

Invited Review

Insulin receptors and signal transduction proteins in the hypothalamo-hypophyseal system: a review on morphological findings and functional implications

J.W. Unger and M. Betz

Department of Anatomy, University of Munich, Munich, Germany

Summary. Receptors for insulin are widely distributed in the brain and pituitary. The current hypothesis on receptor function in these regions points to a role of insulin as a mediator in the communication of the peripheral endocrine system with the brain via various steps of the neuroendocrine axis. Recent data demonstrate that receptor-positive neurons in the brain, i.e. in the hypothalamus, and secretory cells in the anterior pituitary gland possess specific proteins that are thought to be involved in key steps of post receptor signal transduction, in particular insulin receptor substrate-1 and phosphatidylinositol 3'-kinase (PI3k). PI3k is a critical enzyme of the intracellular signaling pathway that is activated by a number of receptor tyrosine kinases, including receptors for insulin and IGF-1. This information further completes the framework indicating *in vivo* activity of insulin receptors in central neuroendocrine cells and their involvement in one branch of several physiological mechanisms that control body metabolism and nutritional behaviour.

Key words: Insulin receptor, Insulin receptor substrate-1, Phosphatidylinositol 3'-kinase, Hypothalamus, Pituitary gland

Introduction

Receptors for insulin and the closely related insulin-like growth factor-1 are found in numerous cells and tissues of mammals. Because of this close relationship, the two hormones can act on each other's receptor, although in a dose-dependent manner (Ullrich et al., 1985, 1986). In this review we will focus predominantly on insulin receptors. However, it appears necessary to compare the cellular presence of the two receptors and correlate their occurrence with biological functions. In

addition, both are receptor tyrosine kinases that use the same protein substrates in key steps of receptor signal transduction pathways, as demonstrated by *in vitro* studies (Shemer et al., 1987; McElduff et al., 1988). This raises the question of receptor signal specificity in an intact organism. In order to address this issue, the identification of post-receptor substrates at the cellular level provides an important insight into the *in vivo* situation of a biological system, such as the brain and pituitary gland.

Insulin and the brain

The source of insulin, the B-cells of the islets of Langerhans, release the hormone to the well-known target tissues, primarily adipose tissue, liver and muscle (Fig. 1). The brain has been regarded for a long time to be insulin-independent, a dogma which is still true concerning the neuronal glucose metabolism in most parts of the central nervous system (Crone, 1965; Hom et al., 1984; Grunstein et al., 1985). Nevertheless, insulin receptors are present in abundance in a variety of specific brain regions, in particular in areas involved in the regulation of central autonomic activity. Earlier binding assays and autoradiographic investigations as well as more detailed histological mapping studies, carried out with immunocytochemistry using specific receptor antibodies, demonstrated dense receptor staining in neurons in many brain areas including limbic-hypothalamic nuclei (example, see Fig. 5a), such as the arcuate and paraventricular nuclei, amygdala, hippocampus and autonomic brainstem areas, i.e. nucleus of the solitary tract (Baskin et al., 1987; Werther et al., 1987; Unger et al., 1991). These findings, in conjunction with experimental data from animal studies have indicated an endocrine role of insulin in the adult central nervous system (Schwartz et al., 1992a,b).

Insulin entry across the blood-brain-barrier

There are two potential ways for insulin penetration across the blood-brain-barrier (for further review see:

Offprint requests to: Dr. med. Juergen W. Unger, Department of Neuroanatomy, University of Munich, Pettenkoferstrasse 11, D-80336 Munich, Germany. Fax: (49) 89 5160-4857. e-mail: unger@anat.med.uni-muenchen.de

Plata-Salamán, 1990; Schwartz et al., 1992a):

1) Binding studies and kinetic analysis have indicated a saturable receptor-mediated transport across the blood-brain-barrier by transcytosis through the capillary endothelium into the extracellular compartment of the brain (King and Johnson, 1985; Pardridge, 1986). It is also thought that the insulin content within the brain parenchyma may undergo modification by clearance of the hormone from cerebrospinal fluid via the choroid plexus (Manin et al., 1990).

2) Receptor-mediated uptake by axon terminals in circumventricular organs and axonal transport to specific target neurons, i.e. arcuate nucleus of the hypothalamus (Van Houten et al., 1979, 1983). For the latter, an

important morphological correlate for immediate insulin uptake from the peripheral blood may be the presence of insulin receptors in nerve terminals in the external zone of the median eminence (Unger et al., 1993; Fig. 5a). Similarly, other members of the circumventricular organs, i.e. area postrema, subfornical and sub-commisural organ, also contain dense accumulations of insulin receptors (Van Houten et al., 1979; Unger et al., 1989).

