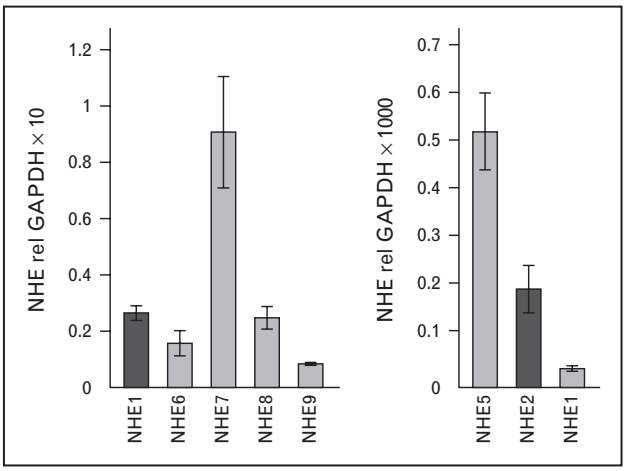

FIGURE 1. Eight different sodium/hydrogen exchanger (NHE) isoforms are present in murine islets. mRNA expression of NHEs was analyzed in freshly isolated murine islets by quantitative real-time PCR. NHE expression is normalized to Gapdh expression. Data are means ± standard deviation. Note that normalized expression levels of NHE1, 6, 7, 8 and 9 (left panel) are ~100-fold higher than those measured for NHE5, NHA2 and NHA1 (right panel). Only the roles of NHE1 and NHA2 (dark grey bars) have been investigated thus far with respect to insulin secretion by β cells.

ROLE OF SODIUM/HYDROGEN EXCHANGER 1 IN β CELLS

High glucose increases the cytoplasmic pH of primary β cells and β-cell lines [14–16]. The underlying mechanisms of this phenomenon and its role in insulin secretion, however, have been a matter of controversy. Juntti-Berggren *et al.* [17] reported that the glucose-induced alkalinization was dependent on extracellular Na^+, sensitive to inhibition by the NHE inhibitor ethylisopropylamiloride (EIPA) and thus likely the result of plasmalemmal NHE activity. Based on their studies in murine islets, Lindstrom *et al.* [14] proposed that glucose-induced alkalinization serves as a direct stimulus of insulin secretion. Other studies, however, reported the contrary in that intracellular alkalinization inhibited insulin secretion [18] and acidification or inhibition of NHE transport stimulated it [19–20].

To address these questions, Stiernet *et al.* investigated $NHE1^{swe/swe}$ mutant mice, which bear a spontaneous premature stop codon within the coding region of the *SLC9A1* gene and thus lack a functional plasmalemmal NHE1 protein [6,21]. Upon intracellular acidification in the absence of HCO_3^-/CO_2, virtually no re-alkalinization in mutant β cells occurred compared with wild-type cells, indicating that NHE1 is the only NHE present at the cell surface in murine β cells activated at low intracellular pH. Under physiological HCO_3^-/CO_2-containing conditions, however, basal intracellular pH as well as the alkalinization produced by high glucose was clearly independent of NHE1 and involved HCO_3^--dependent mechanisms instead. Moreover, all effects of glucose on intracellular Ca^{++} and on the triggering and amplifying pathways of insulin secretion were independent of NHE1, demonstrating that NHE1 has no role in stimulus-secretion coupling. Interestingly, in the presence of HCO_3^-/CO_2, the NHE inhibitors EIPA and dimethylamiloride (DMA), but not the more selective NHE1 inhibitor cariporide, stimulated insulin secretion in both NHE1 wild-type and mutant islets without affecting the intracellular pH [6]. These findings suggested unspecific, NHE1-independent effects of EIPA and DMA in islets, possibly involving

inhibition of another amiloride-sensitive NHE or direct inhibition of K_{ATP} channels [22,23].

