Cell Swelling-induced Peptide Hormone Secretion

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Key Words

Cell Volume • Exocytosis • Ca^{2+} independent hormone secretion • Signaling • Resistance to physiological inhibitor • Specificity • Ischemic preconditioning • SIADH

Abstract

Cell swelling induces peptide exocytosis using unique signaling pathway. Hyposmotic-induced secretion in normal cells is not mediated by specific receptors, is independent from extra and intracellular Ca^{2+} , sodium and potassium channels activity, prostaglandins, leukotriens, does not involve cytoskeleton, cAMP generation, phospholipase A_2 , G proteins, protein kinase C. It is promoted by swelling of the secretory vesicles. Resistance to endogenous inhibitors is frequent attribute of this type of secretion. Swelling-induced secretion involves also secretory vesicles not involved in conventional stimulation. Hyposmosis-induced insulin secretion is more sensitive to high cellular cholesterol than conventional one suggesting substantial difference between mechanisms. Participation of sequential exocytosis as dominating mechanism in swelling-induced exocytosis is hypothesized. Signaling and response in tumor cells

often differs from native cells and varies markedly between cell lines. Pathogenetic implications: cell swelling could be involved in alcohol induced hypoglycemia in diabetic patients and release of peptides from pituitary and neurons. Swelling-induced products could be mediators of ischemic preconditioning involved also in protection of diabetic heart. Swelling-induced exocytosis is an ancient mechanism generally present in cells; in cells engaged in water and salt regulation is covered by specific response mediated by specific signaling. Disturbance of specific response leads to swelling-induced - inappropriate secretion of antidiuretic hormone - SIADH.

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Introduction

Endocrine secretion is under tight control leading to the release of appropriate hormones in adequate amounts in response to increase of endogenous secretagogues or decrease of inhibitors participating in feedback control or

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various responses to some specific stimuli. Relatively unrecognized is the powerful effect of cell swelling to induce exocytosis of material in intracellular secretory vesicles including hormones.

Evolutionary aspect

Keeping volume within certain limits is a prerequisite for cell integrity and proper function. Protective mechanism(s) should have to develop in early forms of life to assure survival in changing environment. A variety of organisms and cell types spanning all taxonomic kingdoms are exposed to osmotic stresses. Diverse experimental approach revealed that phylogenetically divergent organisms employ uniquely adapted mechanisms of cell volume regulation [1]. Considering that changes in extracellular osmolality were physiological stresses faced by the earliest organisms, it is likely that cell volume regulation proceeds by highly conserved physiological processes [1]. A common physiological response to these challenges is maintenance of cell volume through changes in the concentration of intracellular inorganic and organic solutes followed by secondary water movement. Aquaporins facilitating water transport across the cellular membranes in vertebrates are present also in bacteria, fungi, protozoa, nematodes, plants and insect [2].

Alterations in the extracellular tonicity can either promote cell shrinkage or swelling, depending on the direction of the osmolarity gradient. Metabolic activity also leads to the generation of intracellular osmolytes, which tend to cause the cell to swell. Most of the cell reactions to volume changes are aiming at the reestablishment of initial volume status. To drive water flux, which finally accomplishes cell volume adjustment, cells produce an osmotic difference between the intra- and extracellular spaces by inducing osmolyte transport. The defense against excess cell swelling is accomplished by a reduction of the intracellular osmolarity by release of inorganic and organic osmolytes from the cell and by synthesis of osmotically less active macromolecules from their specific subunits [3-7]. In higher organisms cell volume responses to osmotic challenge are integrated into a signal transduction network regulating various cell functions [3-7] including cell proliferation, apoptosis [8], migration, metabolism (for review see [7, 9, 10]) and secretion including exocytosis of peptide hormones [11].

Exocytosis is also a highly conserved process essential to the function of numerous cellular activities (for review see [12]). It occurs constitutively in all eukaryotic cells and is regulated in some cell types like neurons and endocrine cells in response to extrinsic stimuli leading to well-orchestrated series of molecular interactions including increase of cytosolic calcium [13].

Hormones secreted in response to hypotonicity

The release of almost all protein and peptide hormones tested *in vitro* was stimulated in a concentration-related manner by medium hyposmolarity or isosmolar medium containing permeant molecules [14-20] from various tissues including: pituitary - gonadotropins LH, FSH, adrenocorticotropin (ACTH), melanocyte-stimulating hormone (MSH), thyrotropin (TSH), prolactin [15, 21-24], beta endorphin [25], pancreas - insulin [16, 19, 26], thyrotropin releasing hormone (TRH) [27] and glucagon [16], hypothalamus - TRH [28-30] and gonadotropin-releasing hormone (GnRH) [31, 32], heart - TRH [33], atriopeptin [17], atrial natriuretic peptide (ANP) [34], kidney- renin [35].

