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

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ABSTRACT

The inositol 1,4,5-trisphosphate receptor ($InsP_3R$) 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 $InsP_3R$ 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 $InsP_3R$ 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 $InsP_3R$ mRNA were also expressed in rat (RINm5F) and mouse (βHC9) pancreatic β-cell lines, and rat cerebellum. Type III $InsP_3R$ mRNA was quantitatively the most abundant form in rat islets and RINm5F cells. In βHC9 cells, types II and III $InsP_3R$ mRNA were expressed at similar levels, and in much greater abundance than type I mRNA. Type III was the least abundant $InsP_3R$ 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, $InsP_3R$ mRNA in the cells at the higher glucose concentrations. During short-term (0·5–2 h) incubations, βHC9 cell type III $InsP_3R$ 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 $InsP_3R$ 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 $InsP_3R$ 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 $InsP_3R$ subtypes may be unique with each playing a distinct role in β-cell signal transduction and insulin secretion.

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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 ($InsP_3R$) 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 $InsP_3R$ with different primary structures and tissue distributions (Ferris & Snyder 1992). Five $InsP_3R$ 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), $InsP_3Rs$. Full length cDNA clones have been reported for mouse type I $InsP_3R$ (Furuichi *et al*. 1989), and partial sequences for types II, III and putative type IV $InsP_3R$ have been identified (Ross *et al*. 1992).

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Different patterns of InsP$_3$R expression in various tissues suggests that different subtypes may confer distinct functions and that the mechanisms may exist for regulating differential InsP$_3$R expression (Blondel *et al*. 1993, De Smedt *et al*. 1994). Although types I, II and III InsP$_3$R 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 InsP$_3$R has been reported to be predominant in mouse pancreatic islets (De Smedt *et al*. 1994), whereas type III InsP$_3$R is predominantly expressed in rat pancreatic islets (Blondel *et al*. 1993). In the present study, the expression of types I, II and III InsP$_3$R mRNA in freshly isolated rat pancreatic islets and rat (RINm5F) and mouse (βHC9) 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 InsP$_3$R 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 MgCl$_2$, 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 InsP$_3$R 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 InsP$_3$R isoform PCR product was compared with the density of co-amplified β-actin to determine the ratio of InsP$_3$R 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.

TABLE 1. Sense (S) and antisense (A) primer pairs used for specific amplification of rat types I, II and III $InsP_3Rs$, and β-actin cDNAs

cDNA		**Primers**		**PCR product** (bp)
$InsP_3R$				
Type I	S	5′-GAGAGAAAGCGCACGCCGAGAGGAG-3′	(92–116)	
	A	5′-GGACATAGCTTAAAGAGGCAGTCTC-3′	(490–514)	423
Type II	S	5′-CGGGAATTCGGAGCTTCCAACCTCAAAG-3′	(1251–1270)	
	A	5′-CACAAGCTTAGCTTCTTCACCGTGGTGG-3′	(1604–1622)	390
Type III	S	5′-GGCCGGAATTCAGAGAAGATCGCCGA-3′	(377–391)	
	A	5′-GGACGAAGCTTCTTGCCCCGGTACTC-3′	(900–914)	560
β-actin	S	5′-CTACAGATCATGTTTGAGACC-3′	(2152–2172)	
	A	5′-GAAGGAAGGCTGGAAGAGAGC-3′	(2572–2592)	441

TABLE 2. Relative abundance of $InsP_3R$ isoform mRNA in rat pancreatic islets, cerebellum RINm5F cells and murine βHC9 cells. Values are means ± S.E. from three to five independent determinations

	mRNA ($InsP_3R$/β-actin)			
$InsP_3R$ isoforms	Islets	RINm5F cells	βHC9 cells	Cerebellum
Type I	0·08 ± 0·02 (18 ± 3%)	0·17 ± 0·03 (17 ± 2%)	0·12 ± 0·04 (11 ± 3%)	0·32 ± 0·04 (49 ± 2%)
Type II	0·08 ± 0·03 (18 ± 5%)	0·11 ± 0·01 (12 ± 1%)	0·48 ± 0·07 (46 ± 3%)	0·27 ± 0·05 (42 ± 3%)
Type III	0·25 ± 0·04 (64 ± 5%)	0·71 ± 0·05 (72 ± 2%)	0·45 ± 0·02 (42 ± 3%)	0·06 ± 0·004 (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; βHC9 cells were cultured at 25 mM glucose.

