Characterization of inositol 1,4,5-trisphosphate receptor isoform mRNA expression and regulation in rat pancreatic islets, RINm5F cells and βHC9 cells

B Lee, P G Bradford and S G Laychock

Department of Pharmacology and Toxicology, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, New York 14214–3000, USA

(Requests for offprints should be addressed to S G Laychock, 102 Farber Hall, School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, New York 14214–3000, USA)

ABSTRACT

The inositol 1,4,5-trisphosphate receptor (InsP$_3$R) is an intracellular Ca$^{2+}$ channel that plays a role in the regulation of insulin secretion. In rat isolated pancreatic islets the expression of types I, II and III InsP$_3$R mRNA was identified by reverse transcriptase-polymerase chain reaction and confirmed by cDNA cloning and sequencing. The islet ratios of types I, II and III InsP$_3$R mRNA to β-actin mRNA were 0·08 ± 0·02, 0·08 ± 0·03 and 0·25 ± 0·04 respectively. Types I, II and III InsP$_3$R mRNA were also expressed in rat (RINm5F) and mouse (βHC9) pancreatic β-cell lines, and rat cerebellum. Type III InsP$_3$R mRNA was quantitatively the most abundant form in rat islets and RINm5F cells. In βHC9 cells, types II and III InsP$_3$R mRNA were expressed at similar levels, and in much greater abundance than type I mRNA. Type III was the least abundant InsP$_3$R mRNA in cerebellum. Culture of βHC9 cells for 5 days at 2·8 and 25 mM glucose, or RINm5F cells for 7 days at 5·5 and 20 mM glucose, resulted in significantly enhanced expression of type III, but not types I and II, InsP$_3$R mRNA in the cells at the higher glucose concentrations. During short-term (0·5–2 h) incubations, βHC9 cell type III InsP$_3$R mRNA levels increased in response to glucose in a time- and concentration-dependent manner. Actinomycin D inhibited the glucose response. α-Ketoisocaproic acid also stimulated βHC9 cell type III InsP$_3$R mRNA expression in a concentration-dependent manner, whereas 2-deoxyglucose and 3-O-methylglucose were without effect. The different levels of expression of mRNA for three InsP$_3$R isoforms in islets and insulinoma cells, and the influence of glucose and α-ketoisocaproic acid on the expression of type III mRNA, suggests that nutrient metabolism plays a role in the regulation of this gene and that the function of InsP$_3$R subtypes may be unique with each playing a distinct role in β-cell signal transduction and insulin secretion.

Journal of Molecular Endocrinology (1998) 21, 31–39

INTRODUCTION

Inositol 1,4,5-trisphosphate (InsP$_3$) mediates Ca$^{2+}$ mobilization from intracellular Ca$^{2+}$ stores and plays an important role in insulin secretion from pancreatic β-cells (Laychock 1990). InsP$_3$ exerts its action through specific receptors that are ligand-activated, Ca$^{2+}$-selective channels (Berridge 1993, Pozzan et al. 1994). InsP$_3$ receptors (InsP$_3$R) have been localized to endoplasmic reticulum, nucleus, insulin secretory granules, chromaffin granules and plasma membranes (Ross et al. 1989, Malviya et al. 1990, Yoo & Albanesi 1990, Khan et al. 1992, Blondel et al. 1994). Molecular cloning and expression studies have revealed that there is a family of InsP$_3$R with different primary structures and tissue distributions (Ferris & Snyder 1992). Five InsP$_3$R isoforms (types I–V) have been characterized at the molecular level. Full length cDNA sequences have been reported for rat type I (Mignery et al. 1990), type II (Südhof et al. 1991), and type III (Blondel et al. 1993), and partial sequences for type IV (Ross et al. 1992) and type V (De Smedt et al. 1994), InsP$_3$Rs. Full length cDNA clones have been reported for mouse type I InsP$_3$R (Furuichi et al. 1989), and partial sequences for types II, III and putative type IV InsP$_3$R have been identified (Ross et al. 1992).
Different patterns of InsP₃R expression in various tissues suggest that different subtypes may confer distinct functions and that the mechanisms may exist for regulating differential InsP₃R expression (Blondel et al. 1993, De Smedt et al. 1994). Although types I, II and III InsP₃R mRNA are co-expressed in various tissues (Ross et al. 1992, Blondel et al. 1993), their presence and relative abundance have not been fully characterized in rat pancreatic islets. The expression of type I InsP₃R has been reported to be predominant in mouse pancreatic islets (De Smedt et al. 1994), whereas type III InsP₃R is predominantly expressed in rat pancreatic islets (Blondel et al. 1993). In the present study, the expression of types I, II and III InsP₃R mRNA in freshly isolated rat pancreatic islets and rat (RINm5F) and mouse (HC9) clonal β-cell lines was determined by semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Moreover, in βHC9 cells the regulation of expression of type III InsP₃R mRNA is demonstrated to be an early response to glucose stimulation.

