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Voltage-Operated Calcium Channel Heterogeneity in Pancreatic β Cells: Physiopathological Implications

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Voltage-operated calcium channels play crucial roles in stimulus-secretion coupling in pancreatic β cells. A growing body of evidence indicates that these channels in β cells are heterogeneous. In particular, not all the high-threshold calcium channels expressed belong to the best known L-type. In rat insulinoma cells, for example, L, N, and P/Q-type channels are present, while in human β cells L-type and P/Q-type dominate. Where present, N-type and P/Q-type channels participate, alongside with the dominant L-type, in the control of sugar- or depolarization-induced hormone release. Distinct biophysical properties and selective modulation of the channel subtypes are likely to play important physiological roles. T-type channels are involved in beta cell apoptosis, while calcium channel autoantibodies recognizing high-threshold channels in β cells, have been described both in neurological and diabetic patients. Subtype-selective calcium channel drugs have the potential for being beneficial in beta cell pathological states.

KEY WORDS: β Cell; calcium channel; insulin release; diabetes.

INTRODUCTION

Secretagogue-induced insulin release from pancreatic β cells is a Ca^{2+}-dependent process (Prentki and Matschinsky, 1987; Wollheim and Sharp, 1981). Following their electrochemical gradient, Ca^{2+} ions enter β cells through voltage-operated calcium channels (VOCCs), which open in response to the secretagogue-induced depolarization of the plasma membrane (Ashcroft and Rorsman, 1991; Petersen and Findlay, 1987), and induce the Ca^{2+}-dependent exocytotic release of insulin.

β cell firing pattern is indeed characterized by slow waves of "plateau" depolarisations on which bursts of action potentials are superimposed (Bertram and Sherman, 2000; Cook *et al*., 1991). Both electrical events have been shown to be mediated by calcium influx through VOCCs and to be temporally coupled to calcium increases and hormone secretion (Satin, 2000).

In nerve terminals, VOCCs are clustered at specialized membrane regions (the active zones) where synaptic vesicles dock and fuse to release their neurotransmitter content in a calcium-dependent manner (Llinas *et al*., 1995). To allow for rapid and synchronous release, VOCCs and vesicles are very close to each other, and physically linked by a complex set of "SNARE" proteins (Stanley, 1997). In pancreatic β cells (and more generally in secretory neuroendocrine cells such as pituitary, adrenal chromaffin, pulmonary neuroendocrine cells, etc.) the VOCCs and secretory granules reside in the same subcellular regions of the cells (Bokvist *et al.*, 1995), and depolarisation-induced calcium hot spots and exocytosis are also colocalized (Qian and Kennedy, 2001). However, "active zones" are not present and VOCCs are quite distant from the granules, as evidenced by a "secretory delay" and by the high affinity of the secretory process for calcium (for recent reviews see Mansvelder and Kits, 2000; Satin, 2000).

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Key to abbreviations: DHP, dihydropyridine; HVA, high voltage activated; LVA, low voltage activated; NTP, nitrendipine; VOCC, voltage-operated calcium channel; ω-CTx, ω-conotoxin.

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Besides their localization, the following important questions have been recently addressed on beta cell VOCCs. How many calcium channel subtypes are present in β cells? Do they play differential roles in cell excitability, stimulus-secretion coupling, hormonal modulation of release and pathological states? VOCCs have been described in β cell preparations obtained from various species (Abrahamsson *et al*., 1984; Davalli *et al*., 1996; Findlay and Dunne, 1985; Hopkins *et al.*, 1991; Kelly *et al.*, 1991; Misler *et al.*, 1992; Plant, 1988; Pressel and Misler, 1991; Rorsman and Trube, 1986; Satin and Cook, 1988; Sher *et al.*, 1992; Wollheim and Pozzan, 1984), and their biophysical and pharmacological properties have been partially characterized.

Most β cells have been shown to express mainly high voltage activated (HVA) VOCCs (Davalli *et al*., 1996; Findlay and Dunne, 1985; Hopkins *et al.*, 1991; Kelly *et al.*, 1991; Plant, 1988; Pressel and Misler, 1991; Rorsman and Trube, 1986; Satin and Cook, 1988; Sher *et al.*, 1992), although low voltage activated (LVA) VOCCs have also been observed in some cases (Ashcroft *et al.*, 1990; Davalli *et al*., 1996; Misler *et al.*, 1992; Sala and Matterson, 1990; Satin and Cook 1988).

There is general agreement that the L-type, dihydropyridine (DHP)-sensitive, VOCC represents the major HVA VOCC subtype expressed in pancreatic β cells (Satin, 2000, and references therein). This was originally mainly based on the "long lasting" nature and the DHP sensitivity of the high-threshold currents present in all β cells studied. However, both kinetic criteria (Carbone *et al.*, 1990) and DHP sensitivity (Mansverler *et al.*, 1996) could be very misleading.

One component of DHP-insensitive HVA VOCCs and secretagogue-induced insulin release was clearly evident in most of the studied β cell preparations (Hopkins *et al.*, 1991; Misler *et al.*, 1992; Plant, 1988; Rorsman and Trube, 1986; Wollheim and Pozzan, 1984), suggesting the presence of HVA VOCCs other than the L-type. The presence of non-L-type high-threshold channels seems to vary enormously between species, with such extremes as the mouse β cells, where currents are almost fully blocked by DHPs (Gilon *et al*., 1997), and canine β cells which are almost unaffected (Pressel and Misler, 1991). This alone highlights the need, from a therapeutical perspective, for a further analysis of the channel complement of human β cells.

