Phosphatidylinositol 3-kinase (PI 3-kinase) is stimulated by association with a variety of tyrosine kinase receptors and intracellular tyrosine-phosphorylated substrates. We isolated a cDNA that encodes a 50-kDa regulatory subunit of PI 3-kinase with an expression cloning method using $^{32}$P-labeled insulin receptor substrate-1 (IRS-1). This 50-kDa protein contains two SH2 domains and an inter-SH2 domain of p85α, but the SH3 and bcr homology domains of p85α were replaced by a unique 6-aminooic acid sequence. Thus, this protein appears to be generated by alternative splicing of the p85α gene product. We suggest that this protein be called p50α. Northern blotting using a specific DNA probe corresponding to p50α revealed 6.0- and 2.8-kb bands in hepatic, brain, and renal tissues. The expression of p50α protein and its associated PI 3-kinase were detected in lysates prepared from the liver, brain, and muscle using a specific antibody against p50α. Taken together, these observations indicate that the p50α gene actually generates three protein products of 85, 55, and 50 kDa. The distributions of the three proteins (p85α, p55α, and p50α), in various rat tissues and also in various brain compartments, were found to be different. Interestingly, p50α forms a heterodimer with p110 that can as well as cannot be labeled with wortmannin, whereas p85α and p55α associate only with p110 that can be wortmanninabeled. Furthermore, p50α exhibits a markedly higher capacity for activation of associated PI 3-kinase via insulin stimulation and has a higher affinity for tyrosine-phosphorylated IRS-1 than the other isoforms. Considering the high level of p50α expression in the liver and its marked responsiveness to insulin, p50α appears to play an important role in the activation of hepatic PI 3-kinase. Each of the three α isoforms has a different function and may have specific roles in various tissues.

A variety of growth factors and hormones mediate their cellular effects via interactions with cell surface receptors that possess protein kinase activity (1, 2). The interaction of most of these ligands with their receptors induces tyrosine kinase activation and autophosphorylation of the receptor, resulting in physical association of these receptors with several cytoplasmic substrates having SH2 domains. Phosphatidylinositol 3-kinase (PI 3-kinase) has been identified through its ability to associate with cellular protein kinases, including numerous growth factor receptors and oncogene products (3, 4). This lipid kinase phosphorylates phosphatidylinositol at the D-3 position of the inositol ring in response to stimulation with a variety of growth factors and hormones (5). Although the role of this lipid product in cellular regulation remains unclear, recent reports suggest that the activation of PI 3-kinase leads to the activation of c-Akt, Rac, PKC-γ isoform, and p70 S6 kinase (6–9). As a result, PI 3-kinase has been suggested to play essential roles in the regulation of various cellular activities, including proliferation (10, 11), differentiation (12), membrane ruffling (13), prevention of apoptosis (14), and insulin-stimulated glucose transport (10, 15, 16).

PI-3 kinase is composed of a catalytic 110-kDa protein (p110) associated with a regulatory subunit (17–19). The regulatory subunit contains two proline-rich motifs, two Src homology-2 (SH2) domains, and a domain responsible for the binding with p110 between the two SH2 domains (3, 4). Many activated receptors with tyrosine kinase activity interact with the SH2 domain in the regulatory subunit through phosphorylated XXXM motifs in the receptors themselves (20), resulting in the activation or recruitment of PI 3-kinase (21). To date, four regulatory subunits of PI 3-kinase have been identified, two 85-kDa proteins (p85α, p85β) and two 55-kDa proteins (p55α/p85/AS53, p55γ/p55$^{\beta}$) (22–24). The two recently cloned 55-kDa regulatory subunits, p55α and p55γ, are unique because the SH3 and bcr homology domains found in p55α are replaced by a unique 34-amino acid residue NH2 terminus. In this study, we screened a rat liver cDNA library using a $^{32}$P-labeled human IRS-1 protein and obtained a cDNA that encodes a novel 50-kDa regulatory subunit for PI 3-kinase. Sequence analysis of the cDNA revealed that this protein consists of a unique 6-amino acid sequence at its NH2 terminus, as well as two SH2 domains and an inter-SH2 domain of p85α. Neither the SH3
and bcr homology domains, of the p85 regulatory subunit, nor the unique 34-amino acid residue, of the p55 regulatory subunit, were found in this 50-kDa protein. These sequence data indicate that this 50-kDa protein is generated by alternative splicing of the p85α gene product. We suggest that this protein be called p50α.