MATERIALS AND METHODS

Isolation of rat pancreatic islets; culture of islets, RINm5F and βHC9 cells

Pancreatic islets from adult male Sprague–Dawley rats were isolated using collagenase (type P) (Boehringer Mannheim, Indianapolis, IN, USA), as described previously (Xia & Laychock 1993). All animal procedures were approved by the Institutional Animal Care and Use Committee. Cells of the rat insulinoma cell line, RINm5F, were maintained at 35°C in RPMI-1066 medium (Sigma Chemical Co., St Louis, MO, USA) containing a customary maintenance glucose concentration (11 mM), or 5·5 or 20 mM glucose as indicated in the text, and 10% fetal bovine serum (FBS), as described previously (Laychock & Bauer 1996). Murine βHC9 cells were cultured at 35°C in Dulbecco’s modified Eagle’s medium (DMEM) (pyruvate free) (GIBCO, Grand Island, NY, USA) containing the customary maintenance concentration of glucose (25 mM) or 2·8 mM glucose as indicated, essentially as described for RINm5F cells. Other additions to the cultures were made as indicated in the text.

RNA isolations and cDNA synthesis

Total RNA was extracted from rat pancreatic islets, RINm5F cells, βHC9 cells, and rat brain using a monophasic solution of phenol and guanidine isothiocyanate (Chomczynski & Sacchi 1987). cDNA was reverse transcribed from 2 µg total RNA using random hexamer (GIBCO) in 20 µl solution containing 50 mM Tris–HCl (pH 8·3), 75 mM KCl, 3 mM MgCl₂, 10 mM dithiothreitol, 0·5 mM dNTP and 200 U Superscript II RNase H⁻ reverse transcriptase (GIBCO). Reactions were incubated for 1 h at 42°C, and then heated to 70°C for 15 min.

PCR amplification and quantitation of InsP₃R and β-actin transcript levels

Preliminary studies were conducted to determine the optimal amount of cDNA and number of PCR cycles required to maintain reactions in the exponential phase of the amplification. Polymerization reactions were carried out in a Perkin Elmer 2400 Thermocycler using 10·0 µl 1:5 dilution cDNAs as templates in a 25 µl reaction volume containing: 0·2 mM dNTPs, 10 pmol appropriate oligonucleotide primers (see text); PCR buffer (GIBCO); and 1 unit Taq DNA polymerase (GIBCO). In certain experiments, Pfu DNA polymerase (Stratagene, La Jolla, CA, USA) was substituted for Taq. The amplification conditions were 35 cycles with denaturation for 1 min at 94°C, annealing for 2 min at 55°C, and extension for 3 min at 72°C with the final extension for 7 min. The reaction products were separated by electrophoresis in a 2·0% agarose gel in Tris–borate–EDTA buffer. The gel was stained with ethidium bromide and viewed by Gel Doc 1000 (Bio-Rad, Hercules, CA, USA). The quantity of each PCR fragment was determined using Molecular Analyst software (Bio-Rad). The image density of each InsP₃R isoform PCR product was compared with the density of co-amplified β-actin to determine the ratio of InsP₃R expression.