Swelling-induced secretion can be induced in different parts of neurons - similar TRH release was evoked from the hypothalamic paraventricular nucleus (mostly perikarya) and the median eminence or neurohypophysis (exclusively axon terminals) [28, 29, 36-38]. Cell swelling induced exocytosis is not restricted to endocrine cells and hormones. Medium hyposmolarity also induces secretion of exocrine pancreatic enzymes [16] and myeloperoxidase from human polymorphonuclear leukocytes [39].

Relation to cell volume regulation

Relation of swelling-induced peptide secretion to cell volume regulation is vague. To accommodate expanding volume during hyposmotic swelling, animal cells change their shape and increase surface area by drawing extra membrane from surface and intracellular reserves [40]. Exocytosis of intravesicular material may help a swollen cell to meet the challenge by expanding the plasmalemma through the fusion with vesicular membrane [11]. It is of interest that in mammalian cells significant

endomembrane insertion was observed only during extreme swelling [40]. In contrast to electrolytes, export of proteins and peptides out of the cell does not change an osmotic balance between extra- and intra-cellular space insofar as to have a significant effect on the cell volume regulation [14]. Regulatory volume decrease (RVD) is dependent on the transport of osmolytes and swelling-induced peptide secretion could be affected in an opposite way by calcium; when medium Ca^{2+} is depleted or Ca^{2+} influx prevented by Ca^{2+} channel blockers, the amplitude of cell swelling is greater and the RVD slower [11, 15], in contrast, extracellular Ca^{2+} is not required and, in fact, negatively modulates osmotically-induced secretion in many cells [11, 15-19]. Swelling-induced peptide secretion does not seem to be directly engaged in the cell volume regulation.

Mechanism of signal transduction

Peculiarities of swelling-induced secretion indicate an involvement of the unique signaling pathway [11, 16, 19, 41].

Osmolarity

Secretion induced by extracellular hyposmolarity is due to the relative reduction of extracellular compared to intracellular osmolarity and not to dilution of any essential extracellular elements. Substituting sorbitol or choline chloride for the deleted NaCl [16] or medium dilution with aqueous 5% mannitol [23] to maintain isosmolarity on both sides of the plasma membrane, did not induce peptide hormone secretion. Hyperosmolar medium has usually suppressing effect on secretion [22, 23, 36, 37, 39, 42]. However, on return of the cells to an isosmolar medium, there is a prompt dose-related burst of “off response” secretion [16] whose magnitude is proportional to that of the preceding hyperosmolarity [22]. This indicates that it is the relative difference in intracellular vs. extracellular osmolar concentration which causes exocytosis rather than the absolute osmolarity of either compartment [11].

Receptors

The dynamics of secretion induced by cell swelling closely resembles that induced by specific secretagogue (Fig. 1). Perfusion of pituitary cells with 10 nM TRH (natural secretagogue for prolactin), as well as cell swelling

induced by a hypotonic solution (medium dilution with 30% H_2O), stimulates similar immediate dose-related high-amplitude prolactin secretory burst reaching a peak at 1-2 min followed by a decline (Fig. 1) [11, 20]. Repeated stimuli with 30 s long interstimulus intervals also produce the same secretory response. For both types of the stimuli, the secretory response to continuous exposure and refractory periods to repeated stimulation (<1 min) were essentially identical (Fig. 1). An identical high-amplitude secretory burst was induced by exposure to TRH for times varying from 6 to 600 s. In contrast, for 30% H_2O , the secretory amplitude was proportional to the exposure time between 6 and 120 s. While the TRH response was triggered by rapid specific receptor binding, a very short pulse would not have time to produce a sufficient transmembrane osmotic gradient. It is concluded that a hyposmotic medium does not trigger peptide release by the specific receptor-ligand binding [11, 20].

Any changes in cellular physiology caused specifically by cell swelling would be initiated by distortion of the plasmalemma. Pressure-sensitive mechanoreceptors have been identified in a variety of cell types [43]. The trivalent lanthanide GdCl_3 blocks mechanogated channels [44] and also voltage sensitive Ca^{2+} channels [reviewed 45]. GdCl_3 in the culture medium does not negatively modify hyposmolarity- or ethanol-induced TRH release from isolated pancreatic islets, hypothalamic PVN or posterior pituitaries [37, 38], or prolactin release from acutely dispersed anterior pituitary cells induced by hypotonicity [22, 37]. From these experiments it can be concluded that signal leading to exocytosis after cell swelling does not involve GdCl_3 sensitive mechanogated channels.

