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Tissue- and cell-specific expression of Ins(1,4,5)P_3 3-kinase isoenzymes

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The phosphorylation of Ins(1,4,5)P_3 (InsP_3) to Ins(1,3,4,5)P_4 (InsP_4) is catalysed by InsP_3 3-kinase. Molecular-biological data have shown the presence of two human isoenzymes of InsP_3 3-kinase, namely InsP_3 3-kinases A and B. We have isolated from a rat thymus cDNA library a 2235 bp cDNA (clone B15) encoding rat InsP_3 3-kinase B. Northern-blot analysis of mRNA isolated from rat tissues (thymus, testis, brain, spleen, liver, kidney, heart, lung and intestine) revealed that a rat InsP_3 3-kinase B probe hybridized to a 6 kb mRNA in lung, thymus, testis, brain and heart. In contrast, Northern-blot analysis of the same tissues probed under stringent conditions with a rat InsP_3 3-kinase A probe hybridized to a 2 kb mRNA only in brain and a 1.8–2.0 kb mRNA species in testis. Northern-blot analysis of three human cell lines (HL-60, SH-SY5Y and HTB-138) probed with a human InsP_3 3-kinase B probe showed the presence of a 6 kb mRNA in all cell lines, except in the human neuroblastoma cell line (SH-SY5Y), where two mRNA species of 5.7 and 6 kb were detected. Using the same blot, no hybridization signal could be seen with a human InsP_3 3-kinase A probe. Altogether, our data are consistent with the notion that the two InsP_3 3-kinase isoenzymes, A and B, are specifically expressed in different tissues and cells.

INTRODUCTION

Ins(1,4,5)P_3 (InsP_3) is a second messenger responsible for the mobilization of intracellular Ca^{2+} (Berridge and Irvine, 1989). The InsP_3 signal can be inactivated by two pathways: it is either dephosphorylated by an InsP_3 5-phosphatase to produce Ins(1,4)P_2 or phosphorylated by an InsP_3 3-kinase to yield Ins(1,3,4,5)P_4 (InsP_4) (Irvine et al., 1986; Erneux and Takazawa, 1991). InsP_4 may itself regulate intracellular Ca^{2+} concentration by promoting Ca^{2+} sequestration in rat liver cells (Hill et al., 1988) or by synergizing with InsP_3 to mobilize intracellular Ca^{2+} stores in mouse lacrimal acinar cells (Morris et al., 1987). Other studies in endothelial cells provide direct evidence for a distinct second-messenger function of InsP_4 alone in the uptake of Ca^{2+} (Lückhoff and Clapham, 1992). The presence of specific InsP_4-binding sites in a number of tissues further supports a role for InsP_4 in the phosphoinositide pathway (Reiser et al., 1991; Theibert et al., 1991; Cullen and Irvine, 1992; Cullen et al., 1994). An inositol polyphosphate receptor has been purified from bovine cerebellum. When reconstituted into planar bilayers, it displays K^+-channel activity, which is selectively modulated by InsP_4 (Chadwick et al., 1992).

InsP_3 acts as a second messenger by specific binding to its intracellular receptors. InsP_3 receptor (type I) was originally cloned from mouse (Furuichi et al., 1989), rat (Mignery et al., 1990) and later from human (Südhof et al., 1991). Various different forms of the receptor product from an alternatively spliced gene or encoded by different genes have been reported (Danoff et al., 1991; Südhof et al., 1991; Ross et al., 1992). Two cDNAs encoding two isoenzymes of InsP_3 3-kinase (i.e. InsP_3 3-kinase A and B) have been isolated from a human hippocampus cDNA library (Takazawa et al., 1991a, b). These isoenzymes appeared to be composed of a catalytic domain and a region involved in regulation of activity. The C-terminal part of human InsP_3 3-kinase B (residues 187–462) was 68 % identical with the C-terminal part of human InsP_3 3-kinase A (residues 178–455) and has been shown to correspond to the catalytic domain (Takazawa and Erneux, 1991; Communi et al., 1993). They differed, however, in their molecular mass and sensitivity to Ca^{2+}/calmodulin (CaM). Moreover, the human platelet InsP_3 3-kinase was not recognized by immunodetection with anti-(human InsP_3 3-kinase A) or anti-(human InsP_3 3-kinase B) antibodies, suggesting the existence of another InsP_3 3-kinase isoenzyme (Communi et al., 1994).

At present, it is not clear whether InsP_3 3-kinase A or B is expressed at different levels in various tissues or cell types. Here we have assessed, by Northern-blot analysis, the relative distribution of the two isoenzymes A and B in various rat tissues and human cell lines. We report a tissue-specific expression of InsP_3 3-kinase A and B.

MATERIALS AND METHODS

Materials

SH-SY5Y human neuroblastoma-cell monolayers (passages 70–90) were kindly provided by Dr. R. A. Wilcox (Department of Cell Physiology and Pharmacology, University of Leicester, Leicester, U.K.). The human non-differentiated promyelocytic-leukaemia-cell line (HL-60) and the human glioma-cell line (HTB-138, from left temporal lobe) were obtained from the American Type Culture Collection. Cell-culture media were from Gibco Life Technologies (U.K). Materials for enzymic assay were as previously reported (Takazawa et al., 1988). [2-^{3}H]InsP_3 (sp. radioactivity 3.3 Ci/mmol) was from New England Nuclear Corp. Pefabloc was from Pentapharm A. G. (Basel, Switzerland). [α-^{32}P]dATP (sp. radioactivity 800 Ci/mmol) was from Amersham. RNA ladder (0.24–9.5 kb) was from Gibco–BRL. A glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA probe was provided by Dr. J. Content (Faculté de Médecine, University of Brussels, Brussels, Belgium).

