Histol Histopathol (1998) 13: 1215-1224

http://www.ehu.es/histol-histopathol

Histology and Histopathology

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.

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

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:

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 underly 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 subcommisural 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

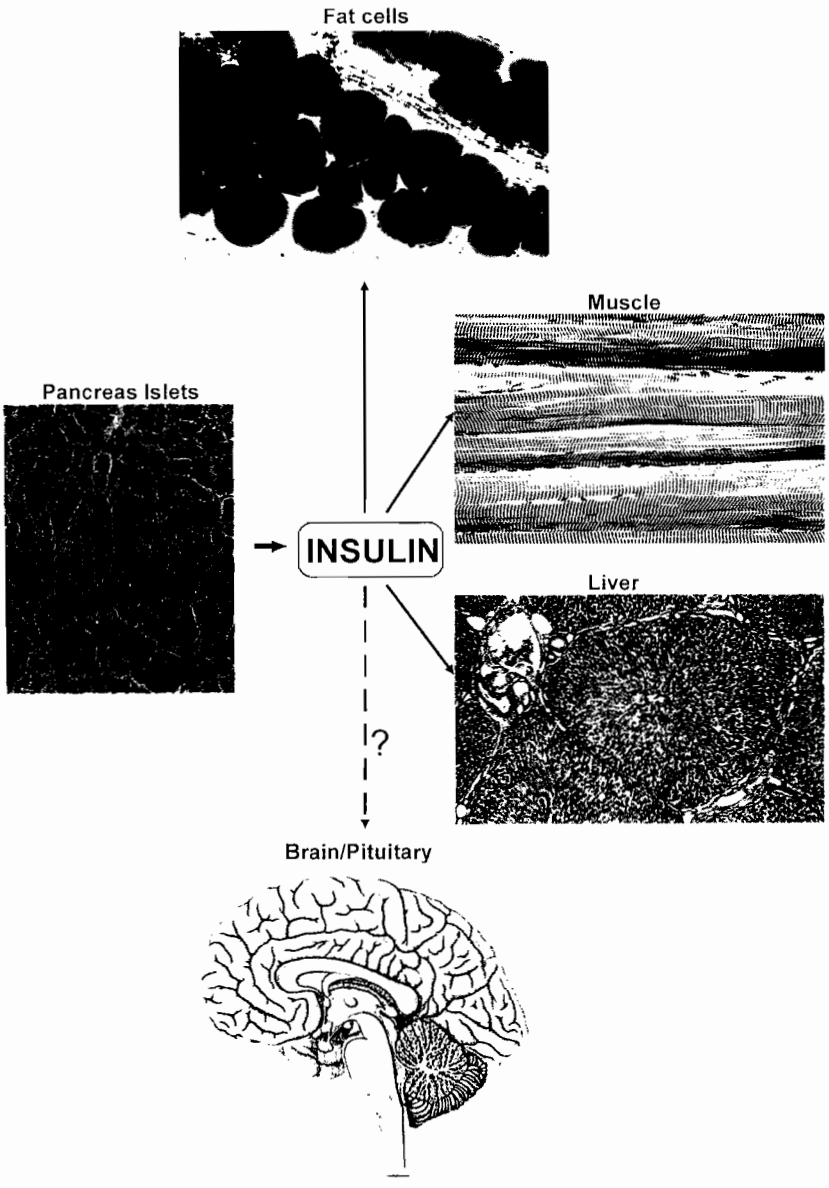

Fig. 1. Schematic overview about the source and most important target regions of insulin, illustrated by histological sections. The role of insulin in the brain and pituitary still contains numerous questions (see text).

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 opiod 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.