Insulin and IGF-1 receptors in the anterior pituitary

Since some of the important relay nuclei of the hypothalamo-hypophyseal system are obviously under influence of peripheral insulin, it has been of interest to expand the "neuroendocrine aspects" of insulin from the

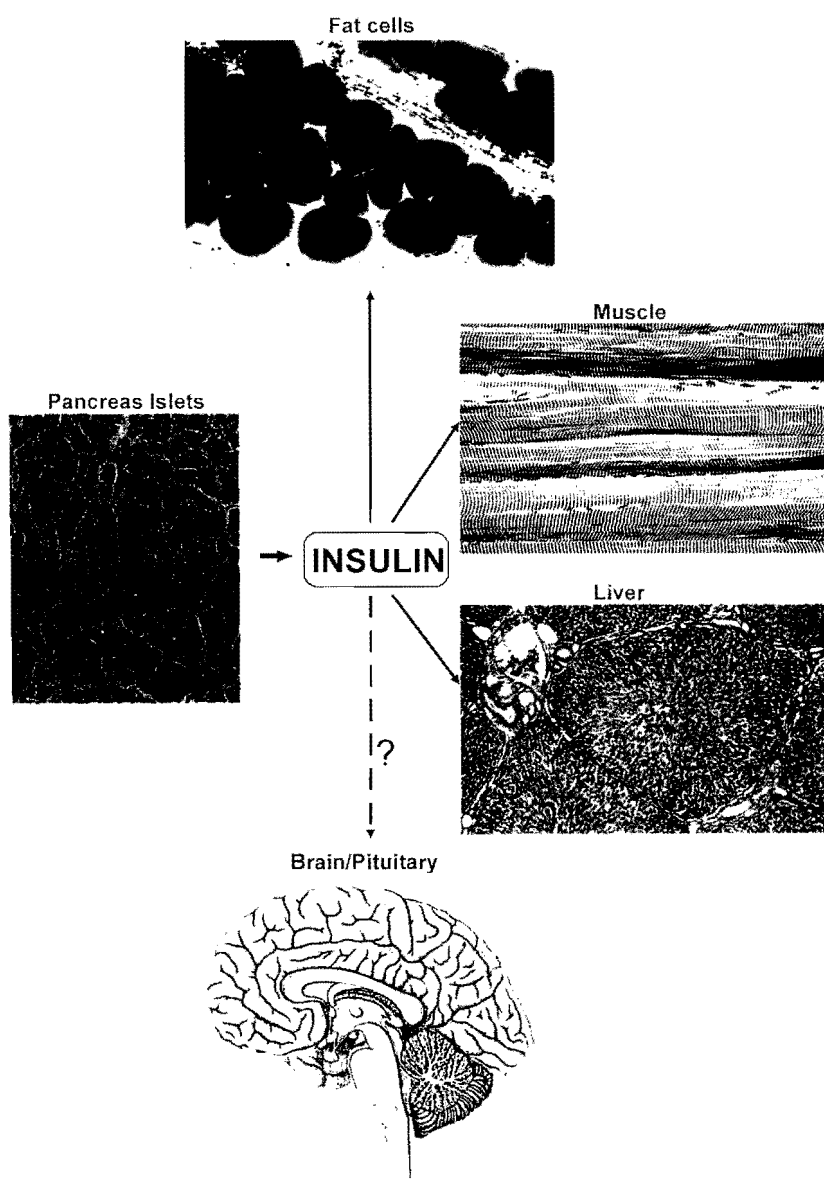


Fig. 1. Schematic overview about the source and most important target regions of insulin, illustrated by histological sections. The role of insulin in the brain and pituitary still contains numerous questions (see text).

brain to the pituitary gland, especially the adenohypophysis, as the next lower important level of neuroendocrine regulation. A few studies have tested ligand binding of insulin and IGF-1 in the rat and human pituitary and they found significant amounts of binding activity (Werther et al., 1987, 1989). Immunocytochemistry revealed that the two receptors are located on separate subpopulations of secretory cells of the pars distalis of the adenohypophysis (Fig. 3a,b). Interestingly, almost 90% of cells containing insulin receptors are also immunoreactive for beta-endorphin (Unger and Lange, 1997). Given the fact that opioid peptides play a

profound role in regulating autonomic functions, including food intake (Johnson, 1995), this finding is an additional support for the interaction of peripheral insulin and central hormones.