Analytical procedures

InsP_3 3-kinase activity was measured in 50 μl of incubation medium as previously reported (Communi et al., 1994). Soluble fractions of rat tissues were prepared as outlined by Takazawa et al. (1988). Protein content was measured as described by Petersen (1977). Cell lysates were prepared as follows: cells from five 100 mm dishes were scraped into 400 μl of a hypotonic ice-cold

Abbreviations used: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CaM, calmodulin; InsP_3, Ins(1,4,5)P_3; InsP_4, Ins(1,3,4,5)P_4.

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buffer containing 1 mM $NaHCO_3$, 50 μg/ml Pefabloc, 5 μM leupeptin and 10 μg/ml calpain inhibitors I and II. After three successive cycles of freeze–thawing and centrifugation at 13000 *g* for 10 min, the supernatant was supplemented with 50 mM Tris/HCl, pH 8, 1 mM EDTA, 1 % sucrose, 12 mM 2-mercaptoethanol and 0.1 % Triton X-100.

Cloning of rat and human InsP_3 3-kinase B

Recombinant phages (5×10^5) from a rat thymus cDNA library (Stratagene) were screened using a 1048 bp *Pst*I--*Pst*I restriction fragment of the clone hh3 MB1 (Takazawa et al., 1991b). After prehybridization in 6 × SSC (1 × SSC = 0.15 M NaCl/15 mM sodium citrate) containing 40 % formamide, 0.25 % non-fat dry milk and 5 mM EDTA at 42 °C for 4 h, hybridization was carried out overnight at 42 °C in the same solution containing the cDNA probe labelled with [α-^{32}P]dATP. The filters were washed three times in 2 × SSC/0.1 % SDS for 30 min at room temperature followed by a 5 min and 15 min wash in 2 × SSC/0.1 % SDS at 42 °C. After four rounds of screening the plaques were judged pure and the insert was rescued as a Bluescript plasmid by *in vivo* excision. The clones were then analysed by restriction mapping and subsequently sequenced on both strands using Sequenase (version 2.0; USB Cleveland) and [α-^{35}S]dATP. The coding nucleotide sequence of the B15 cDNA (2235 bp) isolated from rat thymus in our laboratory differed from that of the published sequence from rat liver (Thomas et al., 1994), in that it contained an A instead of a G at position 541 and a G instead of a T at position 1678.

The same procedure was used to screen a human hippocampus cDNA library (Stratagene) with a 274 bp *Eco*RI–*Pst*I fragment of clone hh3 MB1. This resulted in the isolation of clone hv1 (3880 bp), which had the same coding sequence as hh3 MB1, but which was 1142 bp longer at its 5′ end. The coding region of hv1 contained 575 amino acids.

Rat and human InsP_3 3-kinase B expression

The coding region of rat InsP_3 3-kinase B (i.e. clone B15) was reconstructed to contain a *Bam*HI site at the start codon and a *Hin*dIII site 250 bp downstream of the stop codon. PCR was used to construct an in-frame 5′ end of the rat InsP_3 3-kinase B DNA using the following sense and antisense primers:

5′-GCGCGGATCCAATGGAAAGAGGTTC-3′

and

5′-GGTTCTCTGAGAGCTCTGAGCTTTGC-3′

(*Bam*HI and *Sst*I restriction sites respectively are underlined). Plasmid DNA of B15 was subjected to PCR to generate a 355 bp product. This material was digested with *Bam*HI and *Sst*I. In parallel, plasmid DNA of B15 was digested with *Sst*I and *Hin*dIII. The PCR product and the *Sst*I–*Hin*dIII restriction fragment of B15 were subcloned into the *Bam*HI/*Hin*dIII-digested Bluescript plasmid. Positive clones were identified by restriction mapping. The 5′ end of the new construct (i.e. EB15, 2220 bp) was in-frame with the β-galactosidase part of Bluescript as verified by sequence analysis.

The coding region of human InsP_3 3-kinase B (i.e. clone hv1) was reconstructed to allow the expression of InsP_3 3-kinase B activity. hv1 was digested with *Eco*RI to eliminate the non-coding sequence at the 5′ end and was further subcloned into Bluescript. The 5′ end of the new construct (i.e. hv1R, 3090 bp) was in-frame with the β-galactosidase part of Bluescript as verified by sequence analysis. Restriction sites of both EB15 and hv1R cDNA clones are shown in Figure 2 (below).

InsP_3 3-kinase activity of both EB15 and hv1R was expressed as previously described. A 5 ml portion of Luria–Bertani ('LB') medium containing 50 μg/ml ampicillin was inoculated overnight at 37 °C with the appropriate plasmid; the bacterial lysate was made up in 0.5 ml (Takazawa et al., 1991b).