We will review here the more recent evidence, on the basis of biophysical, pharmacological, molecular, and biochemical data, demonstrating that heterogeneous HVA VOCC subtypes are indeed present and functional in pancreatic β cells of different origin, including human.

L-Type Calcium Channels Are the Major Component of HVA Calcium Channels in β Cells

The L-type has long been identified at the major HVA VOCC subtype in β cells, mainly on the basis of biophysical and pharmacological evidence. More recently, molecular and biochemical studies supported these findings.

DHPs such as nitrendipine (NTP), nimodipine, and nifedipine, as well as phenylalkylamines such as verapamil, at least partially block β cell VOCCs (Findlay and Dunne, 1985; Hopkins *et al.*, 1991; Misler *et al.*, 1992; Plant, 1988; Rorsman and Trube, 1986; Sher *et al.*, 1992; Wollheim and Pozzan, 1984) and secretagogue-induced insulin release (Boschero *et al.*, 1988; Malaisse-Lagae *et al.*, 1984; Wollheim and Pozzan, 1984) in β cells of different origin.

Conversely, L-type VOCC "agonists," such as Bay-K-8644 and CGP-28392 have been shown to increase calcium currents (Davalli *et al*., 1996; Misler *et al.*, 1992; Plant, 1988; Pollo *et al.*, 1993; Sher *et al.*, 1992) or secretagogue-induced insulin release (Davalli *et al*., 1996; Henquin *et al.*, 1985; Malaisse-Lagae *et al.*, 1984) in some of these preparations.

At the molecular level, transcripts encoding both the α_1 C and α_1 D subunits, as well as the accessory α_2 and β subunits have been detected in β cells of different origin (Castellano *et al.*, 1993; Horwath *et al.*, 1998; Ihara *et al.*, 1995; Perez-Reyes *et al*., 1992; Seino *et al.*, 1992; Yaney *et al.*, 1992).

Biochemically, it was shown that in RINm5F insulinoma cells the α_1C subunits couples preferentially to β_{1b} and β_3 to form high affinity ^{3}H-DHP binding (Safayhi *et al.*, 1997). However, the exact stoichiometry of L-type channels in other β cells and in human β cells is not known yet.

Bokwist *et al.* (1995) showed that L-type channels are strongly coupled to exocytosis in mouse pancreatic β cells. The same group has shown that in another secretory cell type, the pancreatic α cell, the coupling between L-type and exocytosis could also be favoured by activation of adrenergic receptors (Gromada *et al.*, 1997). Direct molecular interactions between the different L-type isoforms and proteins of the secretory machinery have also been demonstrated (Wiser *et al.*, 1999; Yang *et al.*, 1999).

Secretion, however, is still slower than transmitter release from nerve terminals (Bokwist *et al.*, 1995; Mansvelder and Kits, 2000) and therefore the association is not as tight as in neurons for N or P/Q channels.

Furthermore, other channel subtypes are also involved in exocytosis from β cells (see below). Kim *et al.* (1998) directly compared L-type and non-L-type

involvement in exocytosis from the same single cell utilizing capacitance measurements, and concluded that both channel families equally contributed to release. A conservative view at this stage would be that even if the overwhelming evidence is in favor of a dominant role for L-type VOCCs in the control of hormone release from β cells, other VOCC subtypes are also involved and the contribution of L-type VOCCs is likely to vary significantly from species to species.

On the basis of its higher level of expression, it is likely that the α_1D subunit plays a major role in normal β cells. And the role of class D containing L-type channels might not be limited to the control of secretion. The class D isoform of the L-type VOCC seems to play an important role in β cell development as evidenced by the hypotrophy of the islets and increased apoptosis of β cells in class D α_1 subunit KO mice (Namkung *et al.*, 2001). Interestingly, in these animals compensatory mechanisms allow class C α_1 subunits to become major players in stimulus-secretion coupling (Namkung *et al.*, 2001).

Iwashima *et al.* (1993) also found that α_1 D mRNA expression is specifically and significantly downregulated after glucose infusions.

A "factor," possibly an autoantibody, able to potentiate L-type VOCCs and to induce apoptosis of β cells was described by Juntii-Berggren *et al.* (1993). This finding, still to be confirmed by other groups, might be an indication that autoimmune, or other humoral factors, affecting L-type channels could be relevant to the pathogenesis of diabetes.

Evidence for N-Type Calcium Channels in β Cells

As mentioned in the Introduction section, for many years definition of channel subtypes in different cell types was largely based on the fact that "long lasting" high threshold channels were automatically classified as L-type (Tsien *et al.*, 1988). Furthermore, block by DHPs was taken as a definitive evidence for the presence of L-type VOCCs. Endocrine cells, in particular, were often considered to express a "pure" population of high-threshold L-type channels. It has to be noted however, that these kinetic criteria were not very robust, and high doses of DHPs have been shown to block also non-L-type calcium channels, specifically in endocrine cells (Mansvelder *et al.*, 1996).

The availability of ω-Conotoxin GVIA (ω-CgTx GVIA), a peptide that selectively targets N-type VOCCs (Olivera *et al.*, 1990) together with more rigorous biophysical analysis, was instrumental in changing the above view (Sher and Clementi, 1991).

Utilizing ω-CgTx GVIA, we found, for example, that this toxin blocked one component of the HVA Ca^{2+} currents in the RINm5F rat insulinoma cell line, and reduced secretagogue-induced insulin release from the same cells (Sher *et al.*, 1992 and Fig. 1). This represented, in our view, a major step in understanding that high-threshold VOCCs in β cells are heterogeneous.