In contrast, IGF-1 receptors are almost exclusively located on FSH-secreting cells (Unger and Lange, 1997). It is, however, not surprising, that the two receptors are expressed on different hypophyseal cell populations, because the functional roles of insulin and IGF-1 in the brain and pituitary are most likely to be quite distinct. Thus, the localization of either receptor in the pituitary may reflect its specific function in the organization of the hypothalamo-hypophyseal system. Beside the classic negative feedback control of growth hormone release by IGF-1 in the hypothalamo-hypophyseal system, recent investigations have suggested that IGF-1 is also involved in the regulation of gonadotropins from anterior pituitary cells, i.e. potentiation of the secretory response to gonadotropin-releasing hormone *in vitro* (Kanematsu et al., 1991; Soldani et al., 1994). In this context, it is also of interest that the IGF-1 system in the rat anterior pituitary lobe is dependent on the estrous cycle, i.e. increase in receptor binding and in the presence of IGF-1 binding proteins under elevated circulating estrogen (Michels et al., 1993).

Proceeding from the correlation of receptor localization in neuroendocrine centers with known biological roles, a further step in understanding their functional activity is the evaluation of markers for post-receptor signal transduction at the cellular level.

Receptor signal transduction (see also Fig. 2)

The receptors for insulin and IGF-1 are transmembrane, heterotetrameric glycoprotein structures that possess ligand binding sites in the extracellular α -subunits ($M_r=135.000$) and tyrosine kinase activity in the cytoplasmic part of the β -subunits ($M_r=95.000$). The subunits are linked by disulfide bonds to provide a $\alpha_2\beta_2$ complex (for review see: Häring, 1991; Lee and Pilch, 1994; Folli et al., 1996). Activation of the tyrosine kinase after insulin binding is a key event in the signal transduction pathway, leading to autophosphorylation of the receptor (White and Kahn, 1989). It is thought that the kinase activity depends on complete phosphorylation of different "tyrosine-clusters" of the β -subunit (Lee and Pilch, 1994). Following activation, the receptor tyrosine kinase phosphorylates major cytosolic substrate proteins, called insulin receptor substrate-1 and -2 (IRS-1, -2; Rothenberg et al., 1991; Sun et al., 1991; Myers et al., 1994). The tyrosine-phosphorylated forms of these adaptor proteins present "docking sites" for other proteins containing src homology 2 (SH2) domains. In this way, other effector proteins are indirectly linked to the signal transduction cascade (Kuhné et al., 1993). Further signal transmission branches into different pathways involving a number of tyrosine-phosphorylated proteins. It is noteworthy that this initial signalling mechanism differs from other tyrosine kinase receptors,