Alterations of channel activity

Several studies showed that neither Na^+ nor K^+ channels play an essential role in hormone secretion induced by cell swelling [11, 15, 16]. Blocking Na^+/K^+ -ATPase sodium pump by ouabain or blocking Na^+ channels with amiloride or K^+ channels with tetraethylammonium did not affect hyposmolarity-induced insulin secretion from pancreatic islets or beta-cells [16, 46] or TSH, LH and prolactin secretion from pituitary cells [15, 47]. On the other hand hypotonicity-induced insulin release was inhibited by blocking outwardly rectifying Cl^- selective conductance in rat β -cells by DIDS [48].

Fig.1. Dynamics of prolactin secretory response of perfused pituitary cells to 10 mM thyrotropin releasing hormone (TRH, prolactin natural secretagogue), hypotonic solution (H2O, medium diluted with 30 % water) or 30 mM KCl (K⁺, membrane depolarizing solution – unspecific stimulus). Continuous stimulation (A) or repeated stimuli lasting 30 sec with 1 min interstimulus interval (B), 1 min stimulation with 6 min interstimulus interval (C) or stimuli lasting 6-60 sec with 6 min interval (D) were compared. Differences were found only if stimulus lasted less than 1 min. (With permission from Štrbák et al. 2004 [20])

Prostaglandins and leukotriens

Inhibition of prostaglandin biosynthesis by indomethacin (cyclooxygenase inhibitor) and that of

leukotrienes by nordihydroguaiaretic acid (NDGA, lipoxygenase inhibitor) did not adversely affect hypotonicity- induced prolactin release from perfused

pituitary cells [38]. Signal transduction leading to exocytosis after cell swelling does not involve indomethacin and NDGA sensitive mediators including prostaglandins and leukotriens.

Cytoskeleton

Microtubules and microfilaments are important mechanical constituents of plasma membrane motion and migration of vesicles within the cell. However, colchicine or cytochalasin, which can respectively disrupt microtubule and microfilament function, have no effect on hyposmolarity-induced secretion [11, 15, 16], suggesting that in normal cells cytoskeleton is not involved.

cAMP generation

Hormone secretion induced by cell swelling is not associated with a rise in cAMP production in either normal or tumor-derived pituitary cells [47].

Role of Ca^{2+}

Increase in intracellular calcium concentration is a key signal to trigger conventional regulated exocytosis in most tissues. The most striking and unusual feature of cell swelling-induced secretion is that it stimulates regulated secretion from native cells independent of extracellular Ca^{2+} [11, 15-17, 25, 49, 50]. Hyposmotic stimulation is independent also from intracellular Ca^{2+} concentration; cell swelling induced insulin release from pancreatic islets [19] or release of atriopeptin from isolated atrial myocytes [17] in calcium free medium containing the membrane permeable calcium chelator BABTA/AM. It is concluded that in contrast to most types of regulated secretion cell swelling-induced secretion is independent from both extracellular and intracellular Ca^{2+} .

Calcium independent mechanisms involved in exocytosis: phospholipase A_2 , G proteins, protein kinase C (PKC)

In contrast to glucose stimulation, osmotically induced insulin secretion from pancreatic islets was not inhibited by bromoenol lactone, an iPLA₂ (Ca^{2+} independent phospholipase) inhibitor [41]. Similarly, preincubation of islets with mycophenolic acid to inhibit GTP synthesis fully abolished glucose-induced insulin secretion but was without effect on hypotonicity stimulated insulin release [41]. Alternative, non osmotic glucose stimulation of insulin release in the absence of extracellular Ca^{2+} requires specific conditions:

simultaneous activation of PKC, PKA as well as glucose metabolism in the *in vitro* system [51]. PKC is also involved in cell response to volume changes [51, 52]. It was therefore of much interest to see if it is involved also in hypotonicity-induced insulin secretion. In both the presence and the absence of Ca^{2+} in the medium, PKC inhibitor bisindolmaleimide VIII did not inhibit cell swelling-induced insulin secretion from pancreatic islets [19]. It is concluded that cell swelling-induced secretion does not involve phospholipase A_2 , G proteins and PKC.