In total, five regulatory subunits for PI 3-kinase have been identified in mammalian cells to date, including two 85-kDa proteins, two 55-kDa proteins, and one 50-kDa protein. In this study, we demonstrated the tissue distributions and different roles in PI 3-kinase activation, via insulin stimulation, of these subunits. Our data suggest that these five regulatory subunits may have different roles in the various responses induced by the numerous growth factors, hormones, and oncogene products with which they interact.

**EXPERIMENTAL PROCEDURES**

**Expression Screening of Rat Liver cDNA Library with Human 32P-IRS-1 Protein**—The recombinant human IRS-1 protein was prepared as described previously (24). The insulin receptor was partially purified from human placenta on wheat germ agglutinin-agarose as described previously (25). The 32P-IRS-1 probe was prepared by incubating IRS-1 with activated insulin receptor in the presence of Mn2+ and γ-32P-ATP (24). An oligo(dT)-primed rat liver cDNA library was prepared in UNI-ZAP XR (Stratagene) according to the manufacturer’s instructions. Sixty 15-cm plates representing 3,000,000 independent plaques were plated and incubated for 7 h at 37 °C. Then the plates were overlaid with nitrocellulose filters that had been impregnated with 10 μm propyl-β-thiogalactopyranoside and incubated for 8 h at 37 °C. The hybridization of the filters with 32P-IRS-1 probe and washing were performed as described previously (24). The cDNA inserts in pBlue-script were prepared by in vitro excision according to the manufacturer’s instructions (Stratagene). The nucleotide sequences were determined using an ABI automated sequencer.

**Northern Blotting**—Northern blotting was performed using a commercially available sheet made by Clontech (Palo Alto, CA). Nucleotides −170–18 of p50α were labeled with [γ-32P]ATP and used as probes. The filter was hybridized and washed according to the manufacturer’s instructions (Clontech). Autoradiography was performed at −80 °C for 24–48 h.

**Antibodies**—A specific antibody against p50α (a50α) was prepared by immunizing rabbits with a 10-amino acid synthetic peptide identical to the unique NH2-terminal region of p50α (MNRRSTLDSSLH, amino acid residues 1–10). An anti-p58β specific antibody (a58β) was prepared by immunizing rabbits with a synthetic peptide identical to the unique SH3 region of p58β (YFPRERPEDLELLPGDLLVSR, amino acid residues 13–44). These peptides were coupled to keyhole limpet hemocyanin and inoculated into rabbits. These anti sera were affinity purified with Affi-Gel 10 covalently coupled to keyhole limpet hemocyanin and inoculated into rabbits. These sera were affinity purified with Affi-Gel 10 covalently coupled to keyhole limpet hemocyanin and inoculated into rabbits. These sera were affinity purified with Affi-Gel 10 covalently coupled to keyhole limpet hemocyanin and inoculated into rabbits. These sera were affinity purified with Affi-Gel 10 covalently coupled to keyhole limpet hemocyanin and inoculated into rabbits. These sera were affinity purified with Affi-Gel 10 covalently coupled to keyhole limpet hemocyanin and inoculated into rabbits. These sera were affinity purified with Affi-Gel 10 covalently coupled to keyhole limpet hemocyanin and inoculated into rabbits.

**Preparation of p50α and p55α RNAs and in Situ Hybridization Histochecmistry**—An antisense RNA probe was prepared by in vitro transcription from the fragment of p50α cDNA that had been used for Northern blot analysis, using T7 RNA polymerase, in the presence of 350 mM digoxigenin (DIG)-linked UTP in a 20-μl reaction mixture, according to the manufacturer’s instructions (Boehringer Mannheim). A sense probe was prepared from a DNA template.