In addition to full-length plasmalemmal NHE1, β cells were found to express a shorter splice variant of NHE1 that localizes specifically to insulin-containing LDCVs [7]. Although full-length NHE1 protein (~100 kDa) was absent from islets in $NHE1^{swe/swe}$ mutant mice, the shorter splice variant (~65 kDa) was still present. The function of this NHE1 splice variant in islets has not been studied.

ROLE OF NHA2 IN β CELLS

Based on chromosomal localization of the *NHA2* gene, tissue distribution, transport characteristics and inhibitor sensitivity, NHA2 was proposed to be the long-sought sodium/lithium countertransporter [24]. Sodium/lithium countertransport is a highly heritable trait that was linked to the development of essential hypertension and diabetes in humans [25–28]. In line with these studies and a potential role in insulin secretion, NHA2 mRNA and protein are expressed in rodent β-cell lines [5▪▪,24,29], as well as in murine and human primary β cells [5▪▪]. Our subcellular fractionation and imaging studies indicate that NHA2 resides in transferrin-positive endosomes and synaptic-like microvesicles (SLMVs), but not in insulin-containing LDCVs in β cells. To search for a putative role of NHA2 in insulin secretion *in vivo*, we generated two different strains of NHA2 knock-out mice [5▪▪]. Both strains of NHA2 knock-out mice exhibited a pathological glucose tolerance with diminished insulin secretion but normal peripheral insulin sensitivity when subjected to intraperitoneal glucose and insulin tolerance tests, respectively. Interestingly, even heterozygous mice were not normal and had an impaired glucose tolerance. In-vitro studies with islets isolated from NHA2 knock-out or heterozygous mice confirmed an insulin secretion deficit upon stimulation with glucose or the sulfonylurea tolbutamide. Insulin secretion, however, was not affected when the β cells were depolarized directly by addition of supraphysiological (50 mmol/l) extracellular K^+. Similar results were obtained when NHA2 was knocked down in the murine β cell line Min6. The observed insulin secretion deficit could be rescued by overexpression of wild-type but not functionally dead human NHA2 in NHA2-deficient Min6 cells, indicating that NHA2 transport is required. Surprisingly, although NHA2 also localizes to SLMVs in β cells, glucose-induced gamma aminobutyric acid (GABA) secretion was not affected by loss of NHA2.

The functional observation that insulin secretion was unaltered by direct K^+-mediated β-cell depolarization but reduced with glucose and sulfonylurea stimulation indicated that the defect induced by the loss of NHA2 was between K_{ATP} channel closure and the final exocytotic event. This suggested disturbed intracellular Ca^{++} homeostasis as a likely cause. Measurements of cytosolic Ca^{++} in β cells of intact islets, however, demonstrated that loss of NHA2 had no impact on intracellular Ca^{++} levels, indicating that the NHA2 effect observed with secretagogues and sulfonylureas was downstream of K_{ATP} channels and Ca^{++} signaling in islets.

It is well known that K^+-induced insulin secretion from islets is far less sustained than with sulfonylurea or glucose stimulation and induces primarily the release of a special pool of predocked vesicles at the plasma membrane [30,31]. Thus, endosomal NHA2 function may not be required during the release of predocked vesicles. To study this in more detail, we performed the following experiment: islets were first incubated in high-glucose medium to partially deplete the granule pool, then subjected to a 15-min low-glucose resting phase and finally depolarized by addition of extracellular K^+. With this protocol, insulin secretion was ~50% reduced in NHA2 knock-out islets compared with wild-type islets, which is similar in magnitude to the reduction we observed when we stimulated knock-out islets with secretatogues or sulfonylureas. The result of this experiment revealed that direct depolarization-induced insulin secretion is not universally conserved in NHA2 knock-out islets. This observation would fit with the hypothesis that direct K^+-mediated depolarization preferentially induces a non-sustained release of predocked vesicles and that only under conditions of continued insulin secretion is NHA2 action required. Alternatively, in the absence of physiological secretagogues, high K^+ may directly stimulate NHA2-independent pathways of insulin secretion that compensate for the loss of NHA2.

