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Insulin Receptor Substrate-1 (IRS-1) Expression in Rat Brain

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ABSTRACT

IRS-1 is phosphorylated on tyrosine residues after insulin stimulation and participates in the early events of signal transduction in peripheral insulin-sensitive tissues. This study determined whether neuronal populations in the rat olfactory bulb and hippocampus (brain regions which have very high concentrations of insulin receptors) also express IRS-1 and contain phosphotyrosine, using in situ hybridization, receptor binding, and immunocytochemistry. IRS-1 mRNA was colocalized with insulin receptor mRNA in neuron cell bodies of hippocampus and olfactory bulb. Similarly, IRS-1 immunoreactivity in hippocampus and olfactory bulb was concentrated in layers that contain synapses of these neurons and have both high insulin binding and phosphotyrosine levels. Thus, IRS-1 and insulin receptors are coexpressed in discrete populations of neurons, suggesting a signal transduction mechanism by which insulin may influence metabolism and gene expression in the brain.

EVIDENCE is growing that insulin plays a role as an afferent metabolic signal to the central nervous system (CNS). The CNS effects of insulin, such as suppression of feeding behavior and stimulation of sympathetic nervous system activity, appear to involve altered gene expression in discrete populations of neurons (1,2), although the intracellular signaling pathways mediating these actions of insulin are not known. Identification of cells expressing molecules involved in the postreceptor signaling events following insulin binding to neurons is, therefore, of fundamental importance for characterizing the nature of insulin action in the brain and the identification of neurons that participate in insulin's CNS effects. An important recent step was immunocytochemical evidence for the presence of insulin receptor substrate-1 (IRS-1) in brain neurons (3). IRS-1 is a 131 kDa phosphoprotein (4) that is phosphorylated on tyrosine residues immediately after cells are stimulated by insulin or IGF-I (5-7). Phosphorylated IRS-1 functions as a docking protein for phosphatidylinositol 3'-kinase and other proteins that contain SH2 domains and which mediate different cellular actions of insulin (8). Both IRS-1 and IRS-1 mRNA are synthesized in classical insulin-sensitive cells (4-11), and IRS-1 mRNA has been found in extracts of rat brain RNA (6). In the present study, we present the first evidence for expression of IRS-1 mRNA by brain neurons. Furthermore, we show that mRNAs for IRS-1 and the insulin receptor are expressed in the same populations of neurons and that IRS-1 and insulin receptor proteins are distributed in parallel with phosphotyrosine.

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Materials and Methods

For in situ hybridization (ISH), sections (16 μm) of unfixed male Wistar rat brain and liver (obtained under Equithesin anesthesia) on RNase-free slides were fixed in 4% paraformaldehyde. Riboprobes to rat insulin receptor mRNA (bases 1647-2291) were prepared from the RIRp16 template of the α-subunit (12). IRS-1 riboprobes (bases 1261-1821) were transcribed from the cDNA template for the c16 clone of rat liver IRS-1 (4). Template DNAs were linearized using 40 U SauI (RIRp16) or ApaI (IRS-1) (Boehringer-Mannheim). Riboprobes were prepared with ^{33}P-UTP (Amersham), using T3 (RIR riboprobe) or T7 (IRS-1 riboprobe) RNA polymerase (Promega). Hybridizations were also done with ^{35}S-UTP. Following treatment with yeast tRNA, sections were hybridized overnight at 60°C. Hybridization and washes were carried out under high-stringency conditions since Tm analysis indicated approximately 10% mismatch, which may have been due to formation of some hybrids with IGF-I receptor mRNA. Controls were treated with RNase. For immunocytochemistry, cryostat sections (16 μm) of brains perfused with 4% paraformaldehyde were incubated in antiserum to IRS-1 (13) or phosphotyrosine (14) (1:100-1000 in PBS with 1% BSA and 0.05% Triton X-100), followed by ABC peroxidase reagents (3). Normal rabbit serum controlled for nonspecific staining. For receptor autoradiography, slide-mounted, cryostat brain sections were used for ^{125}I-insulin binding autoradiography as described previously (15).