Biophysical Effect

A failure to identify specific transduction pathway and resistance to physiological inhibitors suggests that signaling of cell swelling-induced exocytosis bypasses conventional transduction steps and could be effective at the distal end of the cascade [20]. Secretory vesicle swelling is critical for exocytosis [53-55]. Stretching of vesicular and plasma membranes in the region of contact results in exposing areas of hydrophobic acyl chains leading to subsequent merging and fusion. Fusion rates are orders of magnitude higher if an osmotic gradient is applied [53]. The externalization of hormones or transmitters upon exocytosis of vesicles is augmented by secretion of water from the vesicle membrane through the widened fusion pore [55]. Considering these data, we hypothesized that cell swelling triggered exocytosis is a result of a direct biophysical effect of the osmotic gradient on secretory vesicles [20]. Recently [56] we discovered that INS-1E rat tumor beta cells are unable to release insulin in response to cell swelling despite a good response to glucose challenge and appropriate increase in cell volume. These data suggest that the cell and vesicle swelling alone may not be exclusive critical step in hypotonicity-induced exocytosis. We hypothesize therefore, that biophysical effects induced by cell swelling promote exocytosis.

Resistance to Physiological Inhibitors

Noradrenaline (NA) is physiological inhibitor of glucose-induced insulin secretion [57]. Recently it was shown that NA inhibits Ca^{2+} -evoked exocytosis in the β -cell downstream of increased $[Ca^{2+}]_i$ by reducing the number of exocytotic events, without modifying vesicle size; NA induced a marked retardation of the rate of refilling of the readily releasable pool of secretory granules

[58]. Also alternative signaling pathways leading to insulin secretion independently from Ca^{2+} including that induced by black-widow spider neurotoxin α -latrotoxin are inhibited by catecholamines [55, 57-60]. We have shown that noradrenaline does not inhibit cell swelling-induced insulin secretion either in the presence or the absence of Ca^{2+} in the medium [19]. Stimulation by 20 mmol/l glucose or 30% hyposmolarity induced similar increase of insulin secretion and the hypotonic medium had an additive effect on the glucose-induced insulin secretion [19]. When noradrenaline was applied during combined stimulation by glucose and hypotonic medium, its inhibitory effect was partial; only the secretion induced by glucose but not that induced by the cell swelling was inhibited [19]. This indicates that different signaling pathways were used in parallel for glucose and hypotonicity during combined stimulation in Ca^{2+} containing medium and that swelling-induced insulin secretory pathway either bypasses steps affected by noradrenalin or acts at a more distal step of the cascade.

Angiotensin: Angiotensin II inhibits TRH secretion from heart tissue by a mechanism involving AT1 receptors [33]. Hypotonicity-induced TRH secretion from heart slices could not be inhibited by angiotensin II [33]. Similarly as with NA, swelling-induced secretion overrides inhibitory effect of angiotensin II [33]. However, regulation may be different in hormones engaged in water and salt regulation: perfusion of rat atria with hyposmotic solution increased ANP secretion, the addition of Angiotensin-(1-7) to medium decreased the hyposmolarity-induced ANP secretion in a dose-dependent manner [34].

Resistance to various endogenous inhibitors is often a feature of the swelling-induced secretion. This fact could have important pathophysiological implications.

Specificity of cell swelling-induced peptide secretion

In general cell swelling evokes and shrinking inhibits exocytosis of proteins and peptides stored in secretory vesicles from various types of cells (for review see [11, 14]). Cell swelling-induced exocytosis is a broad phenomenon affecting many hormones and enzymes after exposure of the cells to relative hyposmolarity or treatment with permeant agents (for review see [11]). The response could be, however, inappropriate in cells engaged in

water and salt regulation (e.g. stimulation of vasopressin secretion in an organism with hyposmotic blood and hypervolemia), where specific response is desirable [61]. Direct stimulation of the hypothalamic paraventricular (PVN) and supraoptic nuclei (SON) with hyperosmotic saline via microdialysis increases vasopressin and oxytocin levels in extracellular fluid [62-65] by a mechanism involving axons, dendrites, and cell bodies [66]. Levels of these hormones increase also in plasma as a result of their release from axon terminals in the posterior pituitary. These neuropeptides are released within nuclei and into the blood in a Ca^{2+} -dependent manner [62, 67, 68]. The release of vasopressin from the neurohypophysis (NH) is regulated primarily by changes in extracellular fluid osmolarity; mechanism is controlled by osmoreceptors and neuropeptides released within the hypothalamus [69] – the response to excitatory peptides is blunted by reduced osmolality of extracellular fluid. Excitatory effects of angiotensin II, cholecystokinin and neurotensin facilitating secretion from magnocellular neurosecretory cells are mediated by stimulation of stretch-inactivated channels and are Gd^{3+} sensitive [69]. It is of interest that also angiotensin II and III are released from PVN in response to local hyperosmotic stimulation [70] suggesting that they play transmitter role in central osmoregulation [61]. Moreover, the addition of Angiotensin-(1-7) to medium decreased the hyposmolarity-induced ANP secretion in a dose-dependent manner [34].