Given its endosomal localization and the intriguing observations outlined above, we reasoned that NHA2 may primarily affect endocytosis in β cells with an indirect impact on LDCV exocytosis. Previous reports demonstrated that endocytosis and exocytosis are tightly coupled in β cells with inhibition of endocytosis resulting in decreased insulin secretion [32–34]. Our experiments performed with Min6 or primary β cells revealed a reduction of clathrin-dependent, but not clathrin-independent, endocytosis upon loss of NHA2, suggesting that defective endo-exocytosis coupling may be the mechanism for the secretory deficit observed. But what is the function of NHA2 in endosomes and SLMVs in the β cell? Our data indicate that loss of NHA2 has no impact on endosomal steady-state pH and does not affect GABA secretion in β cells. Thus, the role of NHA2 in the endosome may not be

regulation of pH but control of endosomal Na^+ concentration. It is also possible that NHA2 is only present in a subset of specialized endocytotic vesicles and therefore escaped our endosomal pH measurements. In the case of SLMVs, which can bud either directly from the plasma membrane or from endosomal intermediates, the direct plasma membrane-related biogenesis pathway avoiding endosomal intermediates may explain the fact that we could not detect differences in GABA secretion between wild-type and knock-out islets [35,36]. Thus, although our data clearly indicate a critical role of the NHE NHA2 for clathrin-mediated endocytosis and insulin secretion in β cells, many fundamental questions remain unanswered at the moment. Given the peculiar phenotype of NHA2 knock-out mice, future work should also address the potential involvement of NHA2 in the pathogenesis of β-cell disorders in humans. Interestingly, several single-nucleotide polymorphisms (SNPs) affecting the transport function of human NHA2 were recently described [37]. Whether human subjects carrying functionally relevant SNPs have defective insulin secretion has not been studied.

WHAT ABOUT OTHER SODIUM/HYDROGEN EXCHANGERS IN ISLET CELLS?

Our data indicate significant expression of intracellular NHEs 6–9 in islets on mRNA level (Fig. 1). Although β cells account for the large majority of islet cells, it is still possible that these data reflect NHE expression by non-β cells. Publicly available microarray data (http://www.t1dbase.org), however, indicate that NHEs 6–9 are indeed present in primary rodent β cells. Interestingly, in the same database, NHEs 6–9 are also reported to be expressed in human islets.

NHE7 localizes to the trans-Golgi network and is insensitive to inhibition by amiloride [38,39]. The physiological function of NHE7 is not known and the phenotype of a knock-out mouse has not been reported. Given the residence in the Golgi, NHE7 may have a role in insulin synthesis or maturation. NHE8 is present at the plasma membrane of epithelial cells in the kidney and intestine, whereby in HeLa and COS7 cells it was found to localize to the mid-Golgi and trans-Golgi [38,40–42]. In contrast to NHE7, NHE8 is amiloride and EIPA-sensitive [43]. As plasmalemmal NHE transport is absent in NHE1-deficient islets, it seems unlikely that functionally active NHE8 is present at the plasma membrane of β cells [6]. Instead, NHE8 may localize to the Golgi in β cells, as reported in HeLa and COS7 cells, and affect synthesis or maturation of insulin. The differential effects of DMA and EIPA (stimulation of insulin secretion) versus cariporide (no effect on insulin secretion) in islets described earlier could theoretically be caused by inhibition of NHE8 [6]. NHE8 knock-out mice have been generated recently and could be employed to address these questions [44,45].