Insulin and IGF-1 Receptor Signal Transduction

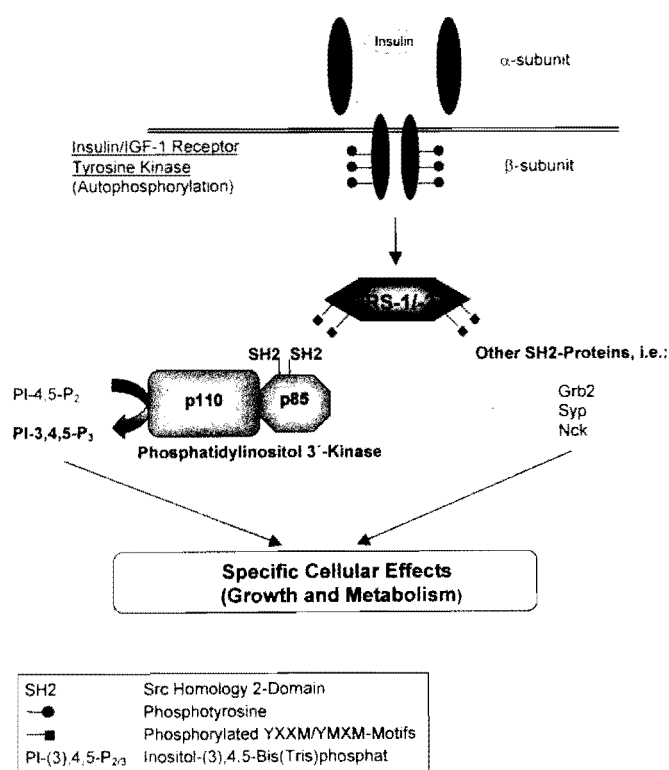


Fig. 2. Model of insulin and IGF-1 receptor signal transduction via tyrosine kinase activity. Insulin or IGF-1 binding to the extracellular α -subunit of the respective receptor initiates intramolecular changes with autophosphorylation of tyrosine residues in the β -subunit by the receptor-associated tyrosine kinase which is followed by tyrosine phosphorylation of YXXM/YMXM motifs in the cytosolic protein insulin receptor substrate-1 (IRS-1). IRS-1 itself serves as a "docking protein" that transmits the signal to further intracellular src homology2 (SH2)-domain containing proteins by generating non-covalent bindings between phosphorylated YXXM/YMXM motifs and the SH2 domains. One of the best characterized SH2-proteins is the regulatory p85-subunit of phosphatidylinositol 3-kinase (PI3-K) which phosphorylates inositol-4,5-phosphate at the 3-position of the inositol ring. IRS-1 also binds to other growth factor-stimulated proteins, i.e. Grb-2 (a protein involved in activation of the MAP-kinase pathway and GDP-release), the tyrosinophosphatase Syp or the adapter protein Nck. Activation and cooperation of this cascade of intracellular proteins leads to the specific cellular effects as consequence from growth factor binding (i.e. gene expression, metabolism, growth).

i.e. receptors for epidermal or fibroblast growth factor, EGF or FGF, respectively, which associate directly with SH2 domains containing substrates after ligand binding and receptor phosphorylation (Schlessinger and Ullrich, 1992; Myers et al., 1994; White and Kahn, 1994).

Insulin receptor substrate-1 (IRS-1) and Phosphatidylinositol 3'-kinase (PI3-k)

A relatively early substrate for insulin receptor tyrosine kinase and one of the best investigated proteins