We later found N-type VOCCS in a different insulinoma cell line, INS-1 (Fig. 2, see also Horwarth *et al.*, 1998). Also in INS-1 cells, both glucose-and KCl-induced release were partially dependent on N-type channels (Fig. 3). Contradictory findings on N-type VOCC expression are available on normal, nontumoral rat β cells. ω-CgTx GVIA has been reported as having no (or only minor) effects on secretagogue-induced insulin release from rat islets (Komatsu *et al.*, 1989). However, other authors showed clear effects of this toxin on glucose- and arachidonic acid-induced insulin release from rat β cells (Ramanadham and Turk, 1994).

In both mouse (Gilon *et al.*, 1997) and human (Davalli *et al.*, 1996; Pollo *et al.*, 1993) β cells, N-type VOCCs are either absent or represent a very minor component difficult to identify unambiguously. It is true however, that a minor effect of ω-CgTx GVIA on release can be shown in human β cells (Fig. 4), despite the lack of evidence for the presence of ω-CgTx GVIA-sensitive currents.

We do not know the reasons for this apparent discrepancy. However, different sources of human β cells might express slightly different complements of VOCCs. On the other hand, because of the highly cooperative nature and nonlinearity between calcium influx through the VOCCs and secretion, it is enough to block a small proportion of channels (may be overlooked in the patch clamp studies) to achieve indeed a significant effect on release. This is the case in other secretory cells such as human small cell lung carcinoma (Codignola *et al.*, 1993), and probably the case in our experiments on β cells too, where the sum of release inhibition by the individual toxins is certainly higher than 100% (Figs. 1, 3, and 4), even if they individually block only a small fraction of current.

Different VOCC subtypes, can be subjected to differential modulation by hormones and neurotransmitters (Carbone and Swandulla, 1989). Noradrenaline can inhibit exocytosis in β cells via different mechanisms, including direct effect on the secretory machinery (Sher *et al.*, 1996) and VOCC modulation. The N-type VOCCs of RINm5F cells are selectively inhibited by noradrenaline in a voltage-dependent manner (Aicardi *et al.*, 1991).

Another form of VOCC modulation that we recently described relies on rapid changes in the surface number of VOCCs rather than on modulation of gating of preexisting

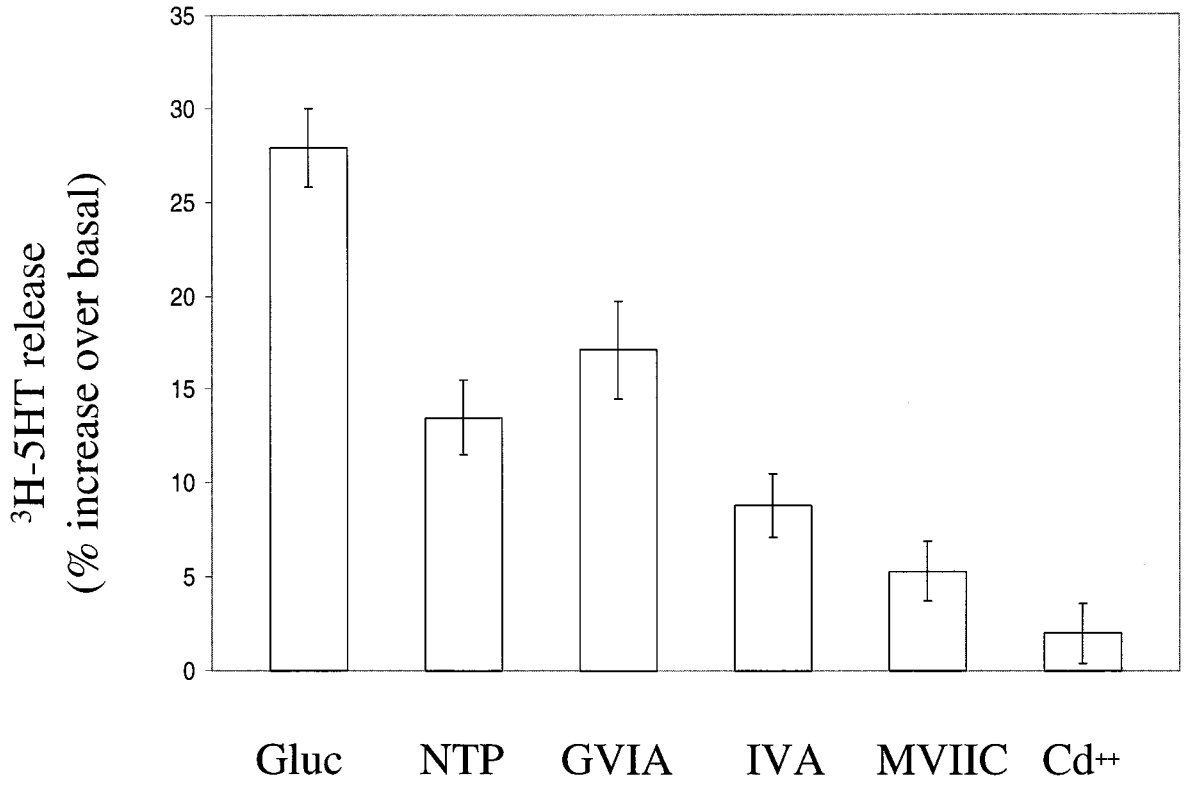

Fig. 1. Pharmacology of hormone release from RINm5F insulinoma cells. Hormone release was studied from RINmF cells as previously described (Sher *et al*., 1996) taking advantage of the corelease of serotonin (5HT) and insulin from the same secretory granules. Control release (Gly) was achieved by stimulating the cells with 20 mM glyceraldehyde, since these cells do not respond to glucose. While Cd^{2+} (200 μM), a nonselective blocker of all VOCCs, almost completely prevented release, Nitrendipine (NTP, 10 μM), ω-conotoxin GVIA (GVIA, 1 μM), ω-agatoxin IVA (IVA, 0.5 μM), and ω-conotoxin MVIIC (MVIIC, 1 μM), all partially inhibited glyceraldehyde-induced hormone release, suggesting that in these cells both L, N and P/Q-type VOCCs participate in the control of exocytosis.

channels. The N-type VOCC has been recently shown to be present in the secretory granule membrane, and to undergo an activity-dependent recruitment to the plasma membrane in different cell types (Sher, 1997).