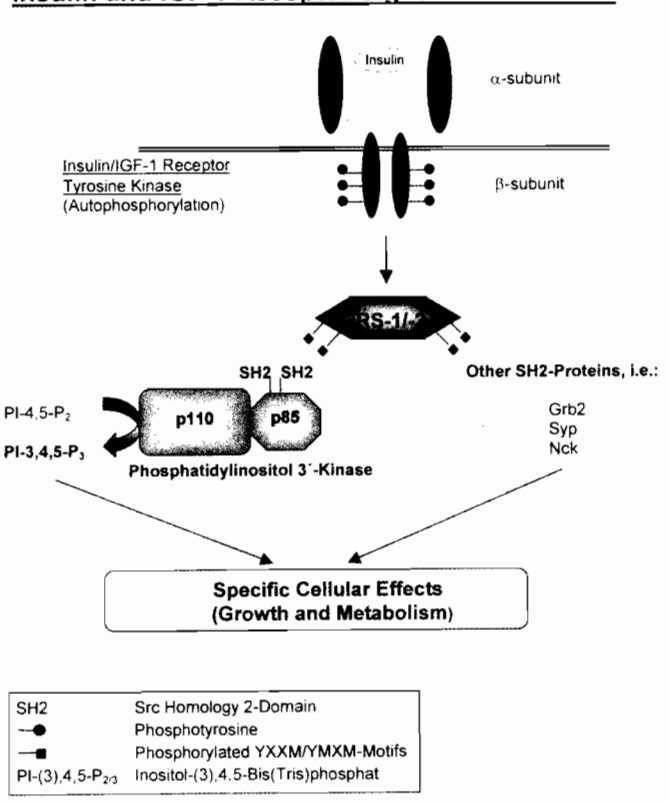

Fig. 2. Model of insulin and IGF-1 receptor signal transduction via tyrosine kinase activity. Insulin or IGF-1 binding to the extracellular α-subunit of the respective receptor initiates intramolecular changes with autophosphorylation of tyrosine residues in the ß-subunit by the receptor-associated tyrosine 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 tyrosinphosphatase 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).

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 ß-subunits (M_r=95.000). The subunits are linked by disulfide bonds to provide a $\alpha_2ß_2$ 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 ß-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,