Cell culture

SH-SY5Y cells were cultured in minimum essential medium supplemented with 10 % (v/v) heat-inactivated foetal-calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), fungizone (5 μg/ml) and 2 mM glutamine (Lambert et al., 1991). HL-60 cells were cultured in RPMI medium supplemented with 10 % (v/v) heat-inactivated foetal-calf serum, penicillin (50 units/ml), streptomycin (50 μg/ml) and 5 mM glutamine (Bradford et al., 1992). HTB-138 cells were cultured in Dulbecco's modification of Eagle's medium with 4.5 g/l glucose and supplemented with 10 % (v/v) heat-inactivated foetal-calf serum, penicillin (50 units/ml), streptomycin (50 μg/ml) and 1 mM sodium pyruvate, according to the culture conditions described by the American Type Culture Collection.

Total RNA extraction

From cells

Confluent cells were scraped from four 100 mm dishes in 1.9 ml of 4 M guanidinium monothiocyanate (Chirgwin et al., 1979). After the addition of 840 mg of CsCl to this homogenate, each sample was layered on the top of a 1.2 ml cushion of 5.7 M CsCl in 0.1 M EDTA. Separation of total RNA was made after centrifugation at 15 °C for 17 h at 35000 rev./min in a Beckman SW55 rotor. The RNA was treated with proteinase K (60 μg/ml) in 0.3 M NaCl/1 % SDS. After two standard phenol extractions, the total amount of RNA was determined spectrophotometrically (A_{260}). The ratio A_{260}/A_{280} was verified and was always more than 1.8.

From tissues

Approx. 1.5 g of tissue was homogenized in 20 ml of 4 M guanidinium monothiocyanate. After the addition of 8 g of CsCl to this homogenate, each sample was layered on the top of a 12.6 ml cushion of 5.7 M CsCl in 0.1 M EDTA. Separation of total RNA was made after centrifugation at 15 °C for 24 h at 25000 rev./min in a Beckman SW28 rotor. The RNA was prepared as described above.

The yield of total RNA was consistently 300–500 μg/g of tissue and 200–300 μg/four dishes of confluent cells.

The RNA quality was assayed spectrophotometrically and by agarose-gel electrophoresis. The gel was stained with ethidium bromide (10 μg/ml), and the staining intensity of the ribosomal RNA visually inspected.

Poly(A) mRNA purification

Poly(A) mRNA from cells or tissues was isolated by using PolyATtract mRNA Isolation System III (Promega). A 500 μg portion of total RNA was used for each poly(A) mRNA purification to obtain between 5 and 14 μg of each poly(A) mRNA.

Northern-blot analysis

After denaturation using glyoxal according to the procedure of MacMaster and Carmichael (1977), approx. 20 μg of total RNA and 4 μg of poly(A) mRNA were fractionated by electrophoresis