Also in RINm5F cells the N-type VOCCs are subjected to the same form of recruitment via regulated exocytosis (Passafaro *et al.*, 2000), although information on a similar form of modulation for the other VOCC subtypes is still not available.

These N-type VOCCs of β cells are also recognized by Lambert–Eaton myasthenic syndrome (LEMS) autoantibodies (Sher *et al*., 1992) not only showing they are related to their neuronal counterparts, but opening the possibility that they might play specific roles in β cell autoimmunity.

Evidence for P/Q-Type Calcium Channels in β Cells

"Non-L, non-N" HVA VOCCs have been found in several β cell types. We have previously shown (Magnelli *et al.*, 1995) that the major part of these channels in RINm5F cells belongs to the P/Q-type being sensitive to both ω-aga IVA and ω-conotoxin MVIIC.

We have now extended this information to INS-1 rat insulinoma cells (Fig. 2), as well as normal human (Fig. 5) and rat β cells. In all these cells ω-aga IVA blocks a substantial percentage of the HVA currents (24 $\pm$ 1.7, 20.5 $\pm$ 4.3, 26.3 $\pm$ 8.1, and 22.5 $\pm$ 12.5% of block at saturating concentrations (200 nM) of ω-agatoxin IVA, in RINm5F, INS-1, normal rat, and normal human β cells, respectively, $n = 6$–25). Interestingly, and at variance to findings in neuronal cells (Mintz *et al.*, 1992, and inset to Fig. 2), the block by ω-aga IVA was found to be rapidly reversible in all the β cell types tested (Figs. 2 and 5; Magnelli *et al*., 1995).

By studying the contribution of VOCC subtypes to insulin or serotonin release from the different β cells we also confirmed that these P/Q-type VOCCs play a significant role in the control of sugar- or KCl-induced hormone release from β cells (Fig. 1, 3, and 4).

