Volume 311, number 2, 85–90 FEBS 11634 October 1992

Minireview

Intracellular signal transduction pathways that control pancreatic β-cell proliferation

Åke Sjöholm

Department of Endocrinology, The Rolf Luft Center for Diabetes Research, Karolinska Institute, Karolinska Hospital, Box 60500, S-104 01 Stockholm, Sweden

Received 14 May 1992; revised version received 3 September 1992

This review focuses on the factors that regulate the proliferation of pancreatic islet β-cells in vitro, and in particular on the intracellular pathways that convey the mitogenic signal into a proliferative response. Substances as diverse as nutrients, polypeptides, cytokines, adrenergic agents, lithium, phorbol esters and cyclic AMP analogs are all able to stimulate or inhibit β-cell proliferation in a time- and concentration-dependent manner. The evidence for involvement of cyclic AMP, cyclic GMP, protein kinase C, inositol polyphosphates, GTP-binding proteins, polyamines and oncogenes is reviewed.

Pancreatic islet; Insulin secretion; Protein kinase C; Cyclic nucleotide; Polyamine; N-protein

1. INTRODUCTION

The pancreatic β-cell has a limited proliferative potential, and the production of new β-cells by division normally does not exceed 3% per day, a figure that rapidly declines with increasing age [1]. Early investigators demonstrated that growth of the islet organ could be stimulated in vivo by diets rich in carbohydrates [2] or in a genetic animal model for diabetes mellitus, viz. the obese-hyperglycemic mouse [3]. Most studies in recent years aiming at elucidating factors controlling the proliferation of β-cells in vitro have utilized pancreatic islets isolated from fetal or neonatal rats containing a high fraction of rapidly growing β-cells, and rely heavily on the use of [^{3}H]thymidine incorporation techniques as measures of β-cell proliferation [1].

Knowledge of the mechanisms that govern the proliferative capacity of the healthy β-cell may be exploited to maximize the growth potential of pancreatic islet grafts prior to transplantation into diabetic patients and thus improve the fate of the graft. Given the fact that virtually all forms of diabetes mellitus are characterized by an insufficient extent of β-cell replication needed to compensate for the loss or dysfunction of β-cells occurring in diabetes [1], elucidation of the growth-regulating pathways might also give rise to novel pharmacological means of expanding the remaining endogenous β-cell population. It should be recognized that the techniques used to assess β-cell proliferation, i.e. [^{3}H]thymidine labeling or bromodeoxyuridine incorporation into DNA, have several shortcomings, including artefactual changes in incorporation due to alterations in isotope uptake, metabolism and specific activity [1]. The regulation of β-cell replication by extracellular factors and by intracellular signaling systems is summarized in Fig. 1.

Correspondence address: Å. Sjöholm, Department of Endocrinology, The Rolf Luft Center for Diabetes Research, Karolinska Institute, Karolinska Hospital, Box 60500, S-104 01 Stockholm, Sweden. Fax: (46) (8) 30 34 58.

Abbreviations: Cyclic AMP, adenosine-3′,5′-cyclic monophosphate; cyclic GMP, guanosine-3′,5′-cyclic monophosphate; DBI, diazepam binding inhibitor; DNA, deoxyribonucleic acid; GABA, γ-aminobutyric acid; GH, growth hormone; GTP, guanosine trisphosphate; IGF-I, insulin-like growth factor I; PDGF, platelet-derived growth factor; Sp-cAMP[S], stimulatory diastereomer of adenosine-3′,5′-cyclic monophosphorothioate; TGF-β, transforming growth factor β; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

2. NUTRIENTS

D-Glucose is a cardinal stimulator of β-cell proliferation in adult and late fetal life, whereas the sugar seems to be inactive at earlier stages of development [4]. Metabolism of the carbohydrate appears essential, because L-glucose, D-fructose and 3-*O*-methylglucose are inactive as β-cell mitogens, and mannoheptulose, an inhibitor of glucose phosphorylation, suppresses β-cell growth [5,6]. When fetal rat islets were maintained in tissue culture at widely different glucose concentrations, it was found that the cell cycle proceeded at constant rate irrespective of ambient glucose concentration [7]. This gave rise to the proposal that glucose enhances β-cell replication by increasing the number of β-cells entering the cell cycle.