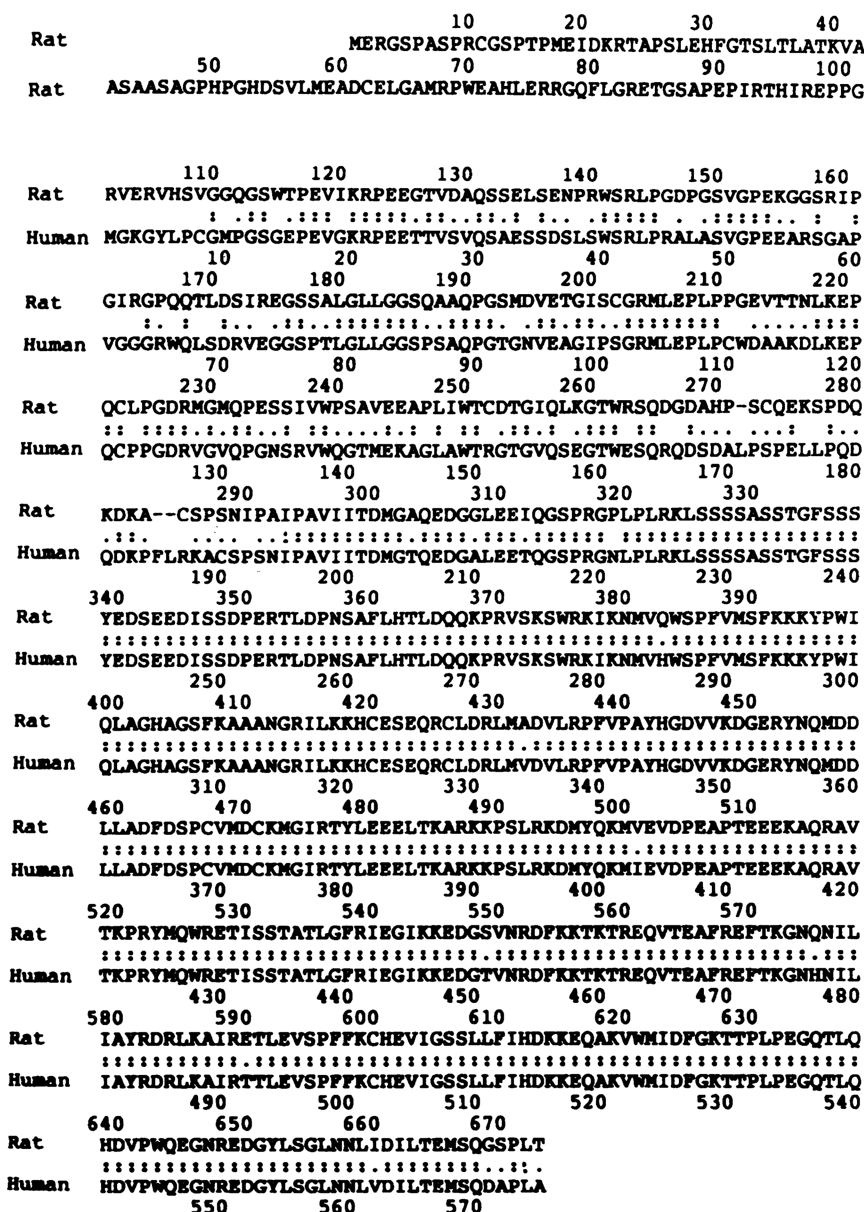

Figure 1 Primary structure of rat thymus InsP_3 3-kinase B as deduced from its cDNA sequence and comparison between primary sequences of rat and human InsP_3 3-kinase B

Gaps (—) were introduced to maximize alignments between sequences.

on a 1%-agarose gel in 10 mM sodium phosphate buffer, pH 7.0. Size RNA markers (4 μg) were applied on independent lanes and treated similarly. Glyoxylated RNAs were transferred by diffusion blotting to a nylon membrane (Pall Biodine B) in 20 × SSC. After baking, the blots were prehybridized for 4 h at 42 °C in a buffer consisting of 50% (v/v) formamide, 5 × Denhardt's solution [100 × Denhardt's = 2% (w/v) Ficoll/ 2% (w/v) poly(vinylpyrrolidone)], 5 × SSPE [20 × SSPE = 0.2 M sodium phosphate (pH 8.3)/3.6 M NaCl/20 mM EDTA], 0.3% SDS, 250 μg of denatured DNA from salmon testis/ml and 200 μg of BSA/ml. Hybridization was carried out overnight at 42 °C in the same buffer as for prehybridization but containing, in addition, 10% (w/v) dextran sulphate, and the cDNA probe labelled with [α-^{32}P]dATP. Filters were washed twice for 10 min in 2 × SSC at room temperature, and then twice for 30 min in 0.2 × SSC/0.1% SDS at 65 °C. Autoradiography was carried out at −70 °C using MP films (Amersham) in the presence of an intensifying screen. After washing, the blot was hybridized with a 1.4 kb *Pst*I–*Pst*I restriction fragment of the GAPDH cDNA clone.

RESULTS

Molecular cloning and expression of rat thymus and human hippocampus InsP_3 3-kinase B

An alignment of both rat and human InsP_3 3-kinase B amino acid sequences is shown in Figure 1. A rat thymus cDNA library was screened with a human InsP_3 3-kinase B cDNA probe (clone

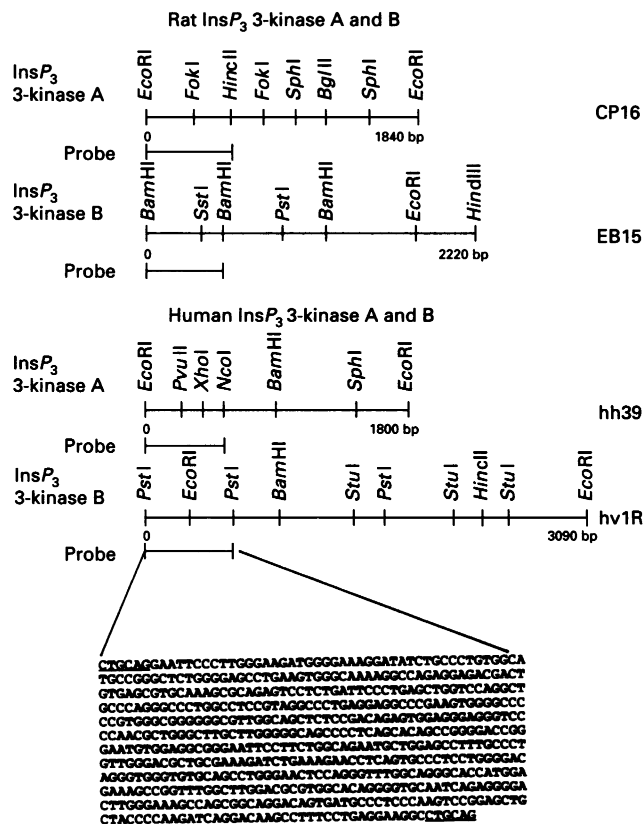

Figure 2 Design of rat and human InsP_3 3-kinase A and B probes used for Northern-blot analysis

Restriction enzymes for each clone are indicated, and the names of the probes are italicized. The nucleotide sequence of human InsP_3 3-kinase B probe (*Pst*I–*Pst*I) is indicated at the bottom and the *Pst*I sites are underlined. The sequences of clone CP16, EB15 (with two changes; see the Materials and methods section) and hh39 are available under accession numbers X56917, X74227 and X54938 respectively from the EMBL, GenBank and DDBJ Nucleotide Sequence Databases.

hh3 MB1) to isolate a cDNA clone B15 of 2235 bp. Sequencing of clone B15 identified a 2019 bp open reading frame encoding a 673-amino-acid protein. The initiation codon was indicated by the presence of highly conserved bases often found to flank the initiating ATG, i.e. AG**A**ATGG versus the consensus sequence (G/A)CC**A**TGG (Kozak, 1986). Since the rat InsP_3 3-kinase B cDNA clone, previously isolated (Thomas et al., 1994), was not expressed, a construction was prepared (EB15; see the Materials and methods section) to express recombinant rat InsP_3 3-kinase B as a β-galactosidase fusion protein. InsP_3 3-kinase activity of EB15 at 10 μM InsP_3 was 5 nmol/min per ml and was stimulated 4-fold by Ca^{2+}/CaM.