RESULTS

Expression of $InsP_3R$ isoform mRNA in islets, insulinoma cells and cerebellum

RT-PCR was used to determine the expression of $InsP_3R$ isoform mRNA in rat pancreatic islets and rat brain. Amplification primers for $InsP_3R$ 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 $InsP_3R$ isoform and for β-actin used in this study are shown in Table 1. Based upon the reported cDNA sequences of the rodent types I, II and III $InsP_3Rs$ (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 $InsP_3R$ mRNA (Fig. 1). Types I, II and III $InsP_3R$ mRNA 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 $InsP_3R$ cDNA clones (data not shown).

The relative abundance of each $InsP_3R$ isoform mRNA was determined by comparing the amount of amplification product for each isoform with amplification of β-actin mRNA expressed in the same sample. Preliminary studies established the linearity of amplification rate under the conditions used for these experiments. In islets, the expression of types I and II $InsP_3R$ mRNA was similar, whereas the expression of type III $InsP_3R$ mRNA was approximately three times greater than types I and II (Table 2).

Expression of $InsP_3R$ isoform mRNA in the clonal pancreatic β-cell lines for rat (RINm5F) and mouse (βHC9) was also determined. Both cell lines expressed types I, II and III $InsP_3R$ mRNA (Figs 1 and 2), confirming subtype expression in a homogeneous population of β-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 $InsP_3R$ mRNA was the most

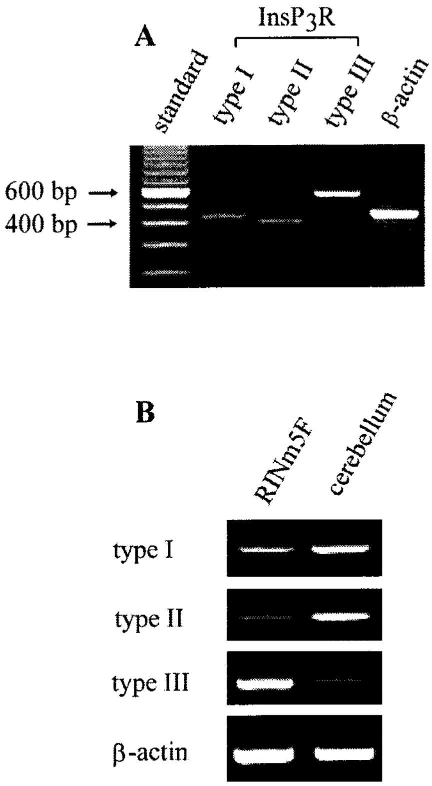

FIGURE 1. PCR amplification of InsP$_3$R mRNA isoforms in (A) rat pancreatic islets and (B) RINm5F cells maintained at 11 mM glucose and rat cerebellum. cDNA was amplified with primers specific for InsP$_3$R types I, II and III, and β-actin; the sizes of types I, II and III products are 423, 390 and 560 bp respectively, and β-actin is 441 bp. In (A), equal amounts of cDNA were used to amplify InsP$_3$R isoforms, and half of the amount of cDNA used for InsP$_3$R was used for amplifying β-actin. In (B), equal amounts of cDNA were used to amplify cDNA for types I, II and III InsP$_3$R and β-actin.

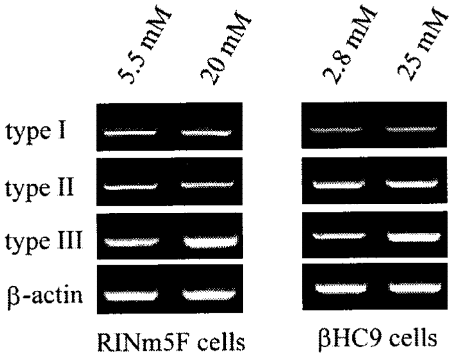

FIGURE 2. Regulation of InsP$_3$R mRNA isoform expression in RINm5F cells and βHC9 cells. RINm5F cells were cultured for 7 days, and βHC9 cells were cultured for 5 days, at the glucose concentrations indicated. cDNA was amplified by PCR for InsP$_3$R types I, II and III and β-actin. Equal amounts of βHC9 cell cDNA were used to amplify InsP$_3$R isoforms and β-actin. In RINm5F cells, the amount of cDNA for amplifying types I and II InsP$_3$R in RINm5F cells was four-times higher than the amount of cDNA used for amplifying type III InsP$_3$R and β-actin.