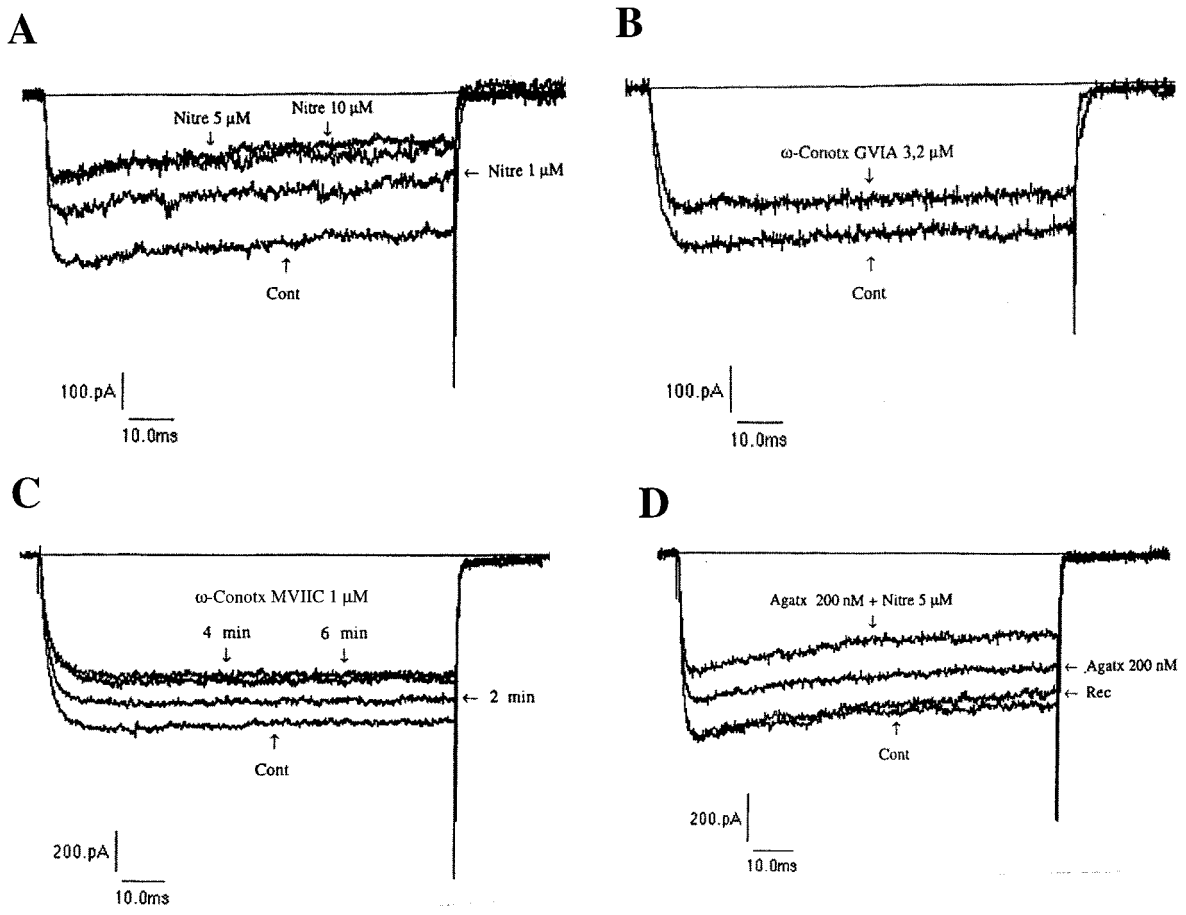

Fig. 2. Sensitivity of barium currents in INS-1 insulinoma cells to different VOCC blockers. Cultured INS-1 cells were patch clamped using standard protocols as described before (Pollo *et al.*, 1993). Barium currents were evoked with depolarizing voltage steps to +10 mV, delivered every 15 s. The traces in (A) represent a clear example where Nitrendipine, dose-dependently and saturably blocks only a proportion of the total currents, revealing the presence of non-L-type VOCCs. In (B) an example of the sensitivity of barium currents to a saturating concentration ω-CgTx GVIA is shown, demonstrating the presence in these cells of N-type VOCCs. In (C) the time-dependent block of a fraction of barium currents by ω-CTx MVIIC is shown, demonstrating, in parallel to (A) that a substantial fraction of currents is carried by non-L-type VOCCs. These non-L-type, ω-conotoxin MVIIC currents include a component of N-type VOCCs (B) and a component of P/Q-type VOCCs reversibly blocked by ω-agatoxin IVA (D). ω-agatoxin IVA and nitrendipine block are additive (D).

Class A α_1 subunits are known to code for P/Q-type channels in neurons. To confirm at the molecular level that class A calcium channels are expressed in pancreatic β cells, we utilized both RT-PCR and in situ hybridization techniques. Protein expression was evaluated with both Western blotting and immunohystochemistry.

As shown in Fig. 6A, all β cells, including human ones, express class A α_1 subunits as evidenced by the presence of amplification fragment of the expected size and with the right restriction fragment pattern. Western blotting revealed that a single band of the expected size was labelled by the selective class A antibodies in all β cell types (Fig. 6B).

Immunohystochemistry with a diverse set of antibodies confirmed that the class A α_1 subunit is highly expressed in pancreatic islets and in the β cells in particular (Fig. 7A). Finally, a specific class A α_1 subunit riboprobe has been found to hybridize selectively to the core of the pancreatic islet, where β cells are concentrated (Fig. 7B).

All together these data show, unambiguously, that P/Q-type are present and functional in rodent and human β cells.

Our results on human pancreatic β cells are similar to those obtained by Ligon *et al.* (1998) on rat tissue. Furthermore, they also cloned different class A α_1 subunit isoforms from rat β cells. Interestingly two "unique" isoforms, not previously described in the nervous system

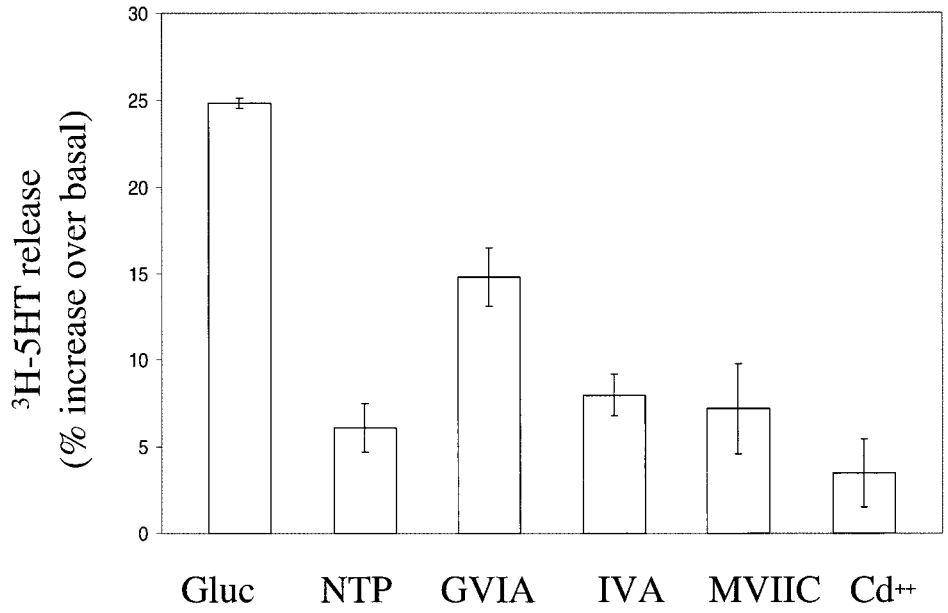

Fig. 3. Pharmacology of hormone release from INS-1 insulinoma cells. Similarly to what we found for RINm5F cells, and in accordance to the presence in these cells also of different VOCCs subtypes (see Fig. 2), all the different VOCC blockers partially depressed glucose (Gluc, 20 mM)-induced hormone release from INS-1 cells.

were found to be expressed in rat β cells. We still do not know if the different isoforms could generate P/Q-type channels with different properties, including the lower sensitivity and reversibility of ω-aga IVA action, as we reported here. This hypothesis is not unlikely, since class A splice variants have already been shown to generate "P/Q"-type VOCCs with different pharmacology (Bourinet *et al.*, 1999). Furthermore, a single amino acid mutation has