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

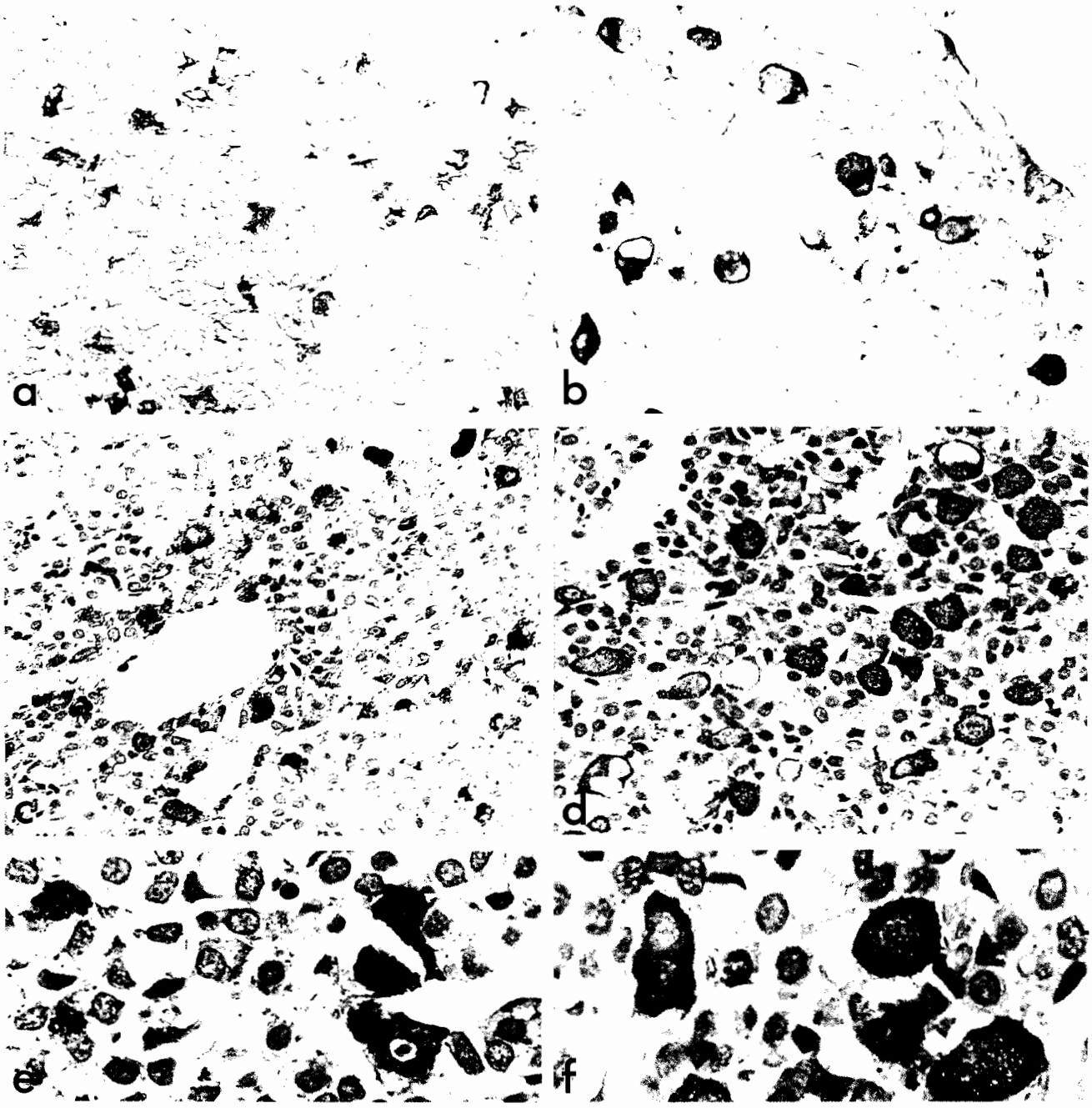

Fig. 3. Histological demonstration of insulin and IGF-1 receptors, IRS-1 and PI3-k in the pars distalis of the pituitary gland. **a.** Insulin receptors. **b.** IGF-1 receptors. Note, that both receptors are located on different subpopulations of secretory cells. **c and e.** PI3-k. **d and f.** IRS-1. 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 p85α subunit of phosphatidyl-inositol 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 p85α 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: p85α and p85ß. PI3-k p85α binds to the catalytic subunit and serves as a link between the enzyme and the ligand activated-receptor tyrosine kinases. p85α 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, ß-PDGF (Downing et al., 1989; Rottapel et al., 1991). However, the precise cellular steps for receptor-specific signal transductions