To shed more light on the interrelations between specific and unspecific responses we studied osmotic effect on exocytosis from tissue explants of hypothalamic PVN and posterior pituitary with simultaneous thyrotropin releasing hormone (TRH) and oxytocin examination [14, 37, 38, 61]. Under our experimental conditions, both neuropeptides are secreted into medium from the same tissue explants, though most likely from different neurons: parvocellular and magnocellular for TRH and oxytocin respectively. As expected TRH secretion was stimulated by cell swelling [29, 36-38, 61], while oxytocin, neurohormone engaged in water and salt regulation, was not released after cell swelling-inducing stimuli; hypotonicity or ethanol in isosmotic medium [38]. This is in good agreement with observations that ethanol inhibits the release of oxytocin from the posterior pituitary induced by high K^{+} [71] or suckling [72] and that hypotonic stimuli hyperpolarize magnocellular neurosecretory cells via stretch-inactivated channels [73]. Excitatory effects of some peptides in these cells are also due to stimulation of stretch-inactivated channels [69]. Therefore, it was

concluded that cell swelling-induced exocytosis possesses limited selectivity; cells specifically engaged in water and salt regulation retain their specific response to osmotic stimuli [37]. The aim of the next study was to ascertain whether the swelling-induced oxytocin secretion could be unmasked by the inhibition of the specific osmotic response using Ca^{2+} -free medium and GdCl_3 , an inhibitor of stretch sensitive channels [43, 74] and a potent blocker of voltage-sensitive Ca^{2+} channels [44]. The presence of GdCl_3 did not change hypotonicity-induced TRH release from PVN and NH. In presence of lanthanide hypotonicity and ethanol stimulated also oxytocin release from PVN and SON suggesting that specific response of magnocellular neurons to osmolarity was inhibited and swelling-induced exocytosis emerged. Ca^{2+} -free medium without the inhibitor was not able to promote swelling-induced oxytocin secretion from SON suggesting that the effect of GdCl_3 on Ca^{2+} transport was not the main mechanism of the GdCl_3 effect.

It is concluded that cell swelling-induced exocytosis possesses limited selectivity, cells specifically involved in water and salt regulation retain their specific response to osmotic stimuli; neurons of the hypothalamic supraoptic and paraventricular nuclei release oxytocin, vasopressin and angiotensin II and III in response to hyperosmotic stimulation. The specific response related to their regulatory role could be obviated by GdCl_3 and at these conditions general unspecific response (exocytosis) to swelling-inducing stimuli emerges. Swelling induced exocytosis is an ancient mechanism generally present in cells, in some of them is covered by specific response mediated by specific signaling.

Exocytosis

SNARE proteins and tetanus toxin.

Mechanism of glucose-induced insulin secretion is relatively well known, therefore comparison with swelling-induced insulin secretion could bring important information. Regulated exocytosis of secretory granules involves exocytosis of a premade pool of stable granules (for review see [75]). In many cell types large reserve pool exists and only a small portion of granules is initially rapidly releasable. The first-phase insulin secretion is attributable to the release of readily releasable pool (RRP) of granules representing less than 50 granules out of more than 10,000 granules [76]. A remarkable feature of the β -cell is that nearly all RRP granules are situated in a close proximity to the few Ca^{2+} channels; they are rapidly

exposed to exocytotic levels of Ca^{2+} and undergo exocytosis within 100-200 ms [76]. Ca^{2+} independent swelling-induced peptide release is also very rapid [11], though using alternative signaling [19, 41]. Exocytotic secretion depends on the functioning of a mechanism involving SNARE proteins. β cells contain several types of SNARE proteins including SNAP-25, syntaxin 1-3, VAMP-2 and synaptotagmin [77]. SNARE proteins are proteolytic substrates for the clostridial neurotoxins [77], particularly their light chain subunit. Tetanus toxin (TeTx) blocks glucose-induced insulin secretion especially at the level of vesicle-associated membrane protein-2 (VAMP-2) [78]. Our study on rat pancreatic islets showed that TeTx inhibits both glucose and hypotonicity-induced insulin secretion [41]. We conclude therefore, that final step of regulatory exocytosis is similar for both types of stimulated secretion.