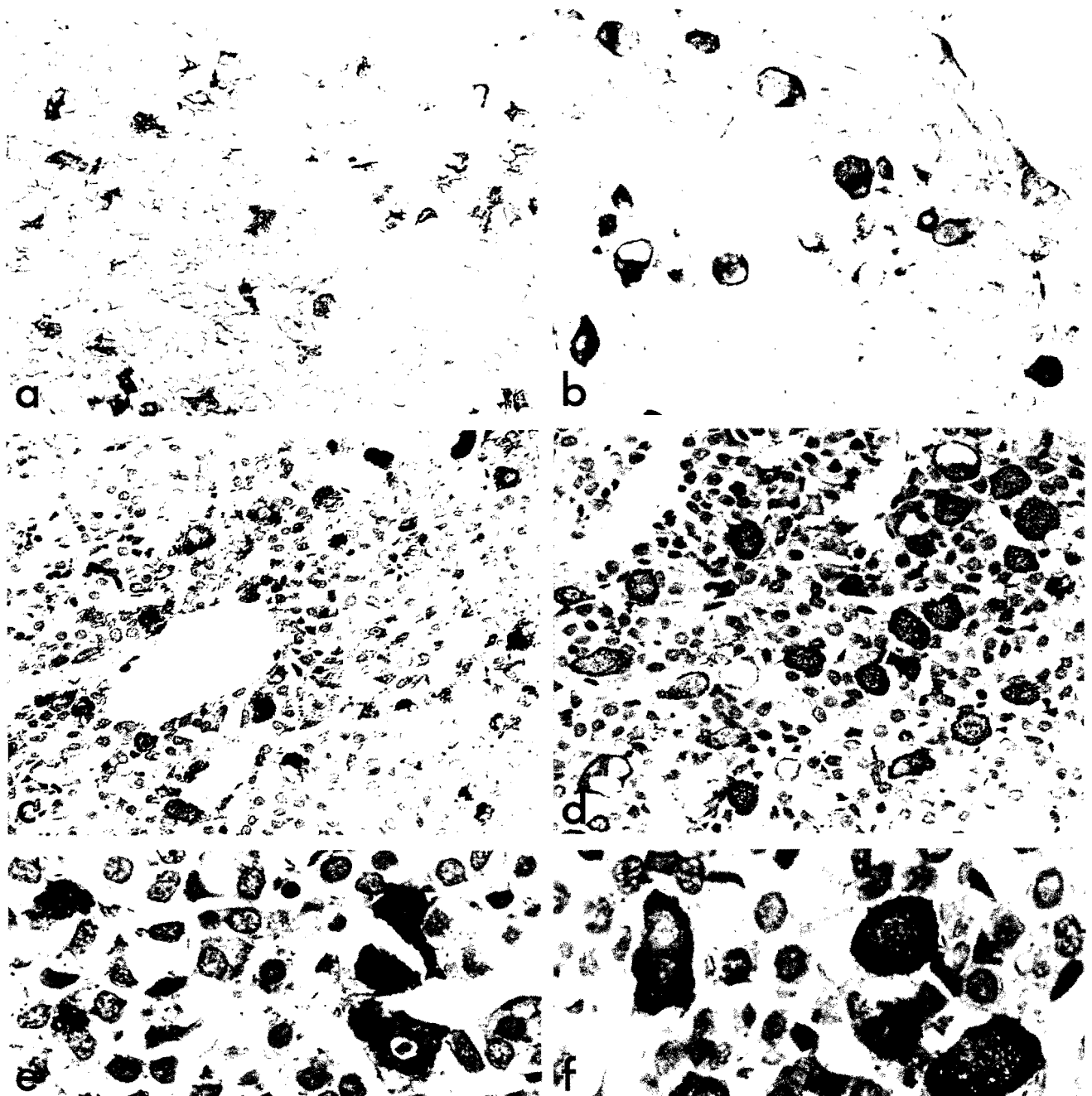


Fig. 3. Histological demonstration of insulin and IGF-1 receptors, IRS-1 and PI3-k in the pars distalis of the pituitary gland. **a.** Insulin receptors. **b.** IGF-1 receptors. Note, that both receptors are located on different subpopulations of secretory cells. **c and e.** PI3-k. **d and f.** IRS-1. The two markers for receptor signal transduction are located in numerous secretory cells that are morphologically similar to the insulin and IGF-1 receptor-positive populations. Higher magnifications (**e, f**) demonstrate that the reaction product is located in the cytosol, where PI3-k immunoreactivity forms distinct clusters (**e**) whereas reaction product representing IRS-1 is found as smaller granules throughout the cytosol with a higher density along the cell surface (**f**). Nuclei as well as large vacuoles are free of immunoreaction. **a-d,** x 400; **e, f,** x 925

is the phosphoprotein insulin receptor substrate-1 (IRS-1). This protein was originally identified in 1985 by White et al. who were able to demonstrate insulin-induced phosphorylation of different tyrosine residues in a 185kDa phosphoprotein, later characterized as IRS-1. Recent studies have confirmed that upon ligand binding IRS-1 is phosphorylated by the insulin or IGF-1 receptor tyrosine kinase (Shemer et al., 1987; Shoelson et al., 1992) and have also shown its central functional importance along the intracellular insulin signalling pathway (Waters et al., 1993; Keller and Lienhard, 1994). The gene for IRS-1 in human cells is located on chromosome 2q35-36 (Nishiyama et al., 1993; Stoffel et al., 1993) and the gene product is expressed in a variety of insulin-sensitive tissues (Rothenberg et al., 1991). Further investigations were able to show that the intensity of activation of IRS-1 depends on the number of phosphorylated tyrosine residues in the protein (Sun et al., 1991, 1993; Keller and Lienhard, 1994; Rordorf-Nikolic et al., 1995). Furthermore, in phosphorylated state YXXM/YMXM-aminoacid sequences of IRS-1 are important for signal transduction, since they are recognition motifs for non-covalent binding to other signal proteins with SH2-containing domains (Shoelson et al., 1992; Rordorf-Nikolic et al., 1995). A number of data suggest that SH2 and SH3 domains participate in the control of intracellular responses to growth factor stimulation (Koch et al., 1991; Lavan et al., 1992; Pawson and Gish, 1992; Chuang et al., 1994). One of these SH2-containing substrates, the p85 α subunit of

phosphatidylinositol 3'-kinase (PI3-k) is activated by docking to IRS-1 (Fig. 2). Molecular interaction between PI3-k and IRS-1 occurs between the phosphorylated YMXM/YXXM-sequences of IRS-1 and p85 α of PI3-k (Folli et al., 1992; Hadari et al., 1992; Lamphere and Lienhard, 1992; Waters et al., 1993; Lamphere et al., 1994; Rordorf-Nikolic et al., 1995; Waters and Pessin, 1996).

Phosphatidylinositol-3' kinase is a cytosolic enzyme that phosphorylates phospho-inositides at the D3-position of the inositol-ring. The enzyme complex forms a heterodimer: a 110kDa protein represents the catalytic subunit, and a 85kDa protein serves as regulatory subunit. Two distinct isoforms of p85 are known: p85 α and p85 β . PI3-k p85 α binds to the catalytic subunit and serves as a link between the enzyme and the ligand activated-receptor tyrosine kinases. p85 α itself contains two SH2 domains which are important for further signal transmission, i.e. regulation of enzyme activity (Carpenter, 1990; Carpenter et al., 1993).