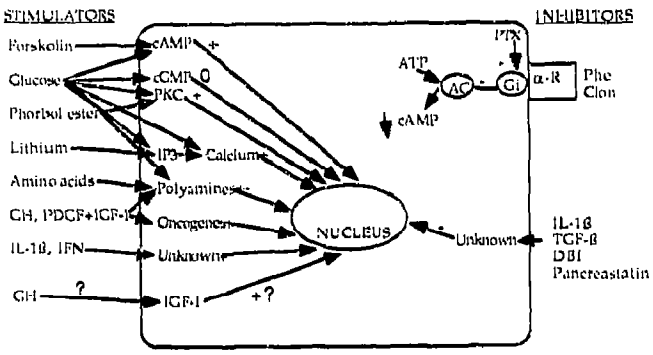

Fig. 1. Summary of the mechanisms that regulate pancreatic β-cell proliferation in vitro. This cartoon depicts extracellular factors that stimulate (left side) or inhibit (right side) β-cell replication. Inside the cell different signal transduction pathways are schematically shown. Arrows indicate stimulatory (+) or inhibitory (−) actions on cell proliferation. '0' denotes no effect, whereas '?' denotes uncertain effect. AC, adenylyl cyclase; α-R, α-adrenergic receptor; ATP, adenosine trisphosphate; cAMP, adenosine-3',5'-cyclic monophosphate; cGMP, guanosine-3',5'-cyclic monophosphate; Clon, clonidine; DBI, diazepam binding inhibitor; GH, growth hormone; Gi, inhibitory GTP-binding protein; IFN, γ-interferon; IGF-I, insulin-like growth factor I; IL-1β, interleukin-1β; IP3, inositol trisphosphate; PDGF, platelet-derived growth factor; Phe, phenylephrine; PKC, protein kinase C; PTX, pertussis toxin; TGF-β, transforming growth factor β. The α-adrenergic agonists clonidine and phenylephrine inhibit β-cell replication by suppressing cAMP synthesis. This effect occurs as a result of interference with an inhibitory GTP-binding protein that couples the receptor and adenylyl cyclase, an effect that is prevented by pertussis toxin. See text for further details.

Amino acids are also able to stimulate β-cell replication, and it appears as if in early fetal life they are more important than is glucose in this respect [4]. Amino acids, and human amniotic fluid, were recently also identified as potent stimulators of cell proliferation in adult mouse islets [8]. Interestingly, in children with certain inborn errors of amino acid metabolism leading to hyperaminoacidemia, an expansion of the pancreatic β-cell fraction has been noted, strongly suggesting that the stimulation of β-cell replication observed in vitro bears clinical significance [9].

3. POLYPEPTIDES

Among the large number of protein hormones existing, growth hormone (GH) and the biologically related lactogenic peptides prolactin and placental lactogen have been extensively investigated with regard to effects on β-cell proliferation. Thus, GH has been reported to stimulate the in vitro replication of fetal [10], neonatal [11,12] and adult [12,13] rat β-cells. In most of these studies there was also a stimulatory effect of GH on the insulin content and/or secretion and the majority of effects were mimicked by prolactin and placental lactogen. It should be noted that the doses of hormones used in the referred studies are several orders of magnitude greater than those encountered in vivo, which sheds some doubt on the physiological significance of the observed effects. On the other hand, it should be borne in mind that in acromegaly and in neonates, as high serum levels of GH as 0.2 μg/ml have been reported [1]. Also during pregnancy, levels of prolactin approach 0.2 μg/ml and those of placental lactogen 8 μg/ml [1]. It is thus conceivable that the elevated serum levels of these hormones may contribute to the expansion of the β-cell mass that occurs during these states by direct interaction with the β-cell. Because GH in many other tissues appears to elicit its biological activities by inducing local production of insulin-like growth factors (IGFs) in target cells [14], the issue of whether a similar paracrine pathway operates also in islets has been addressed. Thus, Swenne et al. [10] and Swenne and Hill [13] reported that in both fetal and adult rat islets GH, but not glucose, stimulated release of IGF-I from islets and that inclusion of a monoclonal antibody to IGF-I partially counteracted the mitogenicity of GH. These results, and the findings that exogenous IGF-I stimulates β-cell replication and that β-cells possess high-affinity IGF-I receptors [15], led Swenne et al. [10,13] to propose that IGF-I, produced by islet cells, participates in a paracrine loop that mediates part of GH's mitogenicity in β-cells. This view has, however, been challenged by others [16], who failed to detect an increase in IGF-I secretion from neonatal rat islets after GH exposure. In addition, Hansson and associates [17] were unable to see a decrease in pancreatic IGF-I mRNA after alloxan administration, and suggested that IGF-I immunoreactivity seen in association with β-cells represents peptide that has either been internalized or receptor-bound. This latter interpretation was also proposed by Nielsen et al. [18], who found a very modest mitogenicity of IGF-I that did not augment the powerful response to GH. A recent preliminary report showed that GH does not affect expression of c-*fos* in RIN5AH insulinoma cells, whereas a stimulatory effect on the expression of this oncogene was noted after exposure to the cyclic AMP-raising agent forskolin [19].