Male Wistar rats, 4 weeks of age (supplied by the Animal Breeding Facility, Gunma University), were anesthetized with an intraperitoneal injection of sodium pentobarbital and perfused through the aorta with an isotonic sodium chloride solution to remove blood, followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4. Brains were then removed, washed with phosphate-buffered saline, pH 7.4, dehydrated through graded alcohols, and embedded in paraplast wax. Serial sections (10 μm thick) were cut, mounted on poly-L-lysine-coated slides, and stored at room temperature until use.

After de-waxing and rehydration, tissue sections were digested with 5 μg/ml proteinase K at room temperature for 20 min. They were then fixed in 0.4% paraformaldehyde at 4 °C for 10 min and incubated at 20 °C overnight with a hybridization solution containing 1 μg/ml DIG-labeled cRNA, 50% formamide, 10 μM Tris-Cl, pH 7.5, 300 mM NaCl, 1 mM EDTA, 0.25% SDS, 1 × Denhardt’s solution, 200 μg/ml yeast tRNA, and 10% dextran sulfate. Following hybridization, the sections were washed in 2 × SSC, 50% formamide at 58 °C for 30 min, incubated in 1 μl/ml RNase A solution at 37 °C for 30 min, and washed once in 2 × SSC and twice in 0.2 × SSC at 50 °C for 20 min each time. The sections were then incubated in a diluted solution of polyvalent sheep anti-DIG Fab antibody conjugated with alkaline phosphatase, before washing and detection of the label with nitro blue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphosphate. Color development was carried out at room temperature for 14 h, and the sections were then washed in TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 7.5). To inhibit further color reaction, the slides were mounted in a mixture containing 24% glycerol, 1% Dextran, and 5% 0.01 vol/vol 0.1% paraformaldehyde.

**Chromatography of Rat Liver and Brain PI 3-Kinase on a DEAE-Sepharose Column and Gel Filtration Chromatography**—The insoluble materials prepared from rat liver and brain (30 ml, 20 and 8 mg/ml, respectively) were directly loaded onto a DEAE-Sepharose Fast Flow column (200 ml). The column was then washed with buffer B, consisting of 20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, 1 μM leupeptin, 0.5 mM PMSF, and 10% glycerol. Once the absorbance eluted at 280 nm had returned to baseline, the column was eluted with a gradient of KCl up to 0.5 M. Aliquots of the individual fractions were assayed for PI 3-kinase activity. The pooled fractions from the peaks (three fractions from liver chromatography, fractions A, B, and C, and one fraction from brain chromatography, fraction D) were concentrated 10-fold with Centricon 10 (Amicon, MA) and subjected to immunoblotting analysis. Concentrated fractions A and B (1 ml) were also applied to the gel filtration column. (Sephracyl S300 column, Pharmacia Biotech Inc.), previously equilibrated with buffer B, and eluted fractions (1 ml) were collected and assayed for PI 3-kinase activity. Molecular weight standards were run under the same conditions as samples.

**PI 3-Kinase Assay**—Eluted fractions from the chromatography column of 50 μM were incubated with 1 μg/ml of the appropriate antiserum for 2 h at 4 °C. Protein A-Sepharose beads were used to precipitate the immune complexes. The presence of PI-3 kinase activity in immune complexes was determined as described previously (24).

**Detection of p110 by Wortmannin Labeling**—All concentrated fractions from the peaks were immunoprecipitated with the appropriate antibodies and pelleted using protein A-Sepharose beads. The beads
were washed three times with lysis buffer A. The beads were then incubated at 25 °C for 30 min with 20 mM Tris- HCl, pH 7.4, 0.5 mM EDTA, 0.5 mM EGTA, and 0.1 mM wortmannin and washed again three times with lysis buffer A. Wortmannin-labeled immunoprecipitates were separated by the beads by boiling in Laemmli buffer. The beads were removed by centrifugation, and the supernatants were subjected to SDS-PAGE.