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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R mRNA expressed in brain (Table 2). Cerebellar types I and II InsP$_3$R 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 Mg^{2+} (data not shown). However, cerebellar type I InsP$_3$R mRNA was detected as being much more abundant than types II or III when Pfu was used with 2 mM Mg^{2+} 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 InsP$_3$R 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 InsP$_3$R mRNA expression. Although types I and II InsP$_3$R mRNA were not different in RINm5F cells cultured at 5·5 mM or 20 mM glucose, type III InsP$_3$R mRNA was 37 ± 4% more abundant ($P<0{\cdot}02$) in the cells cultured at 20 mM glucose (Fig. 2; Table 3). The

TABLE 3. Effects of glucose stimulation on expression levels of InsP$_3$R 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

		mRNA (InsP$_3$R/β-actin)		
		Type I	Type II	Type III
	Glucose			
RINm5F cells	5.5 mM	0·16 ± 0·002	0·13 ± 0·01	0·71 ± 0·05
	20 mM	0·17 ± 0·01	0·11 ± 0·01	0·97 ± 0·05*
βHC9 cells	2.8 mM	0·16 ± 0·02	0·52 ± 0·06	0·28 ± 0·01
	25 mM	0·15 ± 0·02	0·53 ± 0·06	0·46 ± 0·02*

*$P<0·05$ vs 2·8 or 5·5 mM glucose for βHC9 cells or RINm5F cells respectively, as determined by Student's paired *t*-test.

increase in type III InsP$_3$R 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 InsP$_3$R 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).

InsP$_3$R mRNA levels were also investigated in βHC9 cells cultured for 5 days at 2·8 mM and 25 mM glucose. Type III InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R mRNA (Table 3).

Short-term regulation of expression of InsP$_3$R isoform mRNA in βHC9 cells

The short-term effects of glucose stimulation on type III InsP$_3$R mRNA expression was also determined in βHC9 cells which had been previously 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R mRNA expression (Fig. 4A). In contrast, α-ketoisocaproic acid induced a concentration-dependent increase in the expression of type III InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R mRNA are expressed in rat

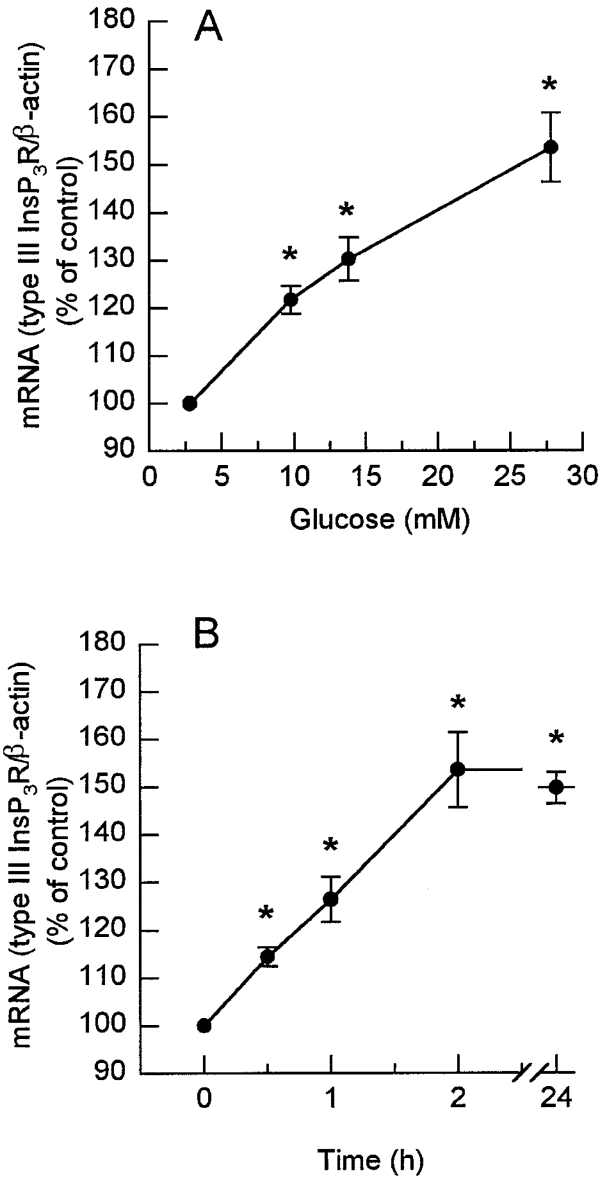

FIGURE 3. Glucose effects on type III InsP$_3$R/ β-actin mRNA ratio in βHC9 cells. βHC9 cells were cultured at 2·8 mM glucose for 4 days (control; 100% values) prior to the introduction of different glucose concentrations or mannitol to achieve equivalent osmolar concentrations between the control group 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) $^*P<0{\cdot}001$ vs control, and (B) $^*P<0{\cdot}05$ vs control at zero time, as determined by one-way ANOVA and multiple comparison test.