Other polypeptides investigated for possible effects on β-cell proliferation include cytokines which are produced by various immune cells that are known to infiltrate the pancreatic islets early in the course of juvenile diabetes mellitus [20]. While interleukin-1β was found to suppress islet cell proliferation in adult mice and rats [21,22], a bimodal pattern was noted in fetal rat islets [23]. Thus, during the initial 24 h interleukin-1β suppressed β-cell growth, an effect that waned by 2 days and was turned into a potent mitogenic response by 3 days of exposure [23]. Interestingly, interleukin-1β has been found to increase the expression of c-*fos* in insulin-producing cells [24] and to stimulate the activity of a tyrosine kinase [25]. Interferon-γ caused a much more moderate enhancement of β-cell replication from day 1 and onwards while at the same time inhibiting insulin secretion [23]. Interleukin-6, tumor necrosis factor α,

and interferon-α were virtually inactive in this system [23].

Naturally, since the islets are comprised of a heterogenous cell population, there is a risk of picking up changes in proliferation of islet cells other than the β-cells. In particular, contamination of rapidly growing mesenchymal cells must be excluded, since this might well obscure changes in β-cell DNA synthesis. To this end, electron microscopical analyses have shown that no fibroblast-like or acinar cells could be detected in cultured fetal islets, which were found to consist of >90% β-cells [10]. In addition, we and others have recently shown that these islets do not increase their rate of DNA synthesis in response to classical fibroblast mitogens such as EGF, TGF-α or PDFG; whereas well-known stimulators of β-cell replication (i.e. glucose or GH) were effective in this respect [26,27]. These combined findings support the notion that most results reported on fetal islets in this review reflect alterations in β-cell DNA synthesis; however, it is advisable that, at least when working with adult islets that contain ≈70–80% β-cells, to identify the DNA-synthesizing cells by immunostaining.

4. POLYAMINES

When β-cell proliferation was stimulated in vitro by glucose, amino acids, GH, PDGF + IGF-I or lithium, it was found that these substances also elevated the islet contents of polyamines [8,26,27], molecules known in other tissues to regulate cell replication [28]. When the increases in polyamines were prevented by addition of selective polyamine synthesis inhibitors together with the mitogenic factors, the elevated rates of β-cell DNA synthesis persisted, or were in some cases even amplified. However, the polyamine content of cell nuclei was not affected by the inhibitors, suggesting that maintenance of polyamines in the nucleus may be an event sufficient to permit a mitotic signal to be translated into a proliferative response. In adult β-cells the bulk of polyamines have been shown by immunochemical staining to be localized in insulin secretory granules [28a]. However, in fetal β-cells as much as 20–25% seem to be associated with the nucleus. It is not known whether changes in cytosolic polyamines may be transduced into the nucleus by the polyamine-dependent casein-kinase II. See Fig. 2 for a view of β-cell polyamine metabolism.

Transforming growth factor β (TGF-β) is a widely distributed polypeptide, which recently was reported to inhibit the mitogenic response of β-cells to a high glucose concentration, but not to GH [29]. This effect by the peptide likely resulted from interference with signal transduction beyond the step of polyamine synthesis, because the islet polyamine content was not affected by TGF-β. Among other regulators of β-cell replication, we recently identified pancreastatin and diazepam binding inhibitor (DBI) as inhibitors of this process [30].