N-ethylmaleimide-sensitive-factor (NSF) and soluble NSF-attachment factor (α SNAP)

are proteins that act to disassemble SNARE complexes. They are sensitive to N-ethylmaleimide (NEM) [79], which blocks disassembly of complexes and their further participation in exocytosis [80]. Although at the beginning the number of vesicles available for exocytosis increases [81], subsequent steps are blocked by NEM [82]. This results in an increase of basal insulin secretion and subsequent absence of the response to glucose stimulation in presence of NEM [41]. In contrast, despite high basal secretion, islets were still able to release more insulin in response to hypotonicity. This response to stimulation suggests the presence of a separate pool of secretory granules available for swelling-induced but not glucose-induced exocytosis after treatment. Interestingly, high insulin secretion from NEM treated cells further increased also after ethanol stimulation [83] suggesting similar pathway for permeant effect. ZnCl_2 was shown to be effective inhibitor of dephosphorylation of phosphotyrosyl residues [84] and affecting exocytosis by similar mechanism as NEM. Essentially the same results received with ZnCl_2 as with NEM [83] strengthen our hypothesis that an extra pool of secretory vesicles is available for exocytosis in this type of stimulation.

Cholesterol is a major component of specialized membrane microdomains known as lipid rafts or caveolae that are involved in exocytosis (for review see [12]). It is rich in clusters of SNARE proteins. Physiological levels of cholesterol are required for the function and stability of the exocytic machinery (SNARE/Cav2.1

complexes). Vikman et al. [85] demonstrated the significance of cholesterol for normal biphasic insulin secretion in mouse β cells by removal of cholesterol from the plasma membrane using methyl- β -cyclodextrin (mCD). They found that desorption of cholesterol leads to the disturbance of a basic exocytotic mechanism partly due to migration of SNAP-25, and concluded that insulin secretion is highly sensitive to changes in plasma membrane cholesterol. In our recent experiments comparison of two insulin secreting tumor cell lines revealed surprising differences: while INS-1 line responded to both glucose and hyposmotic stimulation, INS-1E was unable to respond to osmotic stimulus while response to glucose was regular [56, 86]. Cellular content of cholesterol of INS-1E was much higher than that of INS-1 [87]. When we lowered cholesterol content by desorption with cyclodextrin (for review see [88]), response to hyposmotic stimulus in INS-1E cells emerged [87]. On the contrary supplementation of INS-1 cells with cholesterol inhibited their response to cell swelling [87]. Response to glucose was inhibited by cholesterol desorption in INS-1 cells, while response in INS-1E was more resistant, apparently residual cholesterol content was sufficient for the response. We conclude that cholesterol content substantially affects secretory process; both high and low level negatively affects the response. Optimal cholesterol content is different for either type of stimulation; swelling-induced mechanism is more sensitive to higher cholesterol content than that induced by glucose.

Hypothesis. Sequential exocytosis [89] seems to be dominating mechanism in swelling induced insulin secretion. Apparently, swollen vesicles fuse selectively with other vesicles that have been fused with plasma membrane [89, 90] after swelling but not glucose stimulation. Moreover, it was found that removal of plasma membrane cholesterol facilitates sequential exocytosis by threefold [90] which is in accordance with higher sensitivity of swelling-induced insulin secretion to the inhibitory effect of high cellular cholesterol resulting in absence of the response in intact INS-1E cells.

Tumor cells – diversity in the mechanism and response

Difference in response and/or signal transduction mechanism in tumor cells to cell swelling has been noticed time ago; Sato et al. [23, 49] found that in contrast to

normal cells the rise of intracellular Ca^{2+} is essential in cell swelling-induced secretion in tumor-derived GH4Cl and MMQ rat pituitary cells. They hypothesized that the striking difference in the transduction role of Ca^{2+} in secretion induced by cell swelling between the normal and tumor-derived cells may serve to differentiate normal and tumor cells in general [49]. Straub et al. [50] reported that the hyposmotic shock released insulin in medium with or without Ca^{2+} in betaHC9 cells. They also found that in betaHC9 cells DIDS had no effect on insulin secretory response to hypotonicity, while Beauwens et al. [91] found inhibition by NPPB, another Cl⁻ channel blocker in BRIN-BD11 cells.