The human hippocampus cDNA library was also rescreened to isolate a cDNA clone, i.e. hv1 (3880 bp). A translation-initiation codon was identified in position 883 in the nucleotide sequence. This clone was reconstructed to express human InsP_3 3-kinase B as a fusion protein (hv1R; see the Materials and methods section). The initiation codon was conserved between hv1 and hv1R and it was flanked by a sequence that fits Kozak's criteria in its nucleotide sequence, i.e. AAGATGG (position 22; see Figure 2) versus (G/A)CCATGG (Kozak, 1986). InsP_3 3-kinase activity of hv1R at 10 μM InsP_3 was 11 nmol/min per ml and was stimulated 4–7-fold by Ca^{2+}/CaM.

From the cDNA sequence analysis, the 5′ end (positions 300–912 of the nucleotide sequence of EB15 clone) of the rat InsP_3 3-kinase B was found to be 65% identical with the 5′ end (positions 1–600 of the nucleotide sequence of hv1R clone) of the human InsP_3 3-kinase B. In contrast, the 5′ end of rat (positions 26–619 of the nucleotide sequence of CP16 clone) (Takazawa et al., 1990) and human (positions 1–600 of the nucleotide sequence of clone hh39) (Takazawa et al., 1991a) InsP_3 3-kinase A were 87% identical.

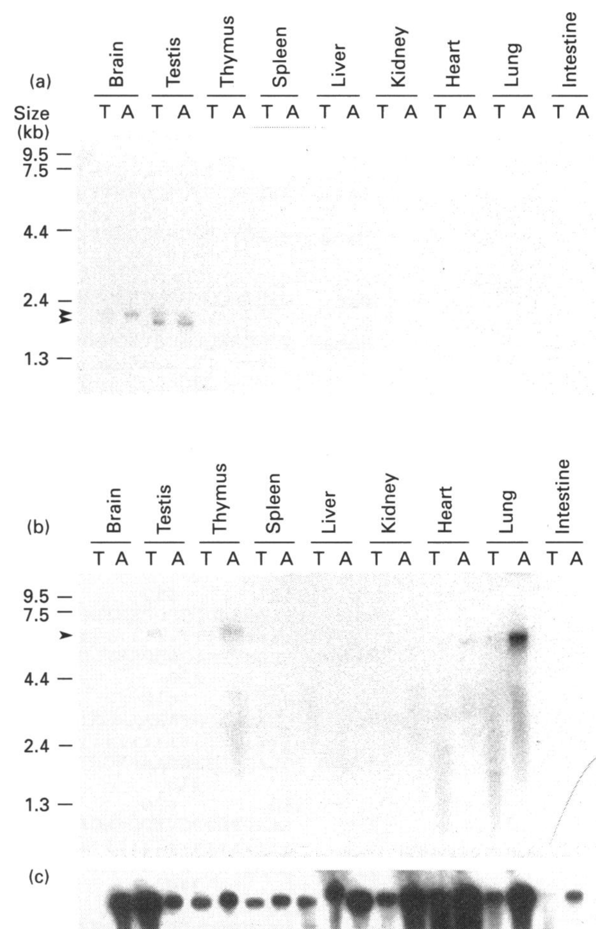

Figure 3 Northern-blot analysis of total RNA and poly(A) mRNA from various rat tissues

(**a**) Northern-blot analysis using an α-^{32}P-labelled cDNA probe synthesized using the 550 bp *Eco*RI–*Hin*cII-restriction-endonuclease fragment of clone CP16 (see Figure 2). The sizes of transcripts encoding the rat InsP_3 3-kinase A were 2 kb in brain and 1.8–2.0 kb in testis (indicated by arrowheads). (**b**) Northern-blot analysis using an α-^{32}P-labelled cDNA probe synthesized using the 517 bp *Bam*HI–*Bam*HI-restriction-endonuclease fragment of clone EB15 (see Figure 2). The sizes of transcripts encoding the rat InsP_3 3-kinase B were 6 kb (indicated by an arrowhead). (**c**) Expression of GAPDH was monitored to correct for possible differences in the amount of RNA loaded per lane. Abbreviations: T, total RNA (approx. 20 μg); A, poly(A) mRNA (approx. 4 μg). The migration of RNA size markers is shown on the left.

Northern-blot analysis of InsP_3 3-kinase isoenzymes A and B in rat tissues

In order to identify the size and tissue distribution of the mRNA corresponding to rat InsP_3 3-kinase A and B, cDNA probes were chosen that were specific to the 5′ end of the coding region of rat InsP_3 3-kinase A and B. An *Eco*RI–HincII digest (i.e. 550 bp) of the rat clone CP16 was prepared and used as a specific rat InsP_3 3-kinase A probe (Figure 2).