In addition to PI3-k, IRS-1 serves as a docking protein for several other intracellular substrates, such as Grb2 (Tobe et al., 1995; Zhang-Sun et al., 1996), the phosphatase Syp (Kuhné et al., 1993), serine kinases, G-proteins or phospholipases (for further review see: Häring, 1991). In turn, activation of PI3-k also occurs through other activated growth factor receptors, i.e. colony-stimulating factor 1, c-kit, β -PDGF (Downing et al., 1989; Rottapel et al., 1991). However, the precise cellular steps for receptor-specific signal transductions

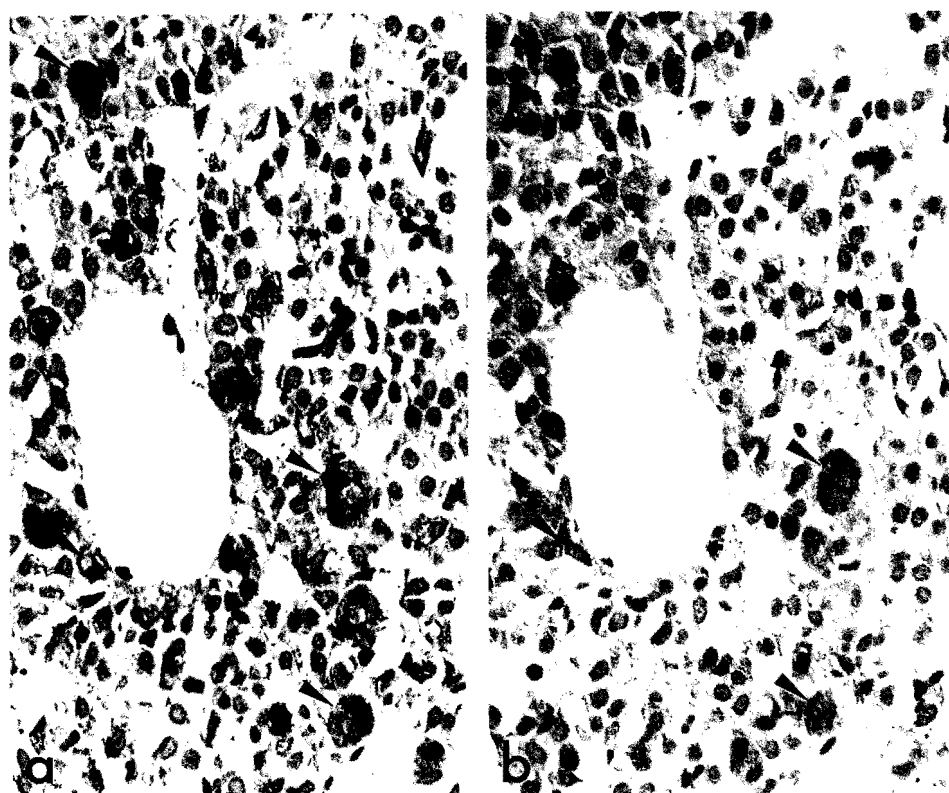


Fig. 4. Colocalization of PI3-k (a) and IRS-1 (b) on consecutive 1 μ m-thick paraffin sections through the pars distalis. Arrowheads point to examples. x 400

are only incompletely known. In general, it is thought that the combination and temporal activation of these intracellular pathways - rather than the occurrence of one or a few "unique" receptor substrates - ultimately defines signal specificity and leads to specific biological effects, i.e. cell growth during development, regeneration or cellular metabolic change in the adult as a response to environmental conditions (Häring, 1991; Myers et al., 1994; Folli et al., 1996; Waters and Pessin, 1996).

Histological demonstration of signal transduction proteins in brain and pituitary

Originally, it was very helpful to use phosphotyrosine (PY) as a general marker for substrates of

tyrosine kinases to identify kinase activity at the cellular level. In these earlier studies, PY-immunoreactivity was detected in numerous neurons throughout the brain and in secretory cells of the anterior and intermediate lobe of the pituitary gland, including the cell populations containing insulin or IGF-1 receptors (Moss et al., 1990; Unger and Lange, 1997). Subsequently, these findings could be refined by the identification of IRS-1, which was also found in numerous neurons, predominantly in hypo-thalamic nuclei that also contain dense accumulations of insulin receptors (Baskin et al., 1993; Folli et al., 1994). Most recently, similar findings were made for IRS-1 in a distinct population of secretory cells in the anterior pituitary (Fig. 3d,f). Furthermore, PI3-k, the post-kinase signal transducer that is activated by