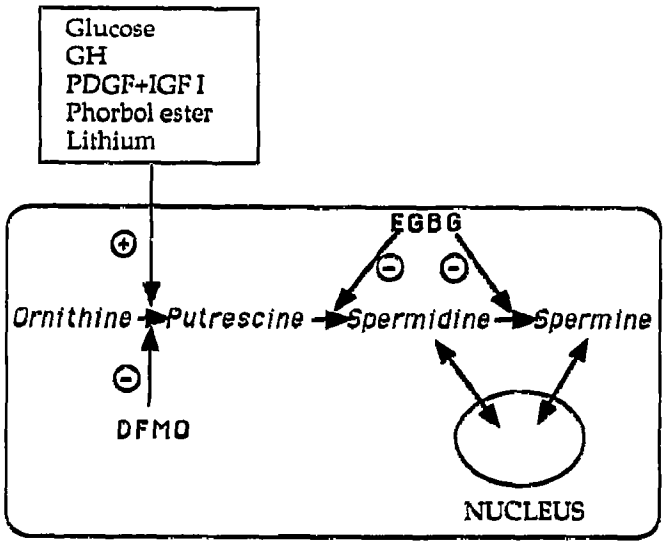

Fig. 2. Polyamine metabolism in pancreatic β-cells. Ornithine is decarboxylated to yield the diamine putrescine in a reaction catalyzed by ornithine decarboxylase, the overall rate-regulating enzyme in this pathway. *S*-Adenosylmethionine decarboxylase catalyzes the formation of decarboxylated *S*-adenosylmethionine, which serves as a donor of aminopropyl moieties used in the further synthesis of spermidine and spermine. In the top, a number of stimulators of β-cell proliferation and polyamine synthesis are shown. '+' denotes stimulation. Compounds that inhibit (−) enzyme activity are DFMO, which specifically and irreversibly inactivate ornithine decarboxylase, and EGBG which is highly selective *S*-adenosylmethionine decarboxylase inhibitor. These inhibitors decrease the cytosolic polyamine content and appear to cause a translocation of amines into the nucleus; this latter event that may explain why β-cell proliferation is not decreased after treatment with DFMO and EGBG. DFMO, DL-α-difluoromethylornithine; EGBG, ethylglyoxal bis(guanylhydrazone); GH, growth hormone; IGF-1, insulin-like growth factor I; PDGF, platelet-derived growth factor.

Pancreastatin, but not DBI, also lowered the islet content of polyamines, an event possibly contributing to the antiproliferative action of this peptide. Since both pancreastatin and DBI are produced by islet cells, it is conceivable that they might function as paracrine inhibitors of β-cell replication in vivo.

5. CYCLIC NUCLEOTIDES AND GTP-BINDING PROTEINS

Among many early events taking place in response to mitogenic stimulation in various cell systems are increases in cyclic AMP synthesis, protein kinase C activation, inositol polyphosphate generation and calcium influx. These events occur also in the β-cell in response to secretagogic stimulation, but it is not known whether these processes play any role in β-cell replication.

In search for intracellular mechanisms operational in transducing the mitogenic message of glucose, a great deal of interest has been focused on the role of cyclic AMP in this context [1]. Glucose is known to increase cyclic AMP in islets, but attempts to utilize various

cyclic AMP analogs as pharmacological probes have resulted in a large body of conflicting results concerning the role of cyclic AMP in β-cell proliferation. Thus, addition of the phosphodiesterase inhibitor theophylline was found to decrease replication of neonatal β-cells in culture [31]. On the other hand, addition of another phosphodiesterase inhibitor, viz. isobutyl-1-methylxanthine produced bimodal effects inasmuch as low concentrations of the drug stimulated [31,32], and high concentrations suppressed β-cell growth [32]. 8-Br-cyclic AMP reportedly enhanced β-cell replication [32], while dibutyryl-cyclic AMP was found either to stimulate [32,33] or inhibit [34] β-cell replication. In addition, the adenylyl cyclase activator forskolin has been reported to stimulate this process [35,36].

All these results should be viewed with a great deal of caution, because none of the analogs used interferes specifically with cyclic AMP metabolism. For instance, for each cyclic AMP molecule split off once dibutyryl-cyclic AMP has entered the cell, two butyryl moities are liberated. Since butyrate is known to affect many cellular functions of its own, including DNA synthesis [37], the true role of cyclic AMP in β-cell replication might be severely obscured by use of dibutyryl-cyclic AMP. Indeed, we have found that high concentrations of sodium butyrate causes a marked inhibition of β-cell growth and also blocks the mitogenic response to the cyclic AMP-raising agent forskolin [36].