cDNA cloning and sequencing

The PCR products were either sequenced directly by Model 373 DNA sequencing system (Applied Biosystems, Foster City, CA, USA) or after subcloning into pGEM-11Zf(+) by dideoxy-chain termination DNA sequencing using Sequenase Version 2·0 DNA sequencing kit (Amersham, Arlington Heights, IL, USA).

Statistical analysis

Significant differences between samples were determined by Student’s t-test (paired, two-tailed), or one-way analysis of variance (ANOVA) with Student/Newman–Keuls multiple comparison test. P values ≤0·05 were accepted as significant.
RESULTS

Expression of InsP$_3$R isoform mRNA in islets, insulinoma cells and cerebellum

RT-PCR was used to determine the expression of InsP$_3$R isoform mRNA in rat pancreatic islets and rat brain. Amplification primers for InsP$_3$R isoform analysis were selected to be distinct for each isoform but to correspond with sequences conserved between mouse and rat. The sequences of primer pairs for each InsP$_3$R isoform and for $\beta$-actin used in this study are shown in Table 1. Based upon the reported cDNA sequences of the rodent types I, II and III InsP$_3$Rs (Mignery et al. 1990, Südhof et al. 1991, Blondel et al. 1993), PCR products of 423, 390 and 560 base pairs respectively, are expected from the amplifications. Results of amplifications using islet cDNA revealed the expression of types I, II and III InsP$_3$R mRNA (Fig. 1). Types I, II and III InsP$_3$R mRNAs were also identified in rat cerebellum extracts (Fig. 1), as reported previously (Ross et al. 1992, Kirkwood et al. 1996). The identity of each PCR product in islet samples was confirmed by cDNA cloning and sequencing. The sequences of these fragments were identical to the reported sequences of rat types I, II and III InsP$_3$R cDNA clones (data not shown).

Expression of InsP$_3$R isoform mRNA in the clonal pancreatic $\beta$-cell lines for rat (RINm5F) and mouse ($\beta$HC9) was also determined. Both cell lines expressed types I, II and III InsP$_3$R mRNA (Figs 1 and 2), confirming subtype expression in a homogeneous population of $\beta$-cells, compared with islets which are composed of endocrine and non-endocrine cells. In RINm5F cells cultured at the customary maintenance concentration of 11 mM glucose, type III InsP$_3$R mRNA was the most

| Table 1. Sense (S) and antisense (A) primer pairs used for specific amplification of rat types I, II and III InsP$_3$Rs, and $\beta$-actin cDNAs |
|-----------------|-----------------|-----------------|-----------------|
| cDNA            | Primers         | PCR product     |
| InsP$_3$R       |                 | (bp)            |
| Type I          | S 5'-GAGAGAAAGCGCACGCCGAGAGGAG-3' | (92–116) |
|                 | A 5'-GGACATAGCTTTAAGAGGCGCATCTTC-3' | (490–514) |
| Type II         | S 5'-CGGGAATTCGGACCTCCTACCTCAGGAGG-3' | (1251–1270) |
|                 | A 5'-CACAGGTCTTCTCCACCCCTGGTGAGG-3' | (1604–1622) |
| Type III        | S 5'-GCCCGGATCTCAGAGATCGCCGGA-3' | (377–391) |
|                 | A 5'-GCCGGAAGCTTTCTTCGGCCCGTAAGCT-3' | (900–914) |
| $\beta$-actin   | S 5'-CTACAGATCATCTTTGAGACC-3' | (2152–2172) |
|                 | A 5'-GAAGGAAGGGTGGAAGAGGC-3' | (2572–2592) |