INS-1 and INS-1E cells

Clonal INS-1E cells were isolated from parental INS-1 based on insulin content and secretory response to glucose [92]. We compared response of freshly isolated rat pancreatic islets with these tumor lines [56]; in Ca^{2+} containing medium glucose induced insulin release in all three cell types. Hypotonicity stimulated insulin release from islets and INS-1 cells. Insulin secretion in INS-1E cells was inhibited despite similar increase in cell volume. Surprisingly, perfusion with Ca^{2+} -depleted medium showed distinct secretory response of INS-1E cells to hypotonicity while that of INS-1 cells was partially inhibited [56]. Inhibition of L-type and/or P/Q-type calcium channels also enabled distinct response to hypotonic medium of INS-1E cells in Ca^{2+} containing medium [86]. At these conditions tetanus toxin inhibited hypotonicity-induced insulin secretion from INS-1 cells but not from INS-1E cells [86]. Interesting enough, Straub et al. [50] found two different exocytotic mechanisms operating in hyposmotic shock in GH4Cl cells in the presence and absence of Ca^{2+} in the medium. They supposed that these two mechanisms may be associated with the release of two distinct pools of insulin-containing granules [50]. It is likely, that cytoskeleton also participates in resistance to swelling-induced insulin secretion in INS-1E cells; the response emerged when cytochalasin B was added to preincubation medium [93].

Response to hypertonicity

Hypertonic medium inhibited glucose-induced insulin secretion from islets while both, INS-1 and INS-1E cell lines were resistant to the inhibitory effects of hypertonicity [56]. A similar effect was observed in HIT-T 15 cells which released insulin in response to hypertonic glucose, although the response was decreased as compared to isotonic conditions [94]. These data suggest different

sensing and/or sensitivity to the effects of osmolarity in insulin secreting tumor cell lines compared to pancreatic islets. Similarly as in pancreatic islets, noradrenaline inhibited glucose-induced but not swelling-induced insulin secretion from INS-1 cells [56].

Permeants

Cell swelling can be induced by hypotonicity or small permeant molecules like e.g. ethanol, urea. Glucose and both permeants induced insulin release from islets [95].

Although ethanol and urea induced similar swelling in tumor cells, they produced opposite effect on insulin secretion; while exposure to ethanol resulted in stimulation, exposure to urea led to suppression of insulin secretion in both, INS-1 and INS-1E tumor cell lines [95]. As expected, noradrenalin did not inhibit ethanol and urea-induced insulin secretion from islets [95]. Surprisingly, stimulating effect of ethanol was completely suppressed by noradrenalin in both tumor cell lines [95]. In contrast to hypotonicity, ethanol stimulated insulin secretion also from INS-1E tumor cells either in presence or absence of Ca^{2+} in the medium [83].

This short survey documents how signaling and response of tumor-derived cells to cell swelling varies markedly between different cell lines. Therefore tumor cell lines could be valuable tool for characterization and analysis of a specific mechanism. However, interpretation of data is limited; any generalization of results found in one cell type could be misleading. Comparison with native or other tumor cell type(s) could give important information and support more general interpretation of the data.

Pathophysiological implications

Secretion

Minor osmotic changes occurring in daily life might participate in regulation of hormone secretion in healthy persons. Hormones, amino acids, glucose and oxidative stress induce cell swelling or shrinkage within a narrow physiological range. Volume changes in different cell types trigger signal transduction events which contribute to the control and regulation of metabolism, transport and gene expression [96]. For example, hepatocyte swelling induced by either hypoosmolarity, glutamine, ethanol, or insulin mediates inhibition of proteolysis in perfused rat liver. Dehydration of hepatocytes as triggered by hyperosmolarity produces

insulin- and cytokine resistance and sensitizes cells to apoptotic stimuli [96]. On the other hand, glucose induces swelling of cells involved in glucose regulation - pancreatic beta- and alpha-cells. GLUT mediated glucose transport is a pre-requisite for this swelling as well as glucose metabolism. [97, 98].

Glycosuria resulting in dehydration with hypertonicity of extracellular fluid common in type 2 diabetic patients will further reduce their β -cell insulin secretory response to hyperglycemia and prevent insulin biological effects connected with inducing cell swelling.

Besides relative anisomolarity, cell swelling and insulin secretion can be also induced by other mechanisms including permeant presence in isosmolar extracellular fluid [11, 26, 36]. Extensive clinical experience indicates that unexpected, frequent, and prolonged hypoglycemia is a substantial problem after alcohol ingestion in diabetic subjects [99]. Ethanol in clinically relevant concentration in isosmotic medium induced dose-dependent release of insulin and TRH from adult rat pancreatic islets *in vitro* [26]. Ethanol as permeant enters also neurons and pituitary cells [28, 29] and induces swelling and release of peptides from neurons and pituitary. It is likely that release of beta-endorphin from pituitary cells induced by hypotonicity [25] could be induced also by ethanol.