In two recent studies it was shown that proliferation (and insulin secretion) of fetal rat β-cells could be significantly suppressed by α-adrenergic stimulation (using the α_1-agonist phenylephrine and the α_2-agonist clonidine) and by the cytokine interleukin-1β [38,39]. In both cases islet cyclic AMP content was found to be lowered as well. Addition of pertussis toxin, which alleviates adenylyl cyclase from an inhibitory constraint by ADP-ribosylating GTP-binding proteins, resulted in an increase in islet cyclic AMP and stimulation of β-cell proliferation and insulin secretion; these effets were mimicked by the membrane-permeant stimulatory cyclic AMP analog Sp-cAMP[S] [38,39]. When the α-adrenergic agonists were given together with Sp-cAMP[S] or to pertussis toxin-pretreated islets, the suppressed β-cell proliferation and insulin secretion were partially prevented, suggesting that α-adrenergic stimulation represses β-cell growth and hormone release in part by interfering with GTP-binding proteins that connect cell surface receptors to adenylyl cyclase [38]. In contrast, no such protection was imposed by pertussis toxin or Sp-cAMP[S] against the inhibitory actions of interleukin-1β [39]; nor was the suppression of RINm5F insulinoma cell proliferation and insulin secretion evoked by interleukin-1β, interferon-γ, or interferon-α affected by pertussis toxin pretreatment [40]. In their entirety, the findings referred to above point to a stimulatory, rather than inhibitory, role for cyclic AMP in regulation of β-cell growth.

The guanylate cyclase activator atrial natriuretic peptide, known to increase β-cell cyclic GMP levels [41], or a membrane permeant cGMP analog failed to affect β-cell growth under basal and stimulatory conditions, suggesting that cyclic GMP has no modulatory role in this process [36], although glucose has been reported to increase islet content of this nucleotide [42].

6. PROTEIN KINASE C AND INOSITOL POLYPHOSPHATES

Some β-cell mitogens, e.g. glucose and polypeptide growth factors, are known in β-cells or other tissues to enhance the activity of protein kinase C and to stimulate synthesis of inositol polyphosphates from plasma membrane phospholipids [43,44]. Protein kinase C is a phospholipid- and Ca^{2+}-dependent enzyme that is activated by diacylglycerol which is formed during agonist-induced polyphosphoinositide hydrolysis [45], an event that occurs early in transduction of the mitogenic signal [44]. Protein kinase C activity is subject to stimulation not only by the endogenous compound diacylglycerol, but also by tumor promoting phorbol esters, such as 12-*O*-tetradecanoylphorbol 13-acetate (TPA, [45]).

Exposure of fetal islets to low concentrations of TPA, considered specific for C-kinase activation, led to a stimulation of β-cell replication, the magnitude of which resembled that of glucose or GH [46]. TPA also elevated the islet polyamine content, but when this increase was precluded by use of enzyme-directed inhibitors of polyamine synthesis, the mitogenic response persisted. It should be noted, however, that in another system of islet cell preparations, this mitogenic action of TPA was not observed [35].

When protein kinase C was down-regulated by prolonged exposure to high concentrations of TPA prior to addition of high glucose, GH or forskolin, the mitogenic and secretory responses to these agents persisted [36]. On the other hand, inclusion of H-7, an inhibitor of protein kinase C and cyclic AMP-dependent kinases, caused a marked inhibition of proliferative activity but it is difficult to tell whether this is due to actions predominantly on the cyclic AMP- or the C-kinase system [46].

Cellular inositol polyphosphate levels can also be increased in a way different from stimulation of their synthesis, viz. by preventing their degradation. One compound capable of preventing inositol polyphosphate breakdown is lithium, which has been shown to elevate a number of inositol polyphosphate species in β-cells and also is a β-cell mitogen [27]. In particular, inositol-1,3,4-trisphosphate was increased by lithium, and has also been reported to be elevated by the potent stimulator of β-cell growth, glucose. It is therefore conceivable that this inositol polyphosphate or others, possibly due to their ability of mobilizing intracellular Ca^{2+}, is important in mediating the mitogenicity of certain

β-cell growth factors. It is clear, however, that this area is in need of further investigations.