| Table 2. Relative abundance of InsP$_3$R isoform mRNA in rat pancreatic islets, cerebellum RINm5F cells and murine $\beta$HC9 cells. Values are means ± s.e. from three to five independent determinations |
|-----------------|-----------------|-----------------|-----------------|
| mRNA (InsP$_3$R/$\beta$-actin) |
| InsP$_3$R isoforms | Islets | RINm5F cells | $\beta$HC9 cells | Cerebellum |
| Type I          | 0·08 ± 0·02    | 0·17 ± 0·03    | 0·12 ± 0·04    | 0·32 ± 0·04 |
|                 | (18 ± 3%)      | (17 ± 2%)      | (11 ± 3%)      | (49 ± 2%)   |
| Type II         | 0·08 ± 0·03    | 0·11 ± 0·01    | 0·48 ± 0·07    | 0·27 ± 0·05 |
|                 | (18 ± 5%)      | (12 ± 1%)      | (46 ± 3%)      | (42 ± 3%)   |
| Type III        | 0·25 ± 0·04    | 0·71 ± 0·05    | 0·45 ± 0·02    | 0·06 ± 0·004|
|                 | (64 ± 5%)      | (72 ± 2%)      | (42 ± 3%)      | (9 ± 1%)    |

The relative abundance of each isoform as a percentage of the total is shown in parentheses. Islets were freshly isolated; RINm5F cells were cultured at 11 mM glucose; $\beta$HC9 cells were cultured at 25 mM glucose.
abundant, with the type II mRNA being the least abundant, among the subtypes (Table 2; Fig. 1). The ratios of types I, II and III InsP3R mRNA to β-actin mRNA in RINm5F cells were 2·1-, 1·4- and 2·8-fold higher respectively, than the ratio in freshly isolated islets, although the relative expression levels as percentage of total were similar between islets and RINm5F cells (Table 2).

Comparison of the expression of InsP3R isoform mRNA in βHC9 cells indicated that the type I isoform was least abundant and that types II and III mRNA were similarly expressed at the customary maintenance concentration of 25 mM glucose (Table 2). The expression level of type I InsP3R mRNA compares favorably between the rat and mouse cell lines, but type II is four times more abundant, and type III is about 30% less abundant, in the βHC9 cells versus RINm5F cells (Table 2).

In contrast to insulinoma cells or islets, cerebellar type III isoform was the least abundant of the subtypes and represented only 9% of the total InsP3R mRNA expressed in brain (Table 2). Cerebellar types I and II InsP3R mRNA were expressed at similar levels under the PCR conditions used in these studies which optimized the detection of each of the three subtypes (Table 2). Similar results were obtained when Pfu DNA polymerase was substituted for Taq polymerase at 4 mM Mg2+ (data not shown). However, cerebellar type I InsP3R mRNA was detected as being much more abundant than types II or III when Pfu was used with 2 mM Mg2+ in the PCR (data not shown), in agreement with a previously published report (De Smedt et al. 1994).

**Long-term effects of glucose on expression of InsP3R isoform mRNA in insulinoma cells**

The long-term culture (7 days) of RINm5F cells in the presence of 5·5 mM glucose or 20 mM glucose was performed to determine the effect of chronic glucose stimulation on InsP3R mRNA expression. Although types I and II InsP3R mRNA were not different in RINm5F cells cultured at 5·5 mM or 20 mM glucose, type III InsP3R mRNA was 37 ± 4% more abundant (P<0·02) in the cells cultured at 20 mM glucose (Fig. 2; Table 3). The
increase in type III InsP₃R mRNA with 20 mM glucose stimulation peaked within 7 days after the onset of stimulation, and was expressed at a similar ratio (1·17) after 4 weeks. The ratio of type III InsP₃R mRNA following culture for 7 days at 5·5 mM glucose was similar to the ratio after 4 weeks of culture (0·78), and the ratio was similar to that observed in RINm5F cells at the customary maintenance glucose concentration of 11 mM (Table 2).