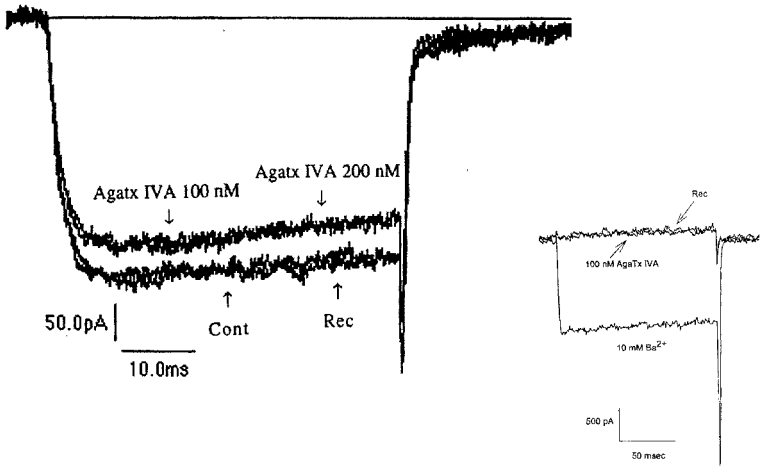

Fig. 4. P/Q-type VOCCs in human β cells. Human β cells were prepared, plated, and patch clamped as previously described (Davalli *et al.*, 1996). We have previously shown that human β cells express L-type VOCCs and a component of DHP-insensitive currents which are also insensitive to ω-CgTx GVIA, excluding a contribution of N-type VOCCs. We have now found that this non-L, non-N current is a P/Q-type current, blocked, as in other β cell types, by ω-agatoxin IVA, in a reversible manner. Inset: As a control, the same batch of ω-agatoxin IVA completely and irreversibly blocked, as expected, the calcium currents of cultured rat cerebellar Purkinje cells.

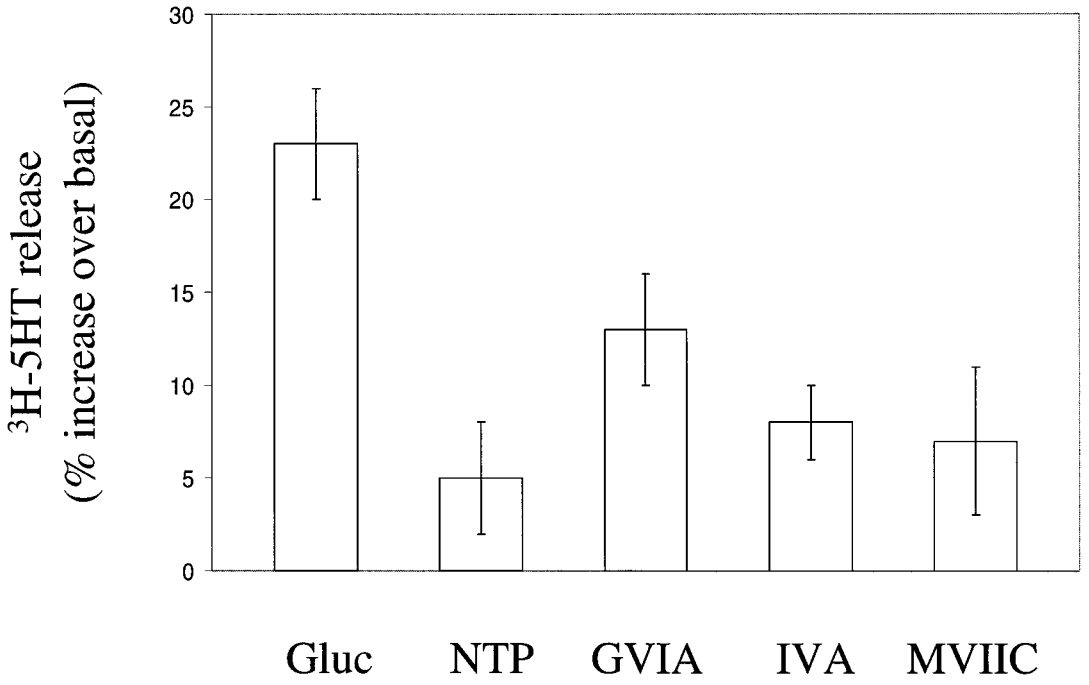

Fig. 5. Pharmacology of hormone release from human β cells. As shown for rat insulinoma cells (Figs. 1 and 3) and for rat normal β cells (see text), we also found that heterogeneous VOCC subtypes contribute to hormone release from human β cells. The contribution of the different VOCC subtypes to glucose (Gluc, 20 mM) induced hormone release is roughly proportional, although not linearly, to the percentage of current they mediate.

been shown to be enough to transform the normally irreversible block of class B VOCCs by ω-CgTx GVIA and ω-Conotoxin MVIIA, to a completely reversible one (Feng *et al.*, 2001).

P/Q-type VOCCs represent the major autoantigen for LEMS patients' autoantibodies (Sher *et al.*, 2000). We have also found that LEMS antibodies recognize and downregulate P/Q-type VOCCs in RINm5F cells (Magnelli *et al*., 1996), suggesting again, like for the N-type, that P/Q-type VOCCs could be primarily involved in autoimmune dysfunctions of β cells.

Other Calcium Channels in β Cells

T-type, LVA VOCCs have been previously described in pancreatic β cells (Ashcroft *et al.*, 1990; Davalli *et al.*, 1996; Misler *et al.*, 1992; Sala and Matteson, 1990; Satin and Cook, 1988).

In both human β cells and rat insulinoma INS-1 cells T-type VOCCs participate to the control of β cell firing and indirectly of hormone release (Bhattacharjee *et al.*, 1997; Misler *et al.*, 1992).

T-type VOCCs have recently been shown to play a crucial role in the sustained increase in cytosolic Ca^{2+} levels that mediate β cell destruction after exposure to pathogenic cytokines (Wang *et al.*, 1999). Furthermore, abnormal expression of T-type VOCCs has been described in both NOD (Wang *et al.*, 1996) and GK (Kato *et al.*, 1996) diabetic mice, as well as in streptozocin-induced diabetic rats (Kato *et al*., 1994).