Table 1 InsP_3 3-kinase activity detected in a crude soluble fraction of rat tissues

The assay was performed in the presence of 5 μM InsP_3, 7.5 mM 2,3-bisphosphoglycerate, 0.1 μM CaM and 10 μM free Ca^{2+}. Results are means ± S.D. ($n = 3$).

Tissue	InsP_3 3-kinase (nmol/min per g of tissue)
Brain	34.98 ± 5.34
Testis	11.44 ± 1.14
Thymus	22.8 ± 1.10
Spleen	3.10 ± 0.52
Liver	4.66 ± 0.88
Kidney	2.44 ± 0.52
Heart	0.57 ± 0.09
Lung	1.92 ± 0.04
Intestine	0.92 ± 0.04

Northern-blot analysis of total RNA and poly(A) mRNA preparations from various rat tissues identified the mRNA for rat InsP_3 3-kinase A as a 2 kb transcript in brain and as a doublet of 1.8–2.0 kb in testis (Figure 3a). The minor 4 kb message seen in testis is probably an artefact, as it was not reproduced in four independent Northern blots. In all other tissues examined (i.e. thymus, spleen, liver, kidney, heart, lung and intestine), no hybridization signal was observed. We verified that all tissues had InsP_3 3-kinase activity (Table 1).

The expression of rat InsP_3 3-kinase B mRNA was studied with a *Bam*HI–*Bam*HI probe (i.e. 517 bp) derived from clone EB15 (Figure 2). The same blot which was hybridized with a rat InsP_3 3-kinase A probe was used for hybridization with a rat InsP_3 3-kinase B probe: a 6 kb transcript was detected predominantly in lung, but also in thymus, testis, brain and heart (Figure 3b).

The presence of RNA was verified by ethidium bromide staining of the same RNA in a parallel lane and by hybridization of the same blot with a GAPDH probe (Figure 3c).

Northern-blot analysis of InsP_3 3-kinase A and B isoenzymes in human cell lines

In all cell lines studied, InsP_3 3-kinase activity could be measured. At 10 μM InsP_3, 0.1 μM CaM and 10 μM free Ca^{2+}, enzyme activity ranged between 0.5 and 0.8 nmol/min per mg of protein. Northern-blot analysis was performed using total RNA and poly(A) mRNA prepared from three human cell lines (SH-SY5Y, HL-60 and HTB-138). Rat thymus, testis and brain total RNA and poly(A) mRNA were run on the same gel and used as internal controls. The nucleotide identity between rat and human InsP_3 kinase A probes was 87%. It is only 61% between rat and human InsP_3 kinase B probes. Specific probes were designed to the 5′ end of the coding region of the human InsP_3 3-kinase A and B: for the isoenzyme A, the probe was an *Eco*RI–*Nco*I fragment from the hh39 clone (526 bp) and for the isoenzyme B, we designed a *Pst*I–*Pst*I fragment of 588 bp from the hv1R clone, as shown in Figure 2. Using a human InsP_3 3-kinase A probe, no hybridization signal was seen in any cell line; in contrast, the expression of rat brain and testis mRNA species, respectively 2 and 1.8–2.0 kb signals, were detected with the same probe (Figure 4a) or with a rat InsP_3 3-kinase A probe (Figure 4b). The high similarity between both probes (87% in terms of nucleic acid) probably explains this cross-hybridization.

The expression pattern of human InsP_3 3-kinase B is shown in Figure 4(c); the human InsP_3 3-kinase B probe hybridized with

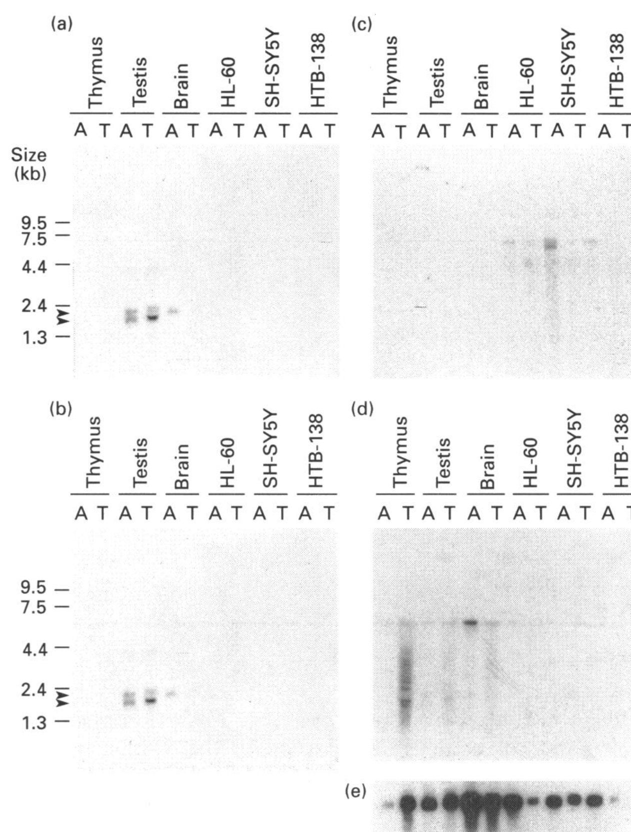

Figure 4 Northern-blot analysis of total RNA and poly(A) mRNA from human cell lines and rat tissues