InsP₃R mRNA levels were also investigated in βHC9 cells cultured for 5 days at 2·8 mM and 25 mM glucose. Type III InsP₃R mRNA expression in βHC9 cells cultured for 5 days at 25 mM glucose increased 66 ± 11% (P<0·02) above values from cells maintained at 2·8 mM glucose (Fig. 2; Table 3). In the same cells, types I and II InsP₃R mRNA expression at 25 mM glucose was 99 ± 15% (P>0·05) and 101 ± 4% (P>0·05) of the expression observed in cells cultured at 2·8 mM glucose (Fig. 2; Table 3). Following only 1 day of culture at 2·8 mM glucose, the expression of types II and III mRNA in βHC9 cells was similar to expression levels in 5-day cultured cells at 2·8 mM glucose (data not shown). It was also observed that following culture of βHC9 cells at low glucose levels the most abundant isoform was type II InsP₃R mRNA (Table 3).

**Short-term regulation of expression of InsP₃R isoform mRNA in βHC9 cells**

The short-term effects of glucose stimulation on type III InsP₃R mRNA expression was also determined in βHC9 cells which had been pre-viously cultured at 2·8 mM glucose for 4 days to attain a low basal, or down-regulated, ratio of expression. When down-regulated βHC9 cells were cultured for 2 h with increasing concentrations of glucose, a concentration-dependent increase in type III InsP₃R mRNA was observed (Fig. 3A). In these experiments, mannitol was included to normalize the osmolarity of the culture medium between treatment groups. In addition, when down-regulated βHC9 cells were cultured with a high concentration of glucose (27·8 mM) there was also a time-dependent increase in the expression of type III InsP₃R mRNA up to 2 h, and thereafter the values did not increase further up to 24 h (Fig. 3B), or even 5 days (data not shown). In down-regulated βHC9 cells, the non-metabolizable glucose analogs 2-deoxyglucose and 3-O-methylglucose failed to affect type III InsP₃R mRNA expression (Fig. 4A). In contrast, α-ketoisocaproic acid induced a concentration-dependent increase in the expression of type III InsP₃R mRNA during a 2-h culture of βHC9 cells (Fig. 4A). At a concentration of 25 mM, glucose and α-ketoisocaproic acid evoked similar changes in type III mRNA expression.

The effects of glucose stimulation on the expression of type III InsP₃R mRNA in down-regulated βHC9 cells was also dependent upon transcription activation as shown by the complete inhibition of the response in the presence of actinomycin D (Fig. 4B). Cycloheximide was without effect on glucose stimulation of type III InsP₃R mRNA expression, indicating that protein synthesis was not involved in the short-term regulatory response (Fig. 4B). Neither actinomycin D nor cycloheximide had an effect on basal type III mRNA expression (Fig. 4B).

**DISCUSSION**

The results of this study indicate that types I, II and III InsP₃R mRNA are expressed in rat

---

**Table 3.** Effects of glucose stimulation on expression levels of InsP₃R isoform mRNA in insulinoma cells. RINm5F cells were cultured for 7 days in RPMI medium containing 5·5 or 20 mM glucose, and βHC9 cells were cultured for 5 days in DMEM medium containing 2·8 or 25 mM glucose, as indicated. Each value is the mean ± s.e. for three independent determinations.

<table>
<thead>
<tr>
<th>mRNA (InsP₃R/β-actin)</th>
<th>Type I</th>
<th>Type II</th>
<th>Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RINm5F cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5·5 mM</td>
<td>0·16 ± 0·002</td>
<td>0·13 ± 0·01</td>
<td>0·71 ± 0·05</td>
</tr>
<tr>
<td>20 mM</td>
<td>0·17 ± 0·01</td>
<td>0·11 ± 0·01</td>
<td>0·97 ± 0·05*</td>
</tr>
<tr>
<td><strong>βHC9 cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2·8 mM</td>
<td>0·16 ± 0·02</td>
<td>0·52 ± 0·06</td>
<td>0·28 ± 0·01</td>
</tr>
<tr>
<td>25 mM</td>
<td>0·15 ± 0·02</td>
<td>0·53 ± 0·06</td>
<td>0·46 ± 0·02*</td>
</tr>
</tbody>
</table>