NHEs 6 and 9 are endosomal NHEs and thus localize to the same organelle as NHA2 [38]. In contrast to NHA2, NHE6 and NHE9 also transport K^+ in addition to Na^+. Experimental evidence indicates that NHE6 and NHE9 function as K^+/H^+ exchangers in endosomes, counteracting endosomal acidification by the V-ATPase [38,46]. As is the case for NHA2 in β cells, NHE6 and NHE9 were shown to be involved in clathrin-mediated endocytosis in nonislets cells [46,47]. Thus, it is tempting to speculate that NHE6 and NHE9 also participate in endo-exocytosis coupling in β cells and therefore have a role in insulin secretion. Clearly, given the phenotype of NHA2 knock-out mice, there is no redundance with NHE6 or NHE9, or both, compensating for the loss of NHA2 in islets. Future investigations of mice lacking one or several endosomal NHE isoforms should shed light on the functional interactions between NHA2, NHE6 and NHE9 in islets. Moreover, the study of patients with mutations in NHE6 or NHE9 will allow the determination of whether these NHEs have a role in insulin secretion in humans *in vivo*.

CONCLUSION

NHE1 defends β cells from intracellular acidification, but has no role in stimulus-secretion coupling. The function of a short NHE1 splice-variant expressed exclusively in insulin-containing LDCVs is not known.

Recent data indicate a critical role of NHA2 for clathrin-mediated endocytosis and insulin secretion in β cells *in vitro* and *in vivo*. The exact function of NHA2 in β cell endosomes and SLMVs, as well as the relationship to insulin secretion, remain currently unknown and warrant further study. Given the peculiar phenotype of NHA2 knock-out mice, future work should also address the potential involvement of NHA2 in the pathogenesis of β-cell disorders in humans. In addition to NHE1 and NHA2, islet cells express significant amounts of the intracellular NHE isoforms 6, 7, 8 and 9, but their role in insulin secretion has not been explored thus far.

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Conflicts of interest

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There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

1. Brett CL, Donowitz M, Rao R. Evolutionary origins of eukaryotic sodium/proton exchangers. Am J Physiol Cell Physiol 2005; 288:C223–C239.
2. Orlowski J, Grinstein S. Diversity of the mammalian sodium/proton exchanger SLC9 gene family. Pflugers Arch 2004; 447:549–565.
3. ■ Fuster DG, Alexander RT. Traditional and emerging roles for the SLC9 Na(+)/H (+) exchangers. Pflugers Arch 2014; 466:61–76.

This is a recent comprehensive review on the biology of mammalian NHEs.

4. Donowitz M, Ming Tse C, Fuster D. SLC9/NHE gene family, a plasma membrane and organellar family of Na(+)/H(+) exchangers. Mol Aspects Med 2013; 34:236–251.
5. ■■ Deisl C, Simonin A, Anderegg M, *et al.* Sodium/hydrogen exchanger NHA2 is critical for insulin secretion in beta-cells. Proc Natl Acad Sci U S A 2013; 110:10004–10009.

This study reveals NHA2 as a critical regulator of insulin secretion and endocytosis in β cells of islets. This is the first description of the biological relevance of a mammalian NHA (SLC9B) transporter.