More recently, two isoforms of the class G α_1 subunit, known to code for T-type VOCCs in other tissues (Perez-Reyes *et al.*, 1998) have also been cloned from rat insulinoma INS-1 cells (Zhuang *et al.*, 2000), but if they play a differential role in β cell excitability, this is still not known.

Small amounts of R-like VOCCs (as defined by their resistance to a cocktail of all known VOCC blockers) have been described in β cell lines (Magnelli *et al*., 1995; Vajna *et al.*, 2001). Class E α1 subunits, known to encode for some R-type VOCCs (Piedras-Renteira and Tsien, 1998) have been detected in INS-1 insulinoma cells as well as in the islets of Langherans in the intact pancreas (Grabsch *et al*., 1999). Although the real role of this minor component of VOCCs in β cell physiology is still not clear, a selective peptide blocker of Class E VOCCs, SNX-482, has been shown to block a component of high calcium currents as well as glucose-induced insulin release from INS-1 cells (Vajna *et al*., 2001).

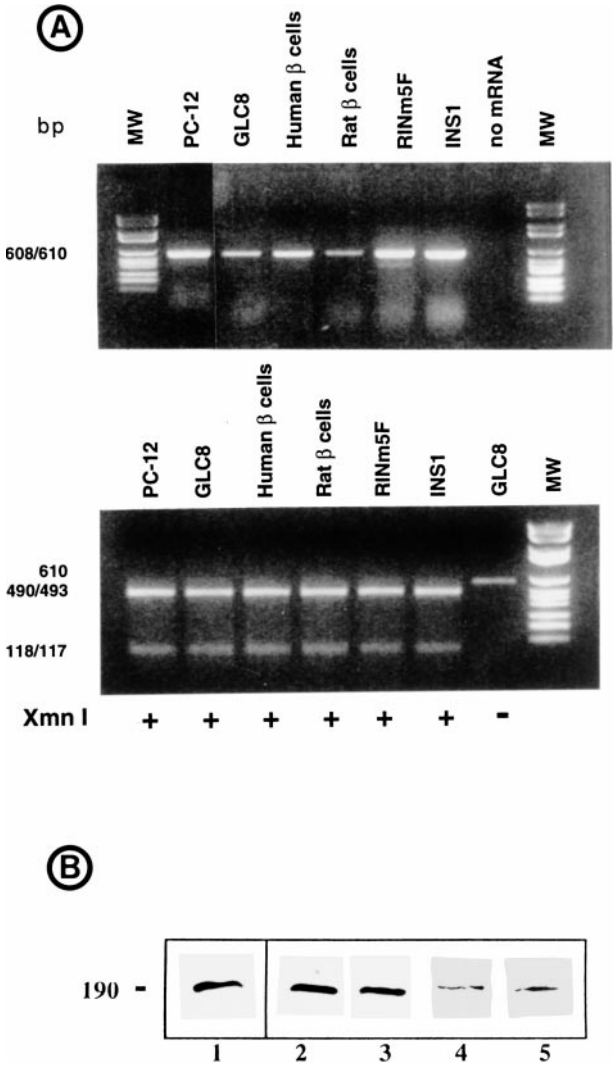

Fig. 6. Pancreatic β cells express class A $\alpha 1$ subunit mRNA and protein. RT-PCR has been performed on extracts of RINm5F, INS-1, normal rat, and normal human β cell, utilizing primers 100% homologous to both rat and human cDNAs encoding the class A α_1 VOCC subunit. Corresponding positions on cDNA sequences were rat-1635 and human-1905 for the forward primer and rat 2242 and human 2510 for the reverse one. An amplification product of the expected size (rat 608 bp, human 610 bp) was obtained in all β cells examined (A, upper panel), as well as in positive control cell lines (GLC8 human small cell lung carcinoma and PC12 rat pheochromocytoma). The specificity of the amplification product was verified by restriction analysis, showing a selective cut by XmnI, generating two fragments of $490 + 118$ and $493 + 117$ bp in rat and human amplified fragments, respectively (A, lower panel). In (B), Western blots of human (2), INS-1 (3), RINm5F (4), and rat (5) β cells are shown. HEK-293 cells stably transfected with the human class A α_1 subunit were used as control (1). Polyclonal antibodies were obtained from Alamone Labs (Israel). In all the samples, a major band of around 190 kDa was clearly recognized by these selective antibodies.