(**a**) Northern-blot analysis using an α-^{32}P-labelled cDNA probe synthesized using the 526 bp *Eco*RI–*Nco*I- restriction-endonuclease fragment of clone hh39 (see Figure 2). No hybridization signal is observed in human cells. (**b**) Northern-blot analysis using an α-^{32}P-labelled cDNA probe synthesized using the 550 bp *Eco*RI–*Hin*cII-restriction-endonuclease fragment of clone CP16 (see Figure 2). The sizes of transcripts encoding the rat InsP_3 3-kinase A were 2 kb in brain and 1.8–2.0 kb in testis (indicated by arrowheadheads). (**c**) Northern-blot analysis using an α-^{32}P-labelled cDNA probe synthesized using the 588 bp *Pst*I–*Pst*I-restriction-endonuclease fragment of hv1R (see Figure 2). The sizes of transcripts encoding the human InsP_3 3-kinase B were 6 kb in HL-60 and HTB-138 and 5.7–6.0 kb in SH-SY5Y (indicated by arrows). (**d**) Northern-blot analysis using an α-^{32}P-labelled cDNA probe synthesized using the 517 bp *Bam*HI–*Bam*HI-restriction-endonuclease fragment of EB15 (see Figure 2). The sizes of transcripts encoding the rat InsP_3 3-kinase B are 6 kb (indicated by an arrow). (**e**) expression of GAPDH. Abbreviations: T, total RNA (approx. 20 μg); A, poly(A) mRNA (approx. 4 μg). The migration of RNA size markers is shown on the left.

a 6 kb mRNA in HL-60 and HTB-138 cells. In SH-SY5Y cells the same probe recognized a doublet of 5.7–6.0 kb. In rat tissues, no signal was observed with the human InsP_3 3-kinase B probe. When the same blot was hybridized with a rat InsP_3 3-kinase B probe, a 6 kb mRNA signal was observed in rat tissues, but there was no signal in the human cell lines (Figure 4d). Hybridization of the same blot with a GAPDH probe is shown in Figure 4(e). Table 1 summarizes the expression pattern of both rat and human InsP_3 3-kinase A and B mRNAs.

DISCUSSION

Biochemical data have provided evidence for the existence of a low-M_r InsP_3 3-kinase in rat brain (50 kDa) and high-M_r isoenzymes in pig aortic smooth muscle (93 kDa), rat liver (61 kDa), human platelets (69–70 kDa), human lymphocytes (70 kDa) and rat thymus (100 kDa) (Takazawa et al., 1990;

Table 2 mRNA species detected in rat tissues and human cells by Northern-blot hybridization

The data are from Figures 3 and 4. Abbreviation: n.d., not detectable.

	Size of transcripts (kb)	
Tissue/cell line	InsP_3 3-kinase A	InsP_3 3-kinase B
Rat tissue		
Brain	2	6
Testis	1.8–2.0	6
Thymus	n.d.	6
Spleen	n.d.	n.d.
Liver	n.d.	n.d.
Kidney	n.d.	n.d.
Heart	n.d.	6
Lung	n.d.	6
Intestine	n.d.	n.d.
Human cell line		
HL-60	n.d.	6
SH-SY5Y	n.d.	5.7–6.0
HTB-138	n.d.	6

Yamaguchi et al., 1988; Conigrave et al., 1992; Communi et al., 1994; D'Santos et al., 1994). Except for the 50 kDa rat brain enzyme, for which internal sequences have been obtained and found in the deduced amino acid sequence of InsP_3 3-kinase A (Takazawa et al., 1990), we do not know to which isoform the human platelet, the human lymphocyte or the rat thymus isoenzymes correspond. From sequence analysis, InsP_3 3-kinase isoenzymes are encoded by a family of different genes; two distinct cDNAs have been isolated after the screening of a human hippocampus cDNA library (Takazawa et al., 1991a,b). More recently, the rat orthologue of InsP_3 3-kinase B was isolated from a rat liver cDNA library (Thomas et al., 1994). We have found a nearly identical coding sequence after the screening of a rat thymus cDNA library and have provided evidence of InsP_3 3-kinase activity when this clone (EB15) was expressed in *Escherichia coli*. From the screening of a human hippocampus cDNA library, we have obtained a new sequence (hv1R) identical with the hh3 MB1 clone (Takazawa et al., 1991b) but containing a longer 5′-end coding sequence. We have also provided evidence that this clone gave rise to InsP_3 3-kinase activity when expressed in *E. coli*.

In the present study we have isolated mRNA from several rat tissues and human cell lines and we have addressed the question of tissue- or cell-specific expression of InsP_3 3-kinase A and B.