7. CONCLUSIONS

Pancreatic β-cell proliferation can be stimulated in vitro by glucose, amino acids, GH, PDGF + IGF-I, lithium, interferon-γ or interleukin-1β. Interleukin-1β, however, caused an initial inhibition of β-cell growth that occurred independent of interaction with pertussis toxin-sensitive GTP-binding proteins or the cyclic AMP signaling pathway. TGF-β and the islet-produced peptides pancreastatin and diazepam binding inhibitor can function as inhibitors of β-cell growth depending on the ambient glucose concentration. α-Adrenergic agonists were found to suppress β-cell proliferation in part by interference with GTP-binding protein(s) connected to adenylyl cyclase. Many of the mitogenic factors caused an increase in the islet content of polyamines; however, when this increase was blocked by enzyme-directed inhibitors the mitogenicity of these compounds persisted. Specific pharmacological activation of protein kinase C or cyclic AMP synthesis increases β-cell DNA synthesis.

8. FUTURE PROSPECTS

While most recent studies on the regulation of β-cell replication have focused on various biochemical pathways, future research efforts should take advantage of molecular biology techniques in order to elucidate in detail the mechanisms that govern β-cell replication. Such approaches would include studies of the expanding family of proto-oncogenes, encompassing analysis of changes in their expression after mitogenic stimulation, clarification of the functional significance of a particular proto-oncogene by addition of specific antisense oligonucleotides and by overexpression of the oncogene in isolated islets or in transgenic animal models. Similar approaches may be used to investigate the role of other regulatory molecules, e.g. isozymes of phospholipases, protein kinases/phosphatases, polyamine synthesizing enzymes, phosphatidyl kinase and various tyrosine kinases. Other biochemical systems that need to be investigated include Ca^{2+} fluxes, signaling through 5-HT, GABAergic and dopaminergic receptors and the inositol polyphosphate system.

Acknowledgements: Own work referred to herein was supported financially by the Swedish Medical Research Council (12X-109), the Cancer Foundation, Funds of the Karolinska Institute, the Medical Faculty at Uppsala University, the Swedish Diabetes Association, the Swedish Society of Medicine, the Clas Groschinsky Memorial Foundation, the Helge Axelson Johnson Foundation, the Torsten and Ragnar Söderberg Foundations, the Hoechst Diabetes Foundation, the Nordic Insulin Fund, the Family Ernfors Fund, the Royal Swedish Academy of Sciences, the Lars Hierta Memorial Foundation, and the Swedish Society for Medical Research.