Results

In the olfactory bulb, ISH to IRS-1 mRNA produced dense images corresponding to the granule cell body layer, mitral cell body layer, and the glomerular layer, which have closely packed neuronal cell bodies (Fig. 1). The pattern of ISH to RIR mRNA was identical to that seen

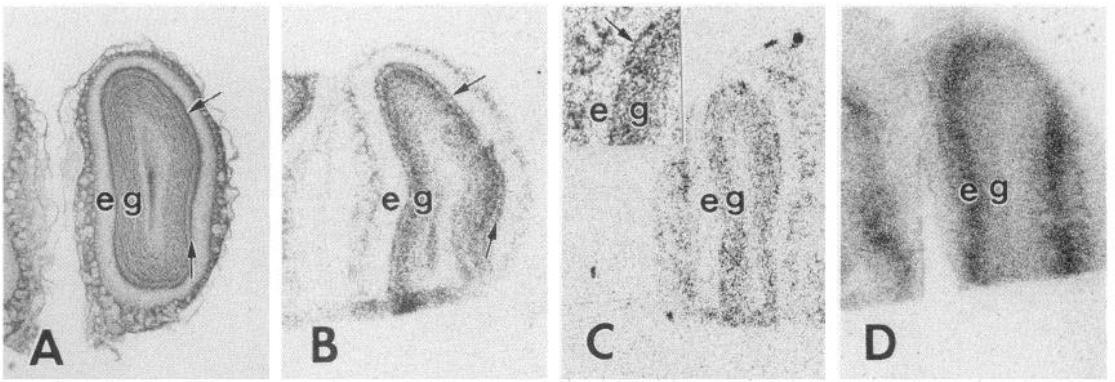

Fig. 1. Olfactory bulb. A. Cresyl violet stain, showing external plexiform layer (e), (mostly synapses), and granule cell body layer (g). Arrows indicate mitral cell body layer. B. ISH (^{33}P) of insulin receptor mRNA in granule cell bodies (g) and mitral cell body layer (arrows) but not in external plexiform layer (e). C. ISH (^{33}P) of IRS-1 mRNA shows same localization as insulin receptor mRNA, including mitral cell body layer (arrow in inset). Glomerular layer (left of the external plexiform layer) also has insulin receptor and IRS 1 mRNA. D. Receptor autoradiography, showing high levels of insulin binding sites in the external plexiform layer (e). 20x

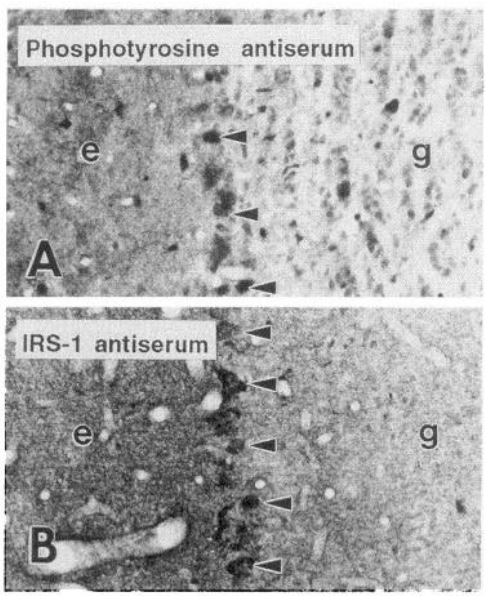

Fig. 2. Immunocytochemical detection of phosphotyrosine and IRS-1 in olfactory bulb. Highest levels of both phosphotyrosine immunoreactivity (A) and IRS-1 immunoreactivity (B) are in external plexiform layer (e), the location of most insulin receptors. Mitral cell bodies, whose dendrites branch profusely in the external plexiform layer, show immunoreactivity to both antisera. The granule cell body layer (g), which has fewer insulin receptors, also has less immunoreactive phosphotyrosine and IRS-1. 100x

with the IRS-1 riboprobe. In the synaptic neuropil of the external plexiform layer, ISH to both IRS-1 and RIR mRNA was much weaker than in layers containing cell bodies. In contrast, insulin binding to the olfactory bulb was greatest in the external plexiform layer and much weaker in the cell body layers that contained the insulin receptor mRNA and IRS-1 mRNA. Likewise, immunocytochemistry revealed that the highest immunoreactivity for both IRS-1 and phosphotyrosine was present in the external plexiform layer (as well as mitral cell bodies, which project dendrites into the external plexiform layer), whereas cell bodies of the granule cell layers had less staining (Fig. 2).