*P<0·05 vs 2·8 or 5·5 mM glucose for βHC9 cells or RINm5F cells respectively, as determined by Student’s paired t-test.
pancreatic islets and in rat and mouse pancreatic β-cell lines. Previously, only the expression of type III InsP₃R mRNA was demonstrated in rat pancreatic islets (Blondel et al. 1993). Quantitatively, type III InsP₃R mRNA is the predominant isoform expressed in islets and β-cell lines, confirming similar findings reported previously (Blondel et al. 1993, De Smedt et al. 1994). Among the pancreatic islet cell types, type III InsP₃R is not present in α- and pancreatic polypeptide secreting (PP)-cells but is expressed in β- and δ-cells (Blondel et al. 1994). All three isoform mRNAs were also identified in the present study in rat cerebellum, although the type I isoform predominated in this tissue, in confirmation of previous reports (Blondel et al. 1993, De Smedt et al. 1994, Kirkwood et al. 1996). The relative abundance of the isoforms was highly dependent upon the conditions of the PCR, especially the Mg²⁺ concentration and number of cycles. Different conditions of cerebellar InsP₃R cDNA amplification may explain the reports of either the higher abundance of types I and III than type II mRNA (Blondel et al. 1993, Kirkwood et al. 1996), or the very high expression of type I relative to types II and III (De Smedt et al. 1994). A search of Genbank indicated that the sequences amplified in this study had identity only with the InsP₃Rs. The present studies demonstrate that at least three InsP₃R isoform mRNAs are also expressed in rat and mouse insulinoma cells. The type III receptor has been presumed to be primarily responsible for mediating the stimulatory effect of InsP₃ on insulin secretion, however, the evidence for expression of types I and II InsP₃R mRNA in rat islets and insulinoma cells suggests these receptor subtypes may also mediate InsP₃ effects in islets. Expression of type III InsP₃R protein in rat islets and types I and III InsP₃R in RINm5F cells has been reported (Blondel et al. 1993, Wojcikiewicz 1995, Wojcikiewicz & He 1995). Selective expression or co-expression of InsP₃R isoforms may affect the Ca²⁺-mobilizing action of InsP₃ if these isoforms have differences in structure, function, regulation or cellular distribution.

This study shows for the first time that the expression of specific subtypes of InsP₃R mRNA are sensitive to glucose stimulation. Glucose is the primary insulin secretagogue in pancreatic islet β-cells, and glucose metabolism and ATP generation mediate changes in cell Ca²⁺ regulation, including InsP₃ generation and Ca²⁺ mobilization, and insulin secretion (Laychock 1990). Long-term glucose stimulation in RINm5F cells and βHC9 cells resulted in a significant increase specifically in type III InsP₃R mRNA expression when compared with cells cultured at low concentrations of glucose.

**Figure 3.** Glucose effects on type III InsP₃R/β-actin mRNA ratio in βHC9 cells. βHC9 cells were cultured at 2.8 mM glucose for 4 days (control; 100% values) prior to the introduction of different glucose concentrations or mannitol to achieve equivalent osmolar concentrations between the control group and glucose-stimulated samples. (A) Concentration-dependent response of cells to glucose during 2-h culture. (B) Time-dependent response of cells to stimulation by 27.8 mM glucose. Values are means ± s.e. for (A) three or (B) between three and four independent determinations. (A) *P<0.001 vs control, and (B) *P<0.05 vs control at zero time, as determined by one-way ANOVA and multiple comparison test.