We have previously reported the presence, in rat thymus, of a 100 kDa isoenzyme. However, we were unable to demonstrate, by immunodetection, immunoprecipitation or activity assay after SDS/PAGE, the presence of InsP_3 3-kinase A in rat thymus extracts (D'Santos et al., 1994). Consistent with this, the Northern-blot analysis presented here has revealed that a probe designed in the 5′ end of the rat InsP_3 3-kinase B (i.e. a sequence presenting no significant sequence similarity to the 5′ end of the rat InsP_3 3-kinase A) hybridized specifically with a 6 kb mRNA in rat thymus. This result appeared to be specific for InsP_3 3-kinase B, since an InsP_3 3-kinase A probe did not show any hybridization signal in rat thymus. With other rat tissues (brain, testis, spleen, liver, kidney, heart, lung and intestine), no signal could be detected with an InsP_3 3-kinase A probe, except in brain and testis (Table 2). Moreover, isoenzyme B was predominantly expressed in lung and also in brain, thymus, testis and heart. These data therefore suggested that mRNAs encoding the two InsP_3 3-kinase isoenzymes, A and B, were expressed in a tissue-specific manner: the isoenzyme A was specifically expressed in brain and testis, whereas the isoenzyme B was predominantly expressed in lung and also in thymus, heart, testis and brain. In some tissues no transcript could be detected with either probe. Assay of InsP_3 3-kinase activity in spleen, liver, kidney and intestine crude soluble fractions indicated that this pathway is present in these cells. The absence of any signal (by Northern-blot analysis) could be due to a very low expression level of the transcript or to the presence of another predominant isoenzyme. A previously unidentified isoenzyme is suggested by the cloning of a cDNA from a screening of a human thyroid cDNA library (K. Takazawa, personal communication). Our data are comparable with the expression of various isoforms of the InsP_3 receptor, which also indicated differential tissue localization and therefore heterogeneity of InsP_3 action. Northern-blot hybridization of human haematopoietic- and lymphoma-cell lines revealed that the type I receptor was expressed relatively weakly in human haematopoietic and lymphoma cells, except in THP-1 monocytes. In contrast, the type II receptor was expressed in large amounts in HL-60, Namalwa KJM-1 cells and Jurkat cells (Yamamoto-Hino et al., 1994). In conclusion, differential expression of InsP_3 3-kinase isoenzymes and InsP_3 receptors permits both differential InsP_3/InsP_4 regulation and action.

Although InsP_3 3-kinase activity was present in the three human cell lines tested (SH-SY5Y, HL-60 and HTB-138), Northern-blot analysis of poly(A) RNA from these cells did not show any signal with a human InsP_3 3-kinase A probe. Analysis of the same blot with a human InsP_3 3-kinase B probe showed a 6 kb mRNA in HL-60 and HTB-138 cells and a doublet of 5.7–6.0 kb in SH-SY5Y neuroblastoma cells. These data provide the first evidence of a specific expression of InsP_3 3-kinase B in human cell lines. Regulation of InsP_4 production, at least in these cells, occurs via InsP_3 3-kinase B (see below) and not by InsP_3 3-kinase A. The two mRNA species found in RNA isolated from SH-SY5Y cells could result from alternative splicing. However, verification of this will require the isolation of various cDNAs by screening human cDNA libraries.

What are the possible mechanisms that may change InsP_3 3-kinase activities? Ca^{2+}/CaM stimulation appears to be a quite general mechanism of control for various isoenzymes, but this varies depending on the tissue source and species. Rat brain isoenzyme A was stimulated 2–3-fold (Takazawa et al., 1990), rat thymus and human lymphocyte isoenzymes were stimulated 4–6-fold and 10-fold respectively (D'Santos et al., 1994) and human platelet isoenzyme was stimulated 17-fold (Communi et al., 1994). This probably results from the existence of isoenzymes and non-conserved sequences in their regulatory domain. It is possible that this mechanism of stimulation of InsP_4 production will occur in cells possessing a well defined isoenzyme, either to antagonize a physiological process regulated by InsP_3 or alternatively to produce the potential InsP_4 signal. For example, the tissue-specific expression of Ca^{2+}/CaM-sensitive cyclic nucleotide phosphodiesterase isoforms is well defined. They also differ in their abilities to be stimulated by Ca^{2+}/CaM, and this mechanism may, in some cells, antagonize cyclic AMP-regulated processes (Sonnenberg et al., 1993).

Another mechanism of regulation of InsP_3 3-kinase activity is its phosphorylation by protein kinase C. An inhibitory effect of phosphorylation by protein kinase C has been shown for the rat brain isoenzyme A and involved the simultaneous phosphorylation of two major sites: serine-119 and serine-185 (Sim et al., 1990). Serine-119 is conserved in the sequence of both InsP_3 3-kinase A and B from rat (Ser-119 in rat InsP_3 3-kinase A and Ser-

326 in rat InsP_3 3-kinase B) and human (Ser-121 in human InsP_3 3-kinase A and Ser-228 in human InsP_3 3-kinase B); however, serine-185 (in rat InsP_3) is not. It is therefore possible that the phosphorylation of InsP_3 3-kinase by protein kinase C is only seen in tissues specifically expressing isoenzyme A. Other mechanisms of negative regulation of InsP_3 3-kinase activity could be triggered by Ca^{2+} and calpain in rat brain (Lee et al., 1990).

The tissue-specific expression of a given isoenzyme of InsP_3 3-kinase is perhaps comparable with the expression of several isoforms of the multifunctional Ca^{2+}/CaM-dependent protein kinase (Nghiem et al., 1993). In human T lymphocytes, an isoenzyme has been cloned which showed differences with the neuronal form in the variable domain adjacent to the C-terminal end of the CaM-binding site. Consequently, CaM affinity may be affected. As for InsP_3 3-kinase isoenzymes, differential expression suggests that they have become specialized for different roles in multiple tissues.

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