In the hippocampus, dense IRS-1 mRNA expression was present in the granule cell body layer of the dentate gyrus (Fig. 3). Lesser but definitely detectable amounts of IRS-1 mRNA were found in pyramidal cell bodies of Ammon's horn. Similar to the results in the olfactory bulb, layers of the hippocampus that are primarily synaptic, such as the stratum radiatum and stratum oriens as well as the molecular layer of the dentate gyrus, had much lower levels of IRS-1 mRNA. IRS-1 mRNA expression was also low in the dentate gyrus hilus, which shows very low insulin binding. The pattern of insulin receptor mRNA expression was identical to that of IRS-1, with highest concentrations found in the layer of dentate gyrus granule cell bodies and moderate amounts in CA1 and CA3 pyramidal cell bodies. In contrast, highest levels of insulin binding by receptor autoradiography were observed in layers containing high densities of synapses: the molecular layer of the dentate gyrus and stratum oriens and stratum radiatum of Ammon's horn. Greatest immunostaining for both IRS-1 and phosphotyrosine was

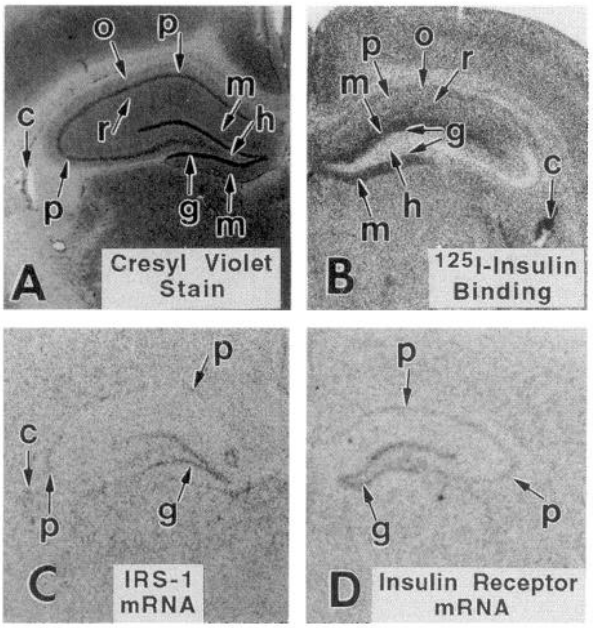

Fig. 3. Hippocampus. A. Cresyl violet, showing layers with cell bodies of pyramidal neurons (p) and dentate gyrus granule cells (g). Synapses are mostly in s. oriens (o) and s. radiatum (r) of Ammon's horn, and molecular layer (m) and hilus (h) of the dentate gyrus. Choroid plexus (c) of a lateral ventricle also is shown. B. Iodoinsulin binding sites are concentrated in s. oriens (o), s. radiatum (r) and dentate gyrus molecular layer (m). Hilus (h), pyramidal neurons (p), and dentate gyrus granule cells (g) show low insulin binding (arrows). Choroid plexus (c) shows high insulin binding. C. ISH for IRS-1 mRNA (^{35}S) in dentate gyrus granule cell bodies (g), pyramidal cells (p), and choroid plexus (c). D. ISH of insulin receptor mRNA (^{35}S) shows same localization as IRS-1 mRNA. 10X

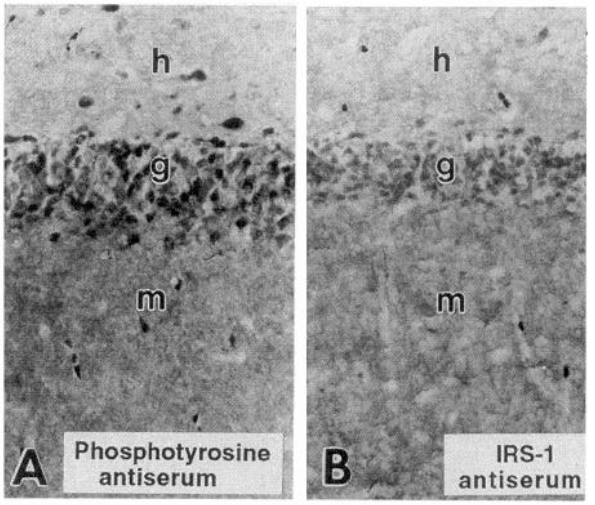

Fig. 4. Immunocytochemical detection of phosphotyrosine and IRS-1 in dentate gyrus. Highest levels of both phosphotyrosine immunoreactivity (A) and IRS-1 immunoreactivity (B) are in molecular layer (m), the location of most insulin receptors. Granule cell bodies (g), whose dendrites branch profusely in the molecular layer, also show immunoreactivity with both antisera. The hilus (h), which has fewer insulin receptors, also has less immunoreactive phosphotyrosine and IRS 1. 100x