The wortmannin was detected by immunoblotting with anti-HA antibody (Fig. 1C, panel a) or specific antibodies against each isoform (Fig. 1C, panels b-f). Bands corresponding to p50α were observed using either the anti-HA antibody or the anti-p50α specific antibody (p50α), with an electric mobility of approximately 50 kDa (Fig. 1C, panel j, lane 6). The results shown in Fig. 1C, panels b-f, indicate that none of the specific antibodies recognize other regulatory subunit isoforms. We were thus able to measure the PI 3-kinase activity associated with each of the regulatory subunit isoforms expressed endogenously in tissues or cell lines.

p50α mRNA Is Most Abundant in Liver, but Is Also Abundant in Brain and Kidney—The levels of p50α mRNA expression in various rat tissues are shown in Fig. 2A. Northern blotting with a 5′-unique 188-nucleotide sequence located in the 5′-untranslated region and a coding region for the NH2-terminal 6 amino acid sequence in the p50α cDNA, neither of which is included in the p58α cDNA nucleotide sequence, revealed two mRNA species of 6.0 and 2.8 kb. The p50α mRNA was most abundant in liver but was also abundant in the brain and kidney. Northern blotting using the cDNA probe coding for the N-terminal SH2 (N-SH2) domain of p85α revealed four bands (7.7, 6.0, 4.2, and 2.8 kb), as previously reported (22) (Fig. 2B). Among them, the 6.0- and 2.8-kb bands matched those of p50α, whereas the 7.7- and 4.2-kb bands matched those of the SH3 p85α domain (22). As we reported previously, a minor portion of the 4.2-kb band and a considerable portion of the 2.8-kb band in the brain correspond to p55α mRNAs (22). The quantities of p58α mRNA and p50α mRNA in liver appeared to be similar, judging from the Northern blotting data.

Although the role of PI 3-kinase in brain and neural cells remains unclear, all five known isoforms are abundantly expressed in brain tissue (Fig. 2B) (22). We further investigated their distributions in various portions of the rat brain using an RNA protection assay. As shown in Fig. 3E, p50α mRNA was abundant in the cerebral cortex (temporal and occipital), putamen, and cerebellum. Although minor differences were observed, the distributions of p85β and p55α were similar to that of p50α (Fig. 3, B and D). p58α mRNA was also abundant in the superior colliculus and brainstem (pons and medulla oblongata) but was detected in every part of the brain (Fig. 3A). On the other hand, the expression of p55y mRNA was particularly prominent in the cerebellum, while being barely detectable in other parts of the brain (Fig. 3C). As to the cerebellum, in situ hybridization histochemistry revealed p50α transcripts to be most abundant in the cytoplasms of Purkinje cells (Fig. 4A). As reported previously (28) the PI 3-kinase and IGF-1 receptor immunoreactivities were detected in almost all Purkinje neurons in the cerebellar cortex, so we assume that p50α plays a specific role in these highly specialized cells.

Immunoblotting of Three Types of Regulatory Subunits (p85α, p55α, and p50α) and Their Associated PI 3-Kinase Ac-
To determine the expression of p85α and its two splice variants in different rat tissues at the protein level, lysates from various rat tissues were immunoprecipitated with protein A-agarose beads covalently coupled to op85PAN-UBI. This antiserum, which is raised against the entire region and the nSH2 region of p85α, recognizes not only p85α but also p55α and p50α. The immunoblot obtained with a p85PAN-UBI revealed the 85-kDa band and a broad 50–55-kDa band (Fig. 5A). The 85-kDa protein was abundantly expressed in every tissue examined, and the 50–55-kDa band was prominent in the brain, liver, and kidney but faint in fat and muscle. By taking into consideration that the antibody used recognizes the entire p85α molecule, the p55α and p50α molecules, which have neither the SH3 nor the bcr homology domain, would be less effectively detected than p85α in the immunoblot using a p85PAN-UBI. Thus, the relative amounts of p55α and p50α proteins, as compared with the amount of p85α, are assumed to be larger than those suggested by the data in Fig. 5A.