These data closely resemble the increase in type III InsP₃R protein levels in RINm5F cells cultured under similar conditions (Blondel et al. 1994). Different isoforms appear to be regulated independently, since long-term glucose stimulation did not affect types I or II InsP₃R mRNA in the βHC9 cells. The maintenance of murine βHC9 cells at the customary 25 mM glucose probably contributes to the discrepancy between the relative levels of types I and III InsP₃R mRNA in these cells versus normal mouse islets where the type I isoform reportedly predominates (De Smedt et al. 1994). However, in βHC9 cells cultured at a low glucose concentration, type II InsP₃R was most abundant in the present study, with types III and I following in relative abundance. In contrast, when RINm5F cells were cultured for 7 days at 5·5 mM glucose, the relative abundance of the InsP₃R isoform mRNAs was similar to that observed in isolated rat islets. Since βHC9 cells are a tumor cell line they may not be identical to normal mouse islets in terms of InsP₃R gene expression; alternatively, the conditions of PCR may contribute to differences in perceived expression levels between laboratories. It remains to be determined whether the relative changes in the InsP₃R mRNAs observed in insulinoma cells exposed to hyperglycemic glucose levels will also be observed in islet β-cells from hyperglycemic/diabetic animals. However, it appears that InsP₃R protein levels are modulated in response to glucose availability and diabetic state (Blondel et al. 1994), suggesting that gene transcription and/or mRNA translation in vivo is regulated by glucose availability. Although there are differences in the relative abundance of the type III mRNA expression between mouse and rat cell lines, it appears that the type III mRNA is regulated in response to glucose in both cell lines.

During short-term culture, glucose was shown to induce a concentration-dependent increase in type III InsP₃R mRNA in βHC9 cells. As low as 9·8 mM glucose induced a significant increase in type III mRNA expression. Such a concentration of glucose might be found during hyperglycemia in humans with non-insulin-dependent diabetes mellitus, suggesting that mildly hyperglycemic concentrations of glucose may alter InsP₃R expression in islet β-cells, Ca²⁺ mobilization and insulin secretory responses. In addition, glucose induced a time-dependent increase in the expression of type III InsP₃R mRNA during a 2-h incubation, suggesting that promotion of InsP₃R gene expression is rapidly induced and attains maximum expression in response to glucose within 2 h. The non-metabolizable glucose analogs 2-deoxyglucose and 3-O-methylglucose did not mimic the glucose response, suggesting that glucose metabolism mediates changes in expression of InsP₃R mRNA. Another nutrient secretagogue, α-ketoisocaproic acid, also induced a concentration-dependent increase in type III InsP₃R mRNA expression.
Maximal secretagogue concentrations of α-ketoisocaproic acid and glucose induced similar expression levels of type III InsP$_3$R mRNA during the 2-h HC9 cell culture, suggesting that an increase in mitochondrial oxidative phosphorylation plays a role in modulating expression of InsP$_3$R mRNA. As evidence that the changes in type III mRNA in response to glucose depended upon DNA transcription, actinomycin D was demonstrated to completely block the glucose-induced changes. Cycloheximide, on the other hand, had no effect on glucose-induced changes in type III mRNA during the short-term incubations, suggesting that protein synthesis did not mediate the glucose response. Glucose has been reported to increase the expression of early response genes, including junB, nur77 and zif268, in the insulin secreting (INS)-1 β-cell line within 60 min (Frödin et al. 1995). The latter response appears to be mediated by the mitogen activated protein (MAP) kinase pathway. Continuing studies will determine if MAP kinase plays a role in glucose effects on InsP$_3$R expression. In addition, glucose induces the acetyl-CoA carboxylase gene (Brun et al. 1993) and the L-type pyruvate kinase gene in INS-1 cells within 2–4 h (Marano et al. 1993). However, it appears that glucose-6-phosphate mediates those glucose effects since 2-deoxyglucose mimicked glucose. The latter responses are unlike that of the InsP$_3$R type III gene where 2-deoxyglucose failed to mimic the glucose response, and stimulation of mitochondrial metabolism by α-ketoisocaproic acid mimicked the glucose response, suggesting that the energy state of the cell is important in InsP$_3$R gene regulation.