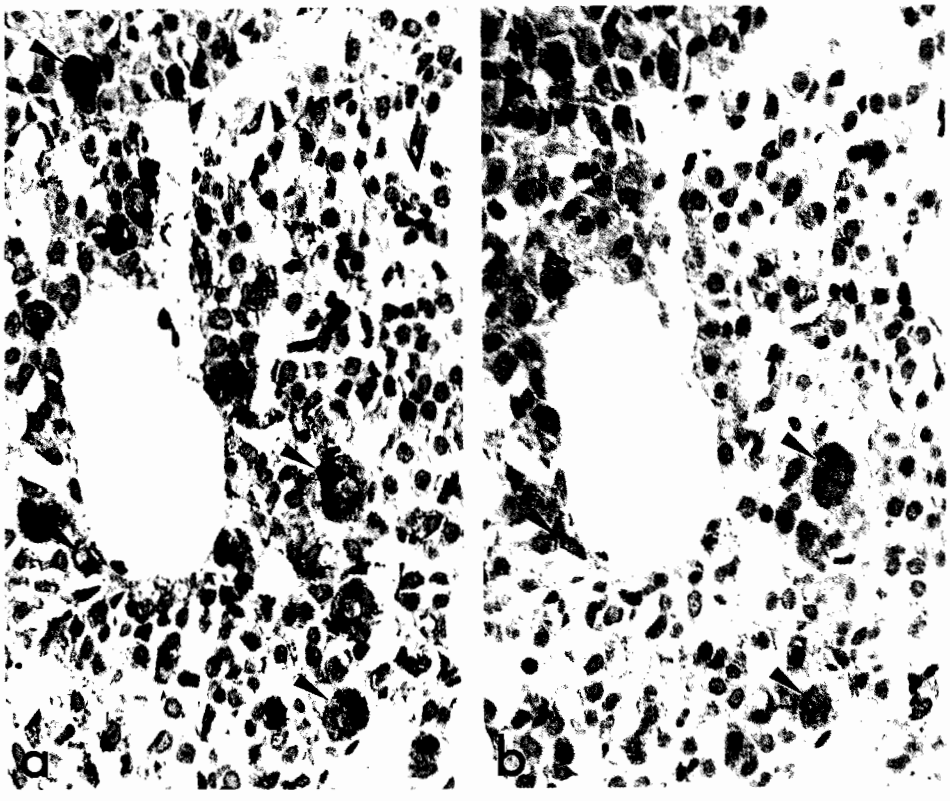

Fig. 4. Colocalization of PI3-k **(a)** and IRS-1 **(b)** on consecutive 1 μm-thick paraffin sections through the pars distalis. Arrowheads point to examples.x 400