6. Stiernet P, Nenquin M, Moulin P, *et al.* 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 2007; 282:24538–24546.
7. Moulin P, Guiot Y, Jonas JC, *et al.* Identification and subcellular localization of the Na+/H+ exchanger and a novel related protein in the endocrine pancreas and adrenal medulla. J Mol Endocrinol 2007; 38:409–422.
8. Mueckler M, Thorens B. The SLC2 (GLUT) family of membrane transporters. Mol Aspects Med 2013; 34:121–138.
9. Campbell JD, Sansom MS, Ashcroft FM. Potassium channel regulation. EMBO Rep 2003; 4:1038–1042.
10. Barg S, Ma X, Eliasson L, *et al.* Fast exocytosis with few Ca(2+) channels in insulin-secreting mouse pancreatic B cells. Biophys J 2001; 81:3308–3323.
11. Komatsu M, Schermerhorn T, Aizawa T, Sharp GW. Glucose stimulation of insulin release in the absence of extracellular Ca2+ and in the absence of any increase in intracellular Ca2+ in rat pancreatic islets. Proc Natl Acad Sci U S A 1995; 92:10728–10732.
12. Yajima H, Komatsu M, Schermerhorn T, *et al.* cAMP enhances insulin secretion by an action on the ATP-sensitive K+ channel-independent pathway of glucose signaling in rat pancreatic islets. Diabetes 1999; 48:1006–1012.
13. Takei M, Dezaki K, Ishii H, *et al.* A new experimental model of ATP-sensitive K(+) channel-independent insulinotropic action of glucose: a permissive role of cAMP for triggering of insulin release from rat pancreatic beta-cells. Endocr J 2013; 60:599–607.
14. Lindstrom P, Sehlin J. Effect of glucose on the intracellular pH of pancreatic islet cells. Biochem J 1984; 218:887–892.
15. Lebrun P, van Ganse E, Juvent M, *et al.* Na+-H+ exchange in the process of glucose-induced insulin release from the pancreatic B-cell. Effects of amiloride on 86Rb, 45Ca fluxes and insulin release. Biochim Biophys Acta 1986; 886:448–456.
16. Doliba NM, Wehrli SL, Vatamaniuk MZ, *et al.* Metabolic and ionic coupling factors in amino acid-stimulated insulin release in pancreatic beta-HC9 cells. Am J Physiol Endocrinol Metab 2007; 292:E1507–E1519.
17. Juntti-Berggren L, Arkhammar P, Nilsson T, *et al.* 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 1991; 266:23537–23541.
18. Nabe K, Fujimoto S, Shimodahira M, *et al.* Diphenylhydantoin suppresses glucose-induced insulin release by decreasing cytoplasmic H+ concentration in pancreatic islets. Endocrinology 2006; 147:2717–2727.
19. Pace CS, Tarvin JT, Smith JS. Stimulus-secretion coupling in beta-cells: modulation by pH. Am J Physiol 1983; 244:E3–E18.
20. Best L, Yates AP, Gordon C, Tomlinson S. Modulation by cytosolic pH of calcium and rubidium fluxes in rat pancreatic islets. Biochem Pharmacol 1988; 37:4611–4615.
21. Cox GA, Lutz CM, Yang CL, *et al.* Sodium/hydrogen exchanger gene defect in slow-wave epilepsy mutant mice. Cell 1997; 91:139–148.
22. Bollensdorff C, Zimmer T, Benndorf K. Amiloride derivatives are potent blockers of KATP channels. Naunyn Schmiedebergs Arch Pharmacol 2001; 364:351–358.
23. Rustenbeck I, Herrmann C, Ratzka P, Hasselblatt A. Imidazoline/guanidinium binding sites and their relation to inhibition of K(ATP) channels in pancreatic B-cells. Naunyn Schmiedebergs Arch Pharmacol 1997; 356:410–417.
24. Xiang M, Feng M, Muend S, Rao R. A human Na+/H+ antiporter sharing evolutionary origins with bacterial NhaA may be a candidate gene for essential hypertension. Proc Natl Acad Sci U S A 2007; 104:18677–18681.
25. Mangili R, Bending JJ, Scott G, *et al.* Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. N Engl J Med 1988; 318:146–150.
26. Canessa M, Adragna N, Solomon HS, *et al.* Increased sodium-lithium countertransport in red cells of patients with essential hypertension. N Engl J Med 1980; 302:772–776.