in the molecular layer of the dentate gyrus (Fig. 4). Thus, insulin receptor and IRS-1 mRNAs were expressed primarily in hippocampal cell bodies, while the insulin receptor and IRS-1 proteins, as well as phosphotyrosines, were concentrated in the areas containing the dendrites and synapses of these neurons.

ISH on slices of rat liver and brain produced dense autoradiographic images with both IRS-1 and insulin receptor riboprobes, and was prevented by RNase treatment prior to hybridization.

Discussion

An unanswered question in the neurobiology of insulin action is the nature of signaling molecules downstream from the insulin receptor. To address this problem, we used ISH for detecting mRNAs encoding rat IRS-1 and insulin receptor in order to determine if cells that express these functionally related proteins are colocalized in the same regions of the brain. The olfactory bulb and hippocampus were chosen because they express high concentrations of insulin receptors and insulin receptor mRNA (16) as well as immunoreactive phosphotyrosine (17) in anatomically discrete layers.

The results showed that the anatomical location as well as relative intensity of expression of IRS-1 mRNA matched that of insulin receptor mRNA in hippocampus and olfactory bulb. Furthermore, IRS-1 and insulin receptor mRNAs were expressed primarily in layers of neuronal cell bodies. This pattern of IRS-1 and insulin receptor mRNA expression is compatible with the coexpression of the IRS-1 and insulin receptor genes by the same populations of neurons in both regions.

In contrast, both insulin receptor and IRS-1 proteins are concentrated in layers of synaptic neuropile where cell bodies are scarce. The differential anatomical distribution of these mRNAs and proteins suggests that the genes encoding IRS-1 and insulin receptor are transcribed in neuronal perikarya and their cognate proteins are subsequently transported to the synaptic regions of the neurons. It is significant that phosphotyrosine immunoreactivity is also concentrated in these same synaptic layers. The common localization of IRS-1 and phosphotyrosine immunostaining in these regions raises the possibility that IRS-1 becomes phosphorylated on tyrosine residues when insulin binds to its receptor on neurons, as occurs in

peripheral insulin-sensitive cells (8). Further work is necessary, however, to characterize the phosphotyrosines observed in this study and their relationship to IRS-1 phosphorylation which might occur following binding of insulin to neurons in vivo. Since IGF-I receptors are also expressed by the same neuronal populations (16,18), it is possible that some of the hybridization was to IGF-I mRNA. Furthermore, IRS-1 is also phosphorylated by the IGF-I receptor, so phosphotyrosine associated with IRS-1 could be related to the action of IGF-I as well as insulin.

The colocalization of insulin receptors, IRS-1, and phosphotyrosine in synaptic layers of the hippocampus and olfactory bulbs suggests that insulin action in these regions may involve signal transduction pathways similar to those found in peripheral insulin-sensitive cells. IRS-1, insulin receptors and phosphotyrosine have also been found in the hypothalamic arcuate nucleus (3), where neuropeptide Y gene expression is inhibited by insulin (1). Moreover, insulin inhibits expression of mRNA encoding the norepinephrine transporter in the locus coeruleus (2). Further study is required to determine if insulin regulation of neuropeptide Y or norepinephrine transporter gene expression involves IRS-1, and whether insulin action in the hippocampus and olfactory bulbs also involves gene transcription. However, the present results are a significant step towards future investigations aimed at the function of insulin in the brain as well as possible brain dysfunction resulting from insulin deficiency or excess in diabetes and obesity.

The similar locations of brain phosphotyrosine, insulin binding, and IRS-1 suggests that phosphorylation of IRS-1 is important in insulin-sensitive neurons. In view of the current hypothesis that IRS-1 is a molecular link between the insulin receptor tyrosine kinase and downstream signal transduction molecules in peripheral insulin-responsive cells, our results suggest that analogous signaling pathways may exist within neurons of the brain.

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