Mammalian types I, II and III InsP$_3$R cDNA have between 60 and 70% identity to each other (Furuichi et al. 1989, Mignery et al. 1990, Südhof et al. 1991, Blondel et al. 1993, Marano 1994, Yamamoto-Hino et al. 1994). Putative types IV and V InsP$_3$R cDNA have been partially sequenced and have a high degree of identity to type II InsP$_3$R cDNA (Ross et al. 1992, De Smedt et al. 1994). It is possible that type II InsP$_3$R in the present study may include type IV and/or type V. However, an insignificant level of type V InsP$_3$R is present in rat cells and RINm5F cells (De Smedt et al. 1994). The functional and regulatory characteristics of these receptors are not known. Type I InsP$_3$R is phosphorylated in response to hormones that activate cyclic AMP, whereas types II and III InsP$_3$R lack the relevant consensus sequences (Joseph & Ryan 1993, Yamamoto-Hino et al. 1994). Significant differences between types I and II InsP$_3$R are found in the region of the Ca$^{2+}$ channel suggesting that the gating properties of the receptor/Ca$^{2+}$ channels may be different (Südhof et al. 1991). Types I, II and III InsP$_3$R have different binding affinities for InsP$_3$; the relative order of affinities are type II>1>III (Newton et al. 1994). In addition, InsP$_3$Rs may have distinct functions related to subcellular localization in endoplasmic reticulum, nucleus, insulin secretory granules, chromaffin granules, and plasma membranes (Ross et al. 1989, Malviya et al. 1990, Yoo & Albanesi 1990, Khan et al. 1992, Blondel et al. 1994). Thus, co-expressed InsP$_3$Rs are regulated independently and may have different InsP$_3$ binding characteristics and subcellular distribution, which could account for the selective regulation of receptor activity in tissues. In addition, glucose effects on InsP$_3$R subtype expression may contribute to islet β-cell insulin secretory responses.

In summary, islets and insulinoma cells from the rat and mouse express three mRNAs associated with the isoforms of InsP$_3$R. At least one isoform, type III, appears to be transcriptionally regulated by metabolic responses of the β-cell. Changes in transcription of this gene are rapid and suggest that InsP$_3$R expression may mediate changes in β-cell responsivity to certain secretagogues, and has the potential to be affected by hyperglycemic concentrations of glucose in vivo.

ACKNOWLEDGEMENTS

This work was supported by a National Institutes of Health Grant DK–25705 awarded to S G L.

REFERENCES


De Smedt H, Missaia L, Parys JP, Bootman MD, Mertens L, Van Den Bosch L & Castells R 1994 Determination of relative amounts of inositol trisphosphate receptor mRNA


Maranto AR 1994 Primary structure, ligand binding and localization of the human type 3 inositol 1,4,5-trisphosphate receptor expressed in intestinal epithelium. *Journal of Biological Chemistry* 269 1222–1230.


Newton CL, Mignery GA & Südhof TC 1994 Co-expression in vertebrate tissues and cell lines of multiple inositol 1,4,5-trisphosphate (InsP3) receptors with distinct affinities for InsP3. *Journal of Biological Chemistry* 269 28613–28619.


Wojcikiewicz RJH 1995 Type I, II and III inositol 1,4,5-trisphosphate receptors are unequally susceptible to down-regulation and are expressed in markedly different proportions in different cell types. *Journal of Biological Chemistry* 270 11678–11683.

Wojcikiewicz RJH & He Y 1995 Types I, II and III inositol 1,4,5-trisphosphate receptor co-immunoprecipitation as evidence for the existence of heterotrimeric receptor complexes. *Biochemical and Biophysical Research Communications* 213 334–341.


**FINAL VERSION RECEIVED 3 March 1998**