Ischemia

Cell swelling could be important mediator of changes which take place at pathophysiological conditions; when tissue becomes ischemic there is a shift of the cells to anaerobic glycolysis and production of metabolites which can increase the intracellular osmolarity by 70 and 130 mOsmol/l after 15 and 60 minutes, respectively, thus increasing transmembrane osmotic pressure differences and producing cell swelling [100]. Exocytotic products released after swelling to surroundings of ischemic tissue could participate in the development of ischemia-reperfusion injury, but could be also mediators of local or remote preconditioning when factors released at the place of ischemia have protective effect [101]. Cell volume regulation was shown to be a key mechanism that accounts for most of the ischemic preconditioning protection in cardiomyocytes [102].

Diabetic heart

Studies focused on preconditioning-mediated cardioprotection in the diabetic heart are controversial [103]. We have, therefore, studied possible osmotic

mechanism of hyperglycemia. Perfusion of isolated rat heart with hyperosmolar high glucose medium followed by washout with isosmolar medium (relative hyposmotic stress) prior to ischemia substantially decreased postischemic contractile dysfunction, size of myocardial infarction and the severity of reperfusion-induced arrhythmias [104]. Sustained perfusion with hyperosmolar high glucose medium without washout period with isosmolar medium did not have any protective effect on ischemia-reperfusion injury thus indicating important role of cell swelling [104]. Apparently, factors released during relative hyposmotic stress (washout period) most likely participate in the mechanism of preconditioning [104]. Episodes of hyperglycemia with glycosuria leading to dehydration and hyperosmolarity followed by normalization of blood glucose and plasma osmolality could be the respective mechanism participating in ischemia preconditioning in diabetic heart.

Disruption of specific osmosensitive mechanisms.

Magnocellular neurons in the PVN and SON, specifically engaged in water and salt regulation, developed their specific response to osmotic stimuli – release of vasopressin and oxytocin in response to hyperosmotic stimulation. This specific response could be obviated by GdCl_3 and at these conditions ancient general secretory response to swelling-inducing stimuli emerged. This might be an explanation of the mechanism of the development of some clinical syndromes: Disruption of mechanosensitive gating in magnocellular neurosecretory cells perturbing specific response to osmotic stimuli could result in an inadequate secretory response of hormones engaged in water and salt regulation (e.g. stimulation of antidiuretic hormone secretion instead of inhibition in patients with hypervolaemic hyponatraemia). This kind of phenomenon could play a role at least in some types of the syndrome of inappropriate secretion of antidiuretic hormone – SIADH. This syndrome [105, 106] often seen by clinicians, could be induced also experimentally in gerbils by brain infarction [107]. SIADH develops due to persistent detectable or elevated plasma AVP concentrations in the presence of continued fluid intake [108]. Due to resulting inadequately high water reabsorption in kidney, hypotonic hyponatraemia is connected with high urine and low plasma osmolality. The major groups of causes of SIADH are: neoplasia, neurological diseases, lung diseases and a wide variety of drugs [108].

Exploitation of the mechanism of swelling-induced peptide secretion could be perspective and promising future for development of new class of drugs. Utilization of different signaling pathway and involvement of extra pool of secretory vesicles could be in some situations advantageous for powerful stimulation.

Abbreviations

ACTH (adrenocorticotropin); ANP (atrial natriuretic peptide); AT1 (angiotensin 1); BABTA/AM (1,2-Bis(2-aminophenoxy)ethane- N,N,N',N' -tetraacetic acid tetrakis (acetoxymethyl ester) – intracellular chelator); cAMP (cyclic adenosine monophosphate); DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulphonate); FSH (follicle stimulating hormone); GTP (guanosine triphosphate); GnRH (gonadotropin-releasing hormone); iPLA₂ (Ca^{2+} independent phospholipase); LH (luteinizing hormone); mCD (methyl- β -cyclodextrin); MSH (melanocyte-stimulating hormone); NA (noradrenaline); NH (neurohypophysis); NDGA (nordihydroguaiaretic acid, lipoxygenase inhibitor); NPPB ((5-nitro-2-(3-phenylpropylamino)-benzoate); SNAP-25 (membrane bound protein anchored to the cytosolic face of membranes); SON (hypothalamic supraoptic nucleus); NEM (N-ethylmaleimide); NSF (N-ethylmaleimide-sensitive-factor); PKA (protein kinase A); PKC (protein kinase C); PVN (hypothalamic paraventricular nucleus); RRP (readily releasable pool); RVD (regulatory volume decrease); SIADH (syndrome of inappropriate secretion of antidiuretic hormone); α SNAP (soluble NSF-attachment factor); SNARE (soluble NSF attachment protein receptor); TeTx (tetanus toxin); TSH (thyrotropin – thyroid stimulating hormone); TRH (thyrotropin releasing hormone); VAMP-2 (vesicle-associated membrane protein-2).

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