As shown by the immunoblot using the specific antibody against p85α (op85PAN-UBI) (Fig. 5B), the expression of p85α protein is ubiquitous. The second isoform, p55α, is expressed abundantly in the brain but only faintly in muscle. On the other hand, the third isoform, p50α, is expressed most abundantly in liver and in relative abundance in the brain and kidney as shown in the immunoblot using the specific antibody against p50α (op50α) (Fig. 5B). These results regarding protein expression levels appear to be consistent with Northern blotting results. Fig. 5C shows the PI 3-kinase activities associated with p85α, p55α, and p50α proteins. p85α-associated PI 3-kinase is ubiquitously detected, whereas p55α-associated PI 3-kinase is detected mainly in brain and muscle, and p50α-associated PI 3-kinase is detected in liver and brain, as well as in muscle.
p50a Binds Two Types of Catalytic Subunits of PI 3-Kinase—Kurosu et al. (29) reported the presence of a 46-/100-kDa heterodimer form of a PI 3-kinase, in rat liver, which was isolated in the flow-through fraction of a DEAE-Sepharose column. In addition, this 46-kDa protein was reported to readily be recognized by the antibody against the whole p85α molecule. To determine whether this 46-kDa protein is identical to p50α, we performed the same chromatographic procedure using DEAE-Sepharose. The soluble fractions prepared from rat liver were applied to a DEAE-Sepharose column, and fractionation was performed according to the reported procedures (29). The obtained fractions were immunoprecipitated with the specific antibodies against p85α or p50α, and the PI 3-kinase activities in these immunoprecipitates were measured.

As shown in Fig. 6A, the p50α-associated PI 3-kinase was detected in two fractions. One was the flow-through fraction, eluted at 0 mM KCl (fraction A), and the other fraction eluted at approximately 0.1 mM KCl (fraction B). On the other hand, the PI 3-kinase activities associated with p85α proteins were observed not in the flow-through fractions but in the fractions eluted at approximately 0.2 mM KCl (fraction C), which is in agreement with the results of Kurosu et al. (29). The apparent molecular masses of the p50α-associated PI 3-kinases in both fractions (fractions A and B) were determined to be 160–180 kDa based on the gel filtration results (Fig. 6B). These fractions (fractions A, B, and C) were then collected and immunoprecipitated with...
op85\(^{\text{PAN-UBI}}\). Western blotting with op85\(^{\text{PAN-UBI}}\) allowed isolation of the p80\(\alpha\) and p50\(\alpha\) proteins with DEAE chromatography (Fig. 6C). With respect to the difference between the two p50\(\alpha\)-associated PI 3-kinase fractions, A and B, we speculate that p50\(\alpha\) may bind to the different catalytic subunits. To ascertain the different characteristics of the catalytic subunits associated with p50\(\alpha\) in the two fractions, we attempted wortmannin labeling, followed by treatment with anti-wortmannin antibody for detection of the catalytic subunit. The catalytic subunit associated with p50\(\alpha\) in fraction B was detected by this procedure as a band of 110 kDa (Fig. 6D, lane 4). In contrast, the catalytic subunit associated with the p50\(\alpha\) in fraction A was not detectable with the same procedure (Fig. 6D, lane 2), despite fraction A containing an amount of p50\(\alpha\) protein similar to that of fraction B. In contrast to the case of p50\(\alpha\), the PI 3-kinase activities associated with p85\(\alpha\) and p55\(\alpha\) were detected only in the eluted fractions containing approximately 0.2 M KCl (fraction C) (Fig. 6A) and 0.15 M KCl (fraction D) (Fig. 7A), respectively, but never in the flow-through fractions. Their associated catalytic subunits were easily detected by the wortmannin labeling and the following immunoblot using wortmannin antibody, as a band of 110 kDa (Fig. 6D, lane 6), respectively.