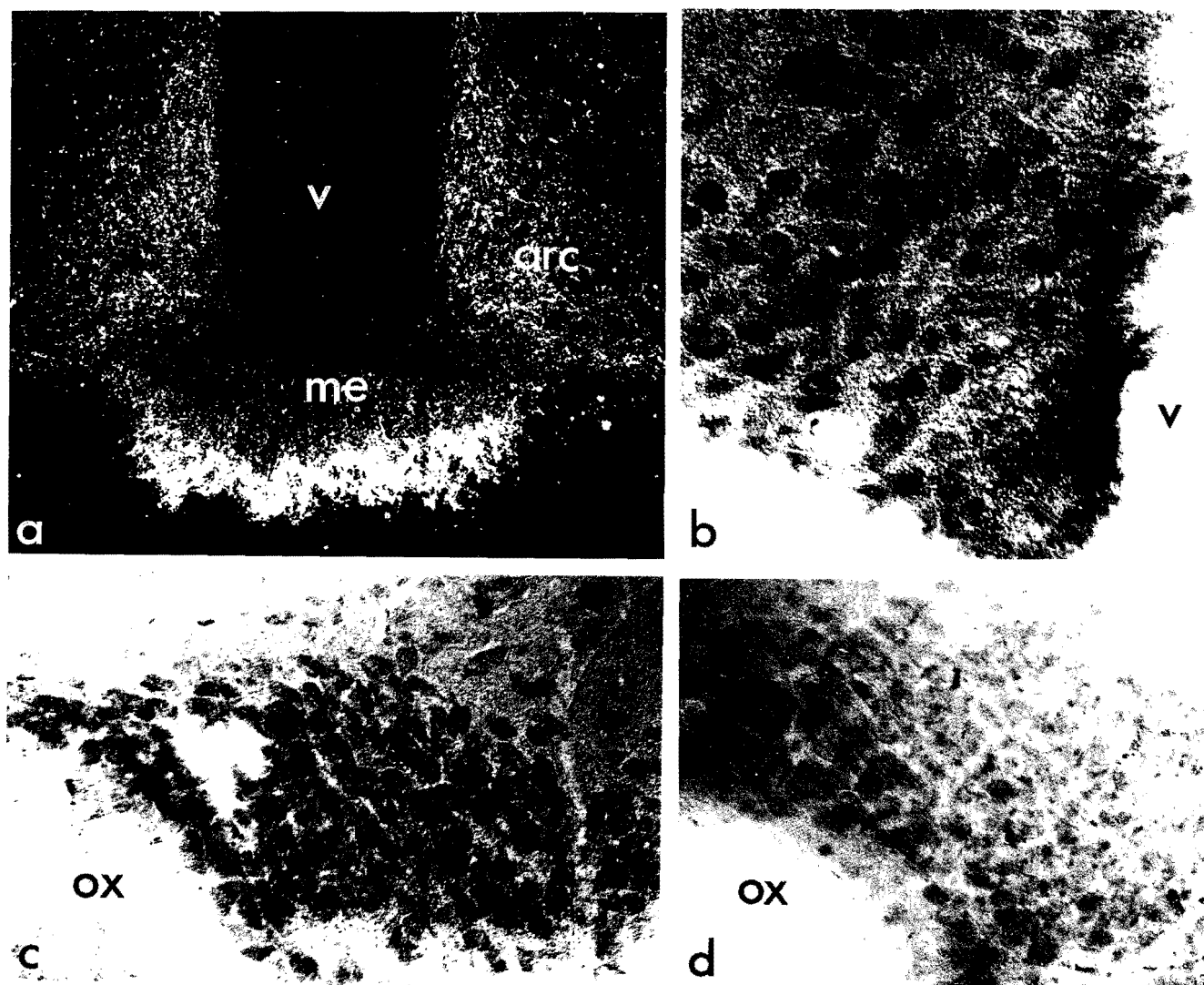


Fig. 5. Representative examples, illustrating the localization of insulin receptors and PI3-k in the hypothalamus of adult rats. **a.** Dense accumulation of insulin receptors is found in the external zone of the median eminence (me) and in the arcuate nucleus (arc) extending to the periventricular regions (dark field photomicrograph). **b and c.** Strong labelling of neurons for PI3-k immunoreactivity is found in the arcuate nucleus (b) and in the magnocellular supraoptic nucleus (c). **d.** Neighbouring section to c, depicting a negative control with absence of specific staining. ox: optic chiasm; v: 3rd ventricle. a, x 120; b-d, x 500