pancreatic islets and in rat and mouse pancreatic β-cell lines. Previously, only the expression of type III InsP$_3$R mRNA was demonstrated in rat pancreatic islets (Blondel *et al.* 1993). Quantitatively, type III InsP$_3$R 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 InsP$_3$R 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 Mg^{2+} concentration and number of cycles. Different conditions of cerebellar InsP$_3$R 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 InsP$_3$Rs. The present studies demonstrate that at least three InsP$_3$R 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 InsP$_3$ on insulin secretion, however, the evidence for expression of types I and II InsP$_3$R mRNA in rat islets and insulinoma cells suggests these receptor subtypes may also mediate InsP$_3$ effects in islets. Expression of type III InsP$_3$R protein in rat islets and types I and III InsP$_3$R in RINm5F cells has been reported (Blondel *et al.* 1993, Wojcikiewicz 1995, Wojcikiewicz & He 1995). Selective expression or co-expression of InsP$_3$R isoforms may affect the Ca^{2+}-mobilizing action of InsP$_3$ 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 InsP$_3$R 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 Ca^{2+} regulation, including InsP$_3$ generation and Ca^{2+} 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 InsP$_3$R mRNA expression when compared with cells cultured at low concentrations of glucose.

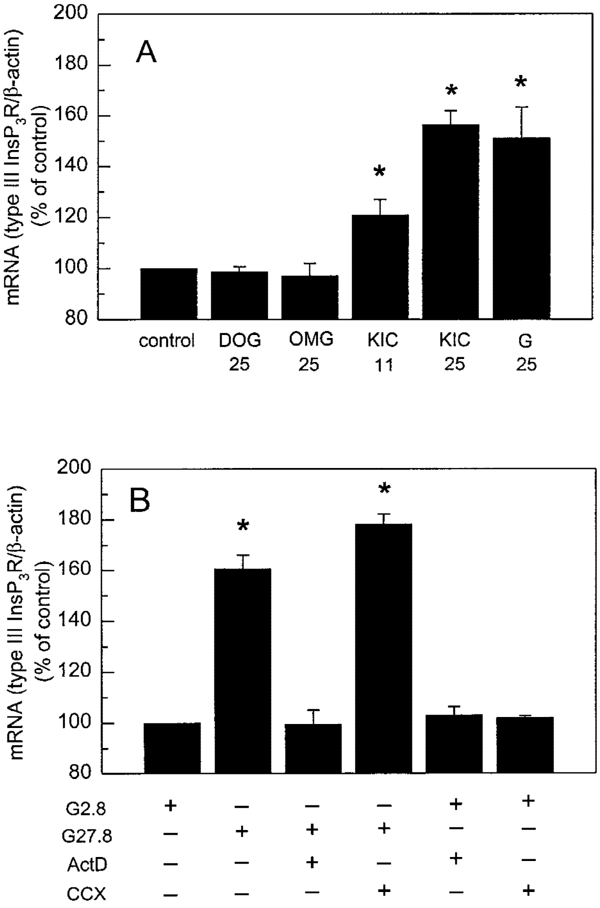

FIGURE 4. Effects of non-metabolizable glucose analogs, inhibitors of transcription and translation, and α-keto-isocaproic acid (KIC) on type III InsP$_3$R/β-actin mRNA ratio in βHC9 cells. βHC9 cells were cultured at 2·8 mM glucose for 4 days (control; 100% values) prior to culture for 2 h with: (A) control media (lacking glucose; ratio 0·25 ± 0·03), 2-deoxyglucose (DOG), 3-O-methylglucose (OMG), KIC, glucose (G) (mM concentration of each agent is indicated below each bar) or mannitol to achieve equivalent osmolarity between all treatment groups; (B) presence (+) or absence (−) of actinomycin D (ActD; 8 μM) or cycloheximide (CCX; 10 μM) during stimulation of cells with glucose at 2·8 mM (G2·8; control) or 27·8 mM (G27·8). Values are means ± S.E. for three different experimental determinations. $^*P<0{\cdot}01$ vs control as determined by one-way ANOVA and multiple comparison test.

These data closely resemble the increase in type III InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R 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 InsP$_3$R expression in islet β-cells, Ca^{2+} mobilization and insulin secretory responses. In addition, glucose induced a time-dependent increase in the expression of type III InsP$_3$R mRNA during a 2-h incubation, suggesting that promotion of InsP$_3$R 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 InsP$_3$R mRNA. Another nutrient secretagogue, α-ketoisocaproic acid, also induced a concentration-dependent increase in type III InsP$_3$R 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 βHC9 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 (Marie *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, Maranto 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>I>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*.

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