The Function of p50\(\alpha\) in the Insulin-induced Activation of Associated PI 3-Kinase—The role of insulin in the induction of the PI 3-kinase activities associated with the various regulatory subunits was evaluated using PC12 and HepG2 cells. PC12 cells express all five regulatory subunit isoforms, but HepG2 cells express only p55\(\alpha\), p50\(\alpha\), and p50\(\beta\) (data not shown). In PC12 cells, insulin caused increases in the PI 3-kinase activities of the op85\(\alpha\), p55\(\alpha\), and p50\(\alpha\) immunoprecipitates up to 1.9-, 1.9-, and 3.3-fold, respectively, whereas the op50\(\beta\) and op55\(\gamma\) immunoprecipitates showed no significant increases in PI 3-kinase activity (Fig. 8A). In HepG2 cells, p85\(\alpha\) and p50\(\alpha\) responded to insulin stimulation with increases of 2.7- and 4.5-fold, respectively, whereas no significant change was observed for p85\(\beta\) (Fig. 8D). In both cell lines, the degree of PI 3-kinase activation was revealed to be highest for the p50\(\alpha\)-associated PI 3-kinase. However, the possibility that the different responses are due to specific antibodies, bound to the different portions of these regulatory subunits, cannot be excluded.

Overexpression of Regulatory Subunits in HepG2 Cells and in CHO Cells Expressing Insulin Receptors or IRS-1—The cDNA
Wortmannin-labeled immunoprecipitates were subjected to SDS-PAGE. The wortmannin labeling was detected by enhanced chemiluminescence.

**A**

![Fractionation of rat brain cytosolic PI 3-kinase activities on a DEAE-Sepharose column and detection of p110 by wortmannin labeling.](Image 60x316 to 295x480)

**B**

![Insulin responsiveness of endogenous regulatory subunit for PI 3-kinase.](Image 157x554 to 457x729)

Construct for each isoform having the HA tag at its COOH terminus was prepared, and the adenoviruses for the transient expression of these isoforms were produced. HepG2 cells and CHO cells, expressing insulin receptors (CHO/IR) or IRS-1 (CHO/IRS-1), were infected with these adenoviruses to achieve similar protein expression levels, as assessed by the immunoblot using anti-HA antibody (data not shown). In CHO cells, expressing insulin receptors (CHO/IR) or IRS-1 proteins, in response to insulin stimulation.

To elucidate the mechanisms accounting for the variability in the extent of PI 3-kinase activation associated with the various regulatory proteins, we investigated the amount of IRS-1 bound to the expressed regulatory subunits in response to insulin. Before and after insulin stimulation, the cells were lysed and immunoprecipitated with the anti-HA antibody. The expressed regulatory subunit proteins of the indicated molecular mass were observed (Fig. 9B), and \[^{35}S\]methionine-labeled IRS-1 proteins (approximately 180 kDa) associated with each of the five regulatory subunits were measured, and the ratios of bound IRS-1/the amount of regulatory subunit expressed were calculated for each of the isoforms (Fig. 9C). p50α proteins apparently associated with larger amounts of phosphorylated IRS-1 protein, as compared with other isoforms. In contrast, p55γ associated with the smallest amount of IRS-1, in response to insulin stimulation, among the five isoforms. The IRS-1 protein was also measured by immunoblotting using the antibody against IRS-1, and no significant difference was observed between the metabolic labeling method and immunoblotting data (data not shown). These data indicate that p50α shows the most efficient IRS-1 binding in response to insulin. This observation may explain the high capacity of this protein to induce PI 3-kinase activity, as compared with other regulatory proteins, in response to insulin stimulation.

In a study utilizing the expression cloning method with \(^{32}P\)-labeled IRS-1, we previously isolated the four regulatory subunit isoforms for PI 3-kinase from a cDNA library prepared from rat brain (22). In this study, we screened a cDNA expression library prepared from rat brain by the same method and isolated cDNA encoding a novel protein, p50α, which appears to be an alternative splicing form of the p85α gene product. Thus, there are five known regulatory subunit isoforms in mammals, including two 85-kDa proteins, two 55-kDa proteins, and one 50-kDa protein, as demonstrated by the results of overexpression experiments using S99 cells (Fig. 1C). In fact, the immunoblot using the antibody that recognizes the entire p85α molecule revealed marked expression of 50–55-kDa proteins in various tissues (Fig. 5A). It appears that these 50–55-kDa proteins have, to date, been regarded as degradation products of p85α or p85β and have thus attracted little attention. In
The 34-amino acid sequence, which shows considerable similarity to the corresponding region of p55, may suggest a specific function of the 34-amino acid portion. p50α contains a unique sequence of only 6 amino acids, apparently too short to associate with other molecules. This would presumably limit the functional capacity of this protein.