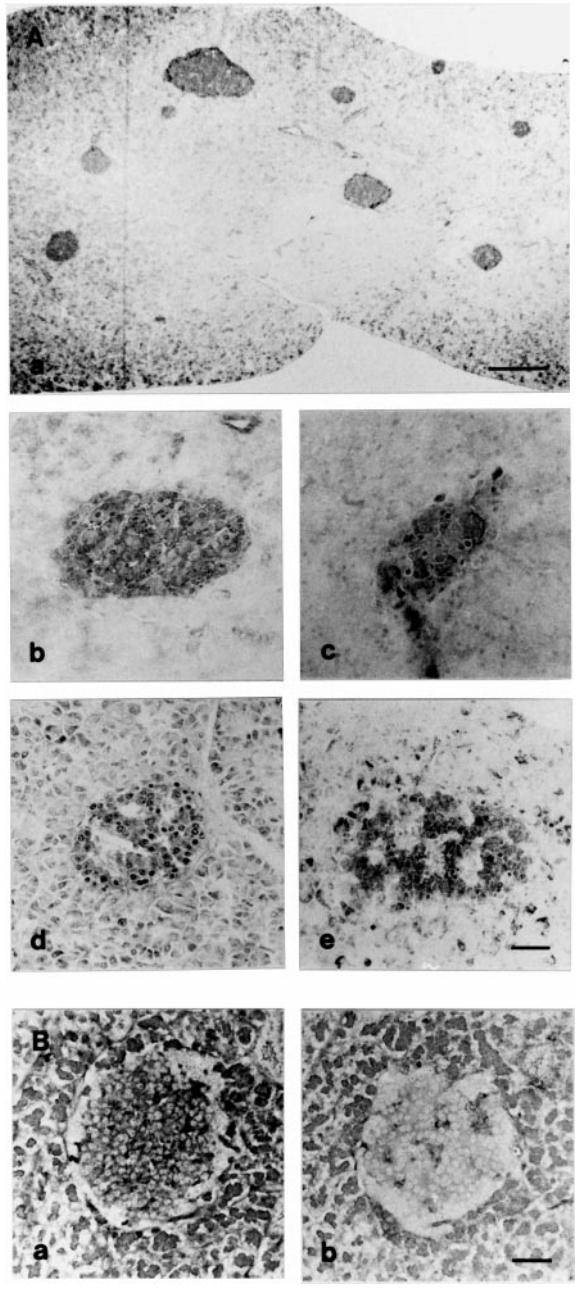

Fig. 7. Immunocytochemistry and in situ hybridization of class A α_1 subunits in rat and human pancreas. Different antibodies were used to detect the class A α_1 subunit protein in pancreas slices (A). In both rat (a) and human (d), pancreas slices, class A antibodies, recognizing an intracellular epitope, labeled specifically the endocrine islets and not the exocrine tissue. The same was true, in rat tissues, for an antibody raised against an extracellular epitope of class A (c). Similar labeling was also obtained when a sandwich of ω-agatoxin IVA plus antitoxin antibodies was utilized in rat (b) and human (e) tissue. In (B), sections of human pancreas were stained with antisense (a) and sense (b) riboprobes specific for the human class A subunit (Volsen *et al.*, 1995). Only islet cells show cytoplasmic labelling with the antisense probe and only background staining with the control probe.

CONCLUSIONS

In most β cell preparations, both electrophysiological and biochemical approaches have shown that DHP-sensitive L-type VOCCs predominate (Boschero *et al.*, 1988; Davalli *et al.*, 1996; Findlay and Dunne, 1985; Henquin *et al.*, 1985; Hopkins *et al.*, 1991; Malaisse-Lagae *et al.*, 1984; Misler *et al.*, 1992; Plant, 1988; Pollo *et al*., 1993; Rorsman and Trube, 1986; Sher *et al.*, 1992; Wollheim and Pozzan, 1984). In line with their ability to affect the HVA VOCCs of β cells, DHPs have also been shown to regulate secretagogue-induced insulin secretion from the same cells.

However, heterogeneity in the VOCC subtypes expressed by β cells has been documented (Ashcroft *et al.*, 1990; Davalli *et al.*, 1996; Hopkins *et al.*, 1991; Marchetti *et al.*, 1994; Misler *et al.*, 1992; Pollo *et al.*, 1993; Sala and Matteson, 1990; Satin *et al.*, 1995; Satin and Cook, 1998; Sher *et al.*, 1992) and, in most β cells, DHP drugs leave a component of HVA Ca^{2+} currents unaffected.

From a therapeutic point of view, a detailed characterization of VOCCs in human β cells is crucial. However, only recently have both voltage-dependent (Barnett *et al.*, 1995; Davalli *et al.*, 1996; Kelly *et al.*, 1991; Misler *et al.*, 1992; Pollo *et al.*, 1993) and voltage-independent (Rojas *et al.*, 1990) calcium channels been described by electrophysiological means in human pancreatic β cells.

Human β cells express both LVA and HVA VOCCs. Furthermore, a major component of the HVA current of human β cells is DHP-sensitive and Ca^{2+} influx through these L-type channels is important for triggering insulin release. (Barnett *et al.*, 1995; Davalli *et al.*, 1996; Misler *et al.*, 1992; Pollo *et al*., 1993).

However, DHPs do not block all HVA VOCCs or glucose-induced insulin release from human β cells. Unlike the above-mentioned results obtained in RINm5F, INS-1, and rat β cells, where ω-CgTx GVIA blocks part of the DHP-insensitive HVA currents and partially blocks secretagogue-induced insulin release, the "non-L" current components in human β cells were insensitive to ω-CgTx GVIA. Since the presence of ω-CgTx-sensitive HVA VOCCs is not a peculiar characteristics of insulinoma cells, but it was confirmed in normal rat β cells (see above), it is reasonabe to believe that these N-type calcium channels could be present in β cells from several species. However, our findings support the view that in human β cells N-type VOCCs only play a minor role, if any.

On the other hand, all the β cells tested, normal and tumoral, rodent and human, express P/Q-type VOCCs that contribute to insulin release and can be the target of calcium channel autoantibodies. In this paper we report original results showing that P/Q-type VOCCs, as well as class A α1 subunit mRNA and protien, are actually expressed and functional also in human β cells.

The coexpression of a multitude of VOCC subtypes in β cells, with very different biophysical and pharmacological properties, could contribute to the complex shaping of β cell bursting activity.

A better understanding of the presence and functional role of the different VOCC subtypes in human pancreatic β cells will lead, hopefully, to the discovery of selective drugs aimed at treating insulin release dysfunctions such as hyperinsulinaemia or diabetes mellitus.

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