IRS-1 was also detected in brain and pituitary. Specifically, PI3-k is present in hypothalamic areas and in secretory cells of the adenohypophysis rich in insulin receptors and IRS-1, respectively (Figs. 3c,e 4a,b, 5b,c). Specificity of immunostaining for both signal transduction markers IRS-1 and PI3-k was demonstrated by blockage experiments after preincubation of the antibodies with a cell lysate from either 3T3 cells or from a human epidermoid carcinoma cell line that contained high amounts of IRS-1 or PI3-kinase, respectively. An example of a negative control is shown in Fig. 5d. The data strongly suggest, that specific neurons and neurosecretory cells possess the machinery that is necessary for tuning receptor signalling.

Functional conclusions

Previous investigations have given strong evidence that interaction of peripheral insulin with neuroendocrine centers may play a key role in the regulation of body energy metabolism, i.e. feeding (Woods et al., 1985). The idea that body weight is regulated around a given setpoint and that the brain may be a sensor for overall body energy balance was developed decades ago. For example, as early as 1953 G.C. Kennedy presented a paper at the Royal Society of London with the title "The role of depot fat in the hypothalamic control of food intake in the rat" (Kennedy, 1953). Despite these early thoughts on regulation of body metabolism, the identification of the various levels of interaction are still a matter of intense research.

It must be emphasized that the hormonal regulatory system of feeding behaviour and body fat masses consists of a number of mechanisms, both in the periphery as well as in neuroendocrine and autonomic brain centers. For example, peripheral changes in blood glucose directly result in an acute increase or decrease in appetite. Furthermore, feedback hormones like the recently discovered protein leptin which is released from adipocytes have been shown to interact directly with the hypothalamus in controlling body fat mass (Elmquist et al., 1997). Neuropeptides that are synthesized in the brain, in particular in hypothalamic and central autonomic nuclei, such as neuropeptide Y (NPY) - which is one of the most powerful central stimulants for carbohydrate intake - or others like galanin or opioid peptides, are equally important in the complex regulatory system of metabolism (Stanley et al., 1986; Leibowitz, 1992; Schick et al., 1993; Johnson, 1995).

In this complex monitoring system, insulin may be regarded as one branch of several integrative control mechanisms. However, it fulfills a number of important prerequisites for being a good candidate as a mediator in the communication between the periphery and central neuroendocrine cell systems in the long-term control of body metabolism and weight; first of all, the presence of specific receptors in target areas of the brain considered to be involved in metabolic regulation. In addition, similar to the recently described leptin, insulin is one of

the few peptide hormones that is most likely not synthesized to a significant amount in the brain (Young, 1986). Therefore, insulin uptake from the peripheral blood into strategic neuroendocrine centers represents one way for controlling long-term changes in body mass via subtle changes in insulin plasma levels. As indicated by several studies, one mechanism of insulin's long-term ability to reduce food intake in this monitoring system is the inhibition of NPY-release from the hypothalamic arcuate-paraventricular pathway (Schwartz et al., 1992b; White, 1993; Cusin et al., 1995). These data have been corroborated by the fact that NPY is upregulated in a state of insulin deficiency, i.e. streptozocin-induced diabetes mellitus (Williams et al., 1989).

Among other neuropeptides, opioid peptides (endorphins and enkephalins) have been shown to play important roles in the complex system of monitoring energy homeostasis, for example regulation of fat and protein balance (Johnson, 1995). There is also evidence that increase of food intake induced by opioid peptides may be caused - at least in part - by interaction with the hypothalamic NPYergic arcuate-paraventricular connection (Kotz et al., 1995) which is known to be regulated by peripheral insulin (Schwartz et al., 1992b). In turn, NPY inhibits release of pro-opiomelanocortin-derived peptide and reduces their mRNA in the intermediate and anterior lobe of the pituitary (Blasquez et al., 1995). Concluding from the morphological data, there is a possibility for a direct interaction between neuropeptides (i.e. B-END, NPY) and insulin at the level of the hypothalamo-hypophyseal system. The data collected on the receptor signal transduction cascade further support this hypothesis, since the findings strongly indicate the *in vivo* ability of cells in the anterior pituitary to transduce biological signals via tyrosine kinase activity originating at least in part from receptors for insulin and IGF-1. Although the current information indicates relatively clearly the way of interaction of peripheral insulin with various steps of the brain-pituitary system, we are still far from understanding the precise biological role, in particular the "cross-talk" of various hormones in the complex regulation of central autonomic activity during health and disease, i.e. endocrine dysregulation.

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