Northern blotting using the cDNA probe coding for the N-SH2 domain of p85α revealed four bands (7.7, 6.0, 4.2, and 2.8 kb), as previously reported (22) (Fig. 2B). Among these bands, those of 6.0- and 2.8-kb bands matched those of p50α, whereas the 7.7- and 4.2-kb bands matched those of the SH3 p85α domain. As we reported previously, a minor portion of the 4.2-kb band and a considerable portion of the 2.8-kb band in brain correspond to p55α mRNA. It should be noted that these three regulatory isoforms are not specific to the rat and are also present in the mouse, hamster, and human, based on the results of immunoblotting using specific antibodies (data not shown).

As shown in our previous report (22) and in this study, all regulatory subunit isoforms for PI 3-kinase are abundantly expressed in brain tissue. Although there is one report describing the important role of PI 3-kinase in neuronal differentiation (14), no study has demonstrated a PI 3-kinase function in neuronal cells after differentiation. The yeast homolog of PI 3-kinase, VPS34, is required for trafficking of proteins from the Golgi apparatus to vacuoles (33, 34). In addition, the activation of PI 3-kinase has been implicated in histamine release (35). Thus, PI 3-kinase may play a major role in the secretion of various neurotransmitters. The data on the distribution of each regulatory isoform in rat brain differed among the isoforms, possibly offering clues as to the mechanisms regulating neurotransmitter secretion.

There has been only one report describing a regulatory subunit with a molecular size of approximately 50 kDa, which was recognized by the antibody against the entire p85α molecule (29). In that study, the 46-kDa regulatory subunit formed a heterodimer with a 100-kDa catalytic subunit in rat liver, and this heterodimer could be separated in the flow-through fraction of a DEAE-Sepharose column. Moreover, the 46-/100-kDa heterodimer with a 100-kDa catalytic subunit in rat liver, and the third regulatory isoform in rat brain differed among the isoforms, possibly offering clues as to the mechanisms regulating neurotransmitter secretion.

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size was determined to be approximately 110 kDa. Thus, there is an apparent difference between the two catalytic subunits associated with p50α in the flow-through fraction and that associated with p50α in the 0.1 M KCl fraction. In contrast to p50α, p55α or p85α having PI 3-kinase was detected only in the 0.15 M KCl or 0.2 M KCl fraction from the DEAE-Sepharose column, respectively, and the molecular sizes of their associated catalytic subunits were determined to be 110 kDa by wortmannin labeling and immunoblotting with the antibody against wortmannin. The binding motif of the p85α regulatory subunit with the p110 catalytic subunit was reported to reside in the inter-SH2 domain (18), and this portion of the protein is identical to the reported 46-kDa protein. Although the reason for this high affinity of p50α for IRS-1, despite p50α, p55α, and p85α sharing the same two SH2 domains, is unknown, we speculate that the NH2-terminal domains of p85α and p55α form complexes with other molecules resulting in an inability to bind IRS-1 as efficiently as p50α. Considering both the high level of p50α expression in the liver and its marked responsiveness to insulin, p50α may play a more critical role than the other isoforms in hepatic insulin-induced activation of PI 3-kinase. p55γ-associated PI 3-kinase was shown to be activated by insulin with a low affinity for IRS-1, whereas p85β did not respond significantly to insulin despite its association with IRS-1. Further study is needed to clarify these issues.

In summary, there are five regulatory subunit isoforms of PI 3-kinase which can be classified into three groups, an 85-kDa protein, a 55-kDa protein, and a 50-kDa protein. Each isoform has a different tissue distribution and was shown to exhibit a different level of activation, of the associated PI 3-kinase, in response to insulin stimulation. Given the idea that PI 3-kinase is involved in a series of systems, it is conceivable that PI 3-kinase plays a variety of roles in response to various stimuli. Further study is required to ascertain which isoform corresponds to which biological phenomenon.

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