27. Vaccaro O, Cuomo V, Trevisan M, *et al.* Enhanced Na-Li countertransport: a marker of inherited susceptibility to type 2 diabetes. Int J Epidemiol 2005; 34:1123–1128.
28. Cirillo M, Laurenzi M, Panarelli W, *et al.* Prospective analysis of traits related to 6-year change in sodium-lithium countertransport. Gubbio Population Study Research Group. Hypertension 1999; 33:887–893.
29. Fuster DG, Zhang J, Shi M, *et al.* Characterization of the sodium/hydrogen exchanger NHA2. J Am Soc Nephrol 2008; 19:1547–1556.
30. Collins SC, Hoppa MB, Walker JN, *et al.* Progression of diet-induced diabetes in C57BL6J mice involves functional dissociation of Ca2(+) channels from secretory vesicles. Diabetes 2010; 59:1192–1201.
31. Hoppa MB, Collins S, Ramracheya R, *et al.* Chronic palmitate exposure inhibits insulin secretion by dissociation of Ca(2+) channels from secretory granules. Cell Metab 2009; 10:455–465.
32. Min L, Leung YM, Tomas A, *et al.* Dynamin is functionally coupled to insulin granule exocytosis. J Biol Chem 2007; 282:33530–33536.
33. Tomas A, Yermen B, Min L, *et al.* Regulation of pancreatic beta-cell insulin secretion by actin cytoskeleton remodelling: role of gelsolin and cooperation with the MAPK signalling pathway. J Cell Sci 2006; 119 (Pt 10):2156–2167.
34. Orci L, Malaisse-Lagae F, Ravazzola M, *et al.* Exocytosis-endocytosis coupling in the pancreatic beta cell. Science 1973; 181:561–562.
35. Hannah MJ, Schmidt AA, Huttner WB. Synaptic vesicle biogenesis. Annu Rev Cell Dev Biol 1999; 15:733–798.
36. Schmidt A, Hannah MJ, Huttner WB. Synaptic-like microvesicles of neuroendocrine cells originate from a novel compartment that is continuous with the plasma membrane and devoid of transferrin receptor. J Cell Biol 1997; 137:445–458.
37. Huang X, Morse LR, Xu Y, *et al.* Mutational analysis of NHAoc/NHA2 in Saccharomyces cerevisiae. Biochim Biophys Acta 2010; 1800:1241–1247.
38. Nakamura N, Tanaka S, Teko Y, *et al.* Four Na+/H+ exchanger isoforms are distributed to Golgi and post-Golgi compartments and are involved in organelle pH regulation. J Biol Chem 2005; 280:1561–1572.
39. Numata M, Orlowski J. Molecular cloning and characterization of a novel (Na+,K+)/H+ exchanger localized to the trans-Golgi network. J Biol Chem 2001; 276:17387–17394.
40. Lawrence SP, Bright NA, Luzio JP, Bowers K. The sodium/proton exchanger NHE8 regulates late endosomal morphology and function. Mol Biol Cell 2010; 21:3540–3551.
41. Xu H, Chen H, Dong J, *et al.* Gastrointestinal distribution and kinetic characterization of the sodium-hydrogen exchanger isoform 8 (NHE8). Cell Physiol Biochem 2008; 21:109–116.
42. Goyal S, Vanden Heuvel G, Aronson PS. Renal expression of novel Na+/H+ exchanger isoform NHE8. Am J Physiol Renal Physiol 2003; 284:F467–F473.
43. Zhang J, Bobulescu IA, Goyal S, *et al.* Characterization of Na+/H+ exchanger NHE8 in cultured renal epithelial cells. Am J Physiol Renal Physiol 2007; 293:F761–F766.
44. Baum M, Twombley K, Gattineni J, *et al.* Proximal tubule Na+/H+ exchanger activity in adult NHE8-/-, NHE3-/-, and NHE3-/-/NHE8-/- mice. Am J Physiol Renal Physiol 2012; 303:F1495–F1502.
45. Xu H, Zhang B, Li J, *et al.* Impaired mucin synthesis and bicarbonate secretion in the colon of NHE8 knockout mice. Am J Physiol Gastrointest Liver Physiol 2012; 303:G335–G343.
46. Kondapalli KC, Hack A, Schushan M, *et al.* Functional evaluation of autism-associated mutations in NHE9. Nat Commun 2013; 4:2510.
47. Xinhan L, Matsushita M, Numaza M, *et al.* Na+/H+ exchanger isoform 6 (NHE6/SLC9A6) is involved in clathrin-dependent endocytosis of transferrin. Am J Physiol Cell Physiol 2011; 301:C1431–C1444.