REFERENCES

[1] Hellerström, C., Andersson, A., Swenne, I., Welsh, M., Welsh, N. and Sjöholm, Å., in: Pathogenesis of Non-Insulin Dependent Diabetes Mellitus (S. Efendić and V. Grill, Eds.) Raven Press, New York, 1988, pp. 79–91.
[2] Tejning, S. (1947) Acta Med. Scand. 128 (Suppl. 198) 1–154.
[3] Westman, S. (1968) Diabetologia 4, 141–149.
[4] Swenne, I., Bone, A.J., Howell, S.L. and Hellerström, C. (1980) Diabetes 29, 686–692.
[5] King, D.L. and Chick, W.L. (1976) Endocrinology 99, 1003–1007.
[6] King, D.L., Kitchen, D.C. and Chick, W.L. (1978) Endocrinology 103, 1321–1329.
[7] Swenne, I. (1982) Diabetes 31, 754–760.
[8] Dunger, A.-M., Sjöholm, Å. and Eizirik, D.L. (1990) Pancreas 5, 639–646.
[9] Milner, R.D.G. and Wirdnam, P.K. (1982) Pediatr. Res. 16, 213–216.
[10] Swenne, I., Hill, D.J., Strain, A.J. and Milner, R.D.G. (1987) Diabetes 36, 288–294.
[11] Nielsen, J.H. (1989) Exp. Clin. Endocrinol. 93, 277–285.
[12] Brelje, T.C., Allaire, P., Hegre, O. and Sorenson, R.L. (1989) Endocrinology 125, 2392–2399.
[13] Swenne, I. and Hill, D.J. (1989) Diabetologia 32, 191–197.
[14] Milner, R.D.G. and Hill, D.J. (1984) Clin. Endocrinol. 21, 415–433.
[15] VanSchravendijk, C.F.H., Foriers, A., Van den Brande, J.L. and Pipeleers, D.G. (1987) Endocrinology 121, 1784–1788.
[16] Romanus, J.A., Rabinovitch, A. and Rechler, M.M. (1985) Diabetes 34, 696–702.
[17] Hansson, H.A., Edwall, D., Löwenadler, B., Nordstedt, G., Palaeus, P. and Skottner, A. (1988) Acta Phys. Scand. 132, 569–576.
[18] Nielsen, J.H., Møller-Petersen, L. and Pedersen, E.E. (1988) Diabetologia 31, 526A (abstract).
[19] Petersen, E.D., Billestrup, N. and Nielsen, J.H. (1992) J. Cell. Biochem. Suppl. 16B, 242.
[20] Sandler, S., Bendtzen, K., Eizirik, D.L., Svensson, C., Strandell, E., Welsh, M. and Welsh, N. (1991) Autoimmunity 10, 241–253.
[21] Southern, C., Schulster, D. and Green, I.C. (1990) Biochem. J. 272, 243–245.
[22] Eizirik, D.L., Welsh, M., Strandell, E., Welsh, N. and Sandler, S. (1991) Endocrinology 127, 2290–2297.
[23] Sjöholm, Å. (1992) Am. J. Physiol., in press.
[24] Hughes, J.H., Watson, M.A., Easom, R.A., Turk, J. and McDaniel, M.L. (1990) FEBS Lett. 266, 33–36.
[25] Martin, M., Lovett, D.H., Szamel, M. and Resch, K. (1989) Eur. J. Biochem. 180, 343–350.
[26] Sjöholm, Å., Welsh, N., Sandler, S. and Hellerström, C. (1990) Am. J. Physiol. 259, C828–C833.
[27] Sjöholm, Å., Welsh, N. and Hellerström, C. (1992) Am. J. Physiol. 262, C391–C395.
[28] Pegg, A.E. (1986) Biochem. J. 234, 249–262.
[28a] Hougaard, D.M., Nielsen, J.H. and Larsson, L.-I. (1986) Biochem. J. 238, 43–47.
[29] Sjöholm, Å. and Hellerström, C. (1991) Am. J. Physiol. 260, C1046–C1051.
[30] Sjöholm, Å., Funakoshi, A., Efendić, S., Östenson, C.-G. and Hellerström, C. (1991) Endocrinology 128, 3277–3282.
[31] Nielsen, J.H. (1985) Biomed. Biochim. Acta 44, 161–166.
[32] Rabinovitch, A., Blondel, B., Murray, T. and Mintz, D.H. (1980) J. Clin. Invest. 66, 1065–1071.
[33] Logothetopoulos, J., Valiquette, N. and Cvet, D. (1983) Diabetes 32, 1172–1176.
[34] Swenne, I. (1982) Biosci. Rep. 2, 867–872.
[35] Mourmeaux, J.L., Remacle, C. and Henquin, J.C. (1989) Endocrinology 125, 2636–2644.

[36] Sjöholm, Å. (1992) J. Cell. Biochem. Suppl. 16B, 231.
[37] Hagopian, H.K., Riggs, M.G., Swartz, L.A. and Ingram, V.M. (1977) Cell 12, 855–861.
[38] Sjöholm, Å. (1991) Biochem. Biophys. Res. Commun. 180, 152–155.
[39] Sjöholm, Å. (1991) FEBS Lett. 289, 249–252.
[40] Sjöholm, Å. (1991) Immunol. Lett. 30, 81–86.
[41] Verspohl, E.J. and Ammon, H.P.T. (1989) Naunyn-Schmiedeberg's Arch. Pharmacol. 339, 348–353.
[42] Laychock, S.G. (1983) Mol. Cell. Endocrinol. 32, 157–170.
[43] Arkhammar, P., Nilsson, T., Welsh, M., Welsh, N. and Berggren, P.-O. (1989) Biochem. J. 264, 207–215.
[44] Rozengurt, E. (1986) Science 234, 161–166.
[45] Kikkawa, U., Kishimoto, A. and Nishizuka, Y. (1989) Annu. Rev. Biochem. 58, 31–44.
[46] Sjöholm, Å. (1991) FEBS Lett. 294, 257–260.