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

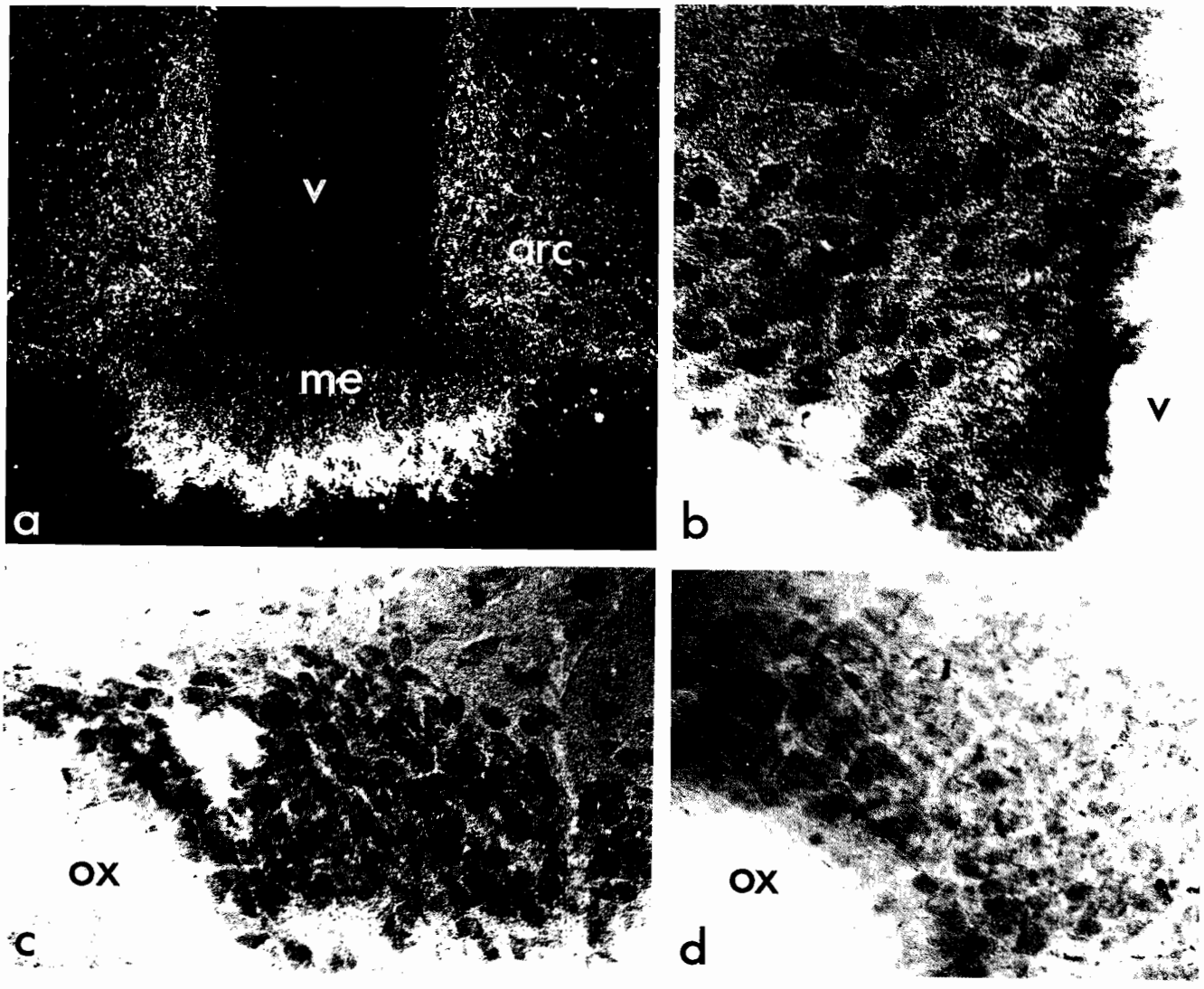

Fig. 5. Representative examples, illustrating the localization of insulin receptors and PI3-k in the hypothalamus of adult rats. **a.** Dense accumulation of insulin receptors is found in the external zone of the median eminence (me) and in the arcuate nucleus (arc) extending to the periventricular regions (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 adenohypopyhsis 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 stongly 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 deficency, 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. ß-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.

Acknowledgements. We thank C. Dittko, I. Holman and I. Wild for excellent technical and photographic support. We also thank Prof. Dr. W. Lange (Department of Anatomy, University of Munich) for helpful discussions. The work was supported by grants from the Deutsche Forschungsgemeinschaft (Un 59/3), Friedrich Baur-Stiftung and by funds from Boehringer Mannheim GmbH.

References

Baskin D.G., Figlewicz D.P., Woods S.C., Porte D. Jr, and Dorsa D.M. (1987). Insulin in the brain. Annu. Rev. Physiol. 49, 335-347.

Baskin D.G., Sipols A.J., Schwartz M.W. and White M.F. (1993). Immunocytochemical detection of insulin receptor substrate-1 (IRS-

1) in rat brain: colocalization with phosphotyrosine. Regul. Peptides 48, 257-266.

Blasquez C., Jégou S., Friard O., Tonon M.C., Fournier A. and Vaudry H. (1995). Effect of centrally administered neuropeptide Y on hypothalamic and hypophyseal proopiomelanocortin-derived peptides in the rat. Neuroscience 68, 221-227.

Carpenter G. (1990). PLC and PKC: a tale of two messengers. Receptor-mediated stimulation of phosphoinositide metabolism and protein kinase C. New Biol. 2, 965-969.

Carpenter C.L., Auger K.R., Chanudhuri M., Yoakim M., Schaffhausen B., Shoelson S. and Cantley L.C. (1993). Phosphoinositide 3-kinase is activated by phosphopeptides that bind to the SH2 domains of the 85-kDa subunit. J. Biol Chem. 268, 9478-9483.

Chuang L.M., Hausdorff S.F., Myers M.G. Jr., White M.F., Birnbaum M.J. and Kahn C.R. (1994). Interactive roles of Ras, insulin receptor substrate-1, and proteins with Src homology-2 domains in insulin signaling xenopus oocytes. J. Biol. Chem. 269, 27645-27649.

Crone C. (1965). Facilitated transfer of glucose from blood into brain tissue. J. Physiol. (Lond.) 181, 103-113.

Cusin I., Dryden S., Wang Q., Rohner-Jeanrenaud F., Jeanrenaud B. and Williams G. (1995). Effects of sustained physiological hyperinsulinemia on hypothalamic neuropeptide Y and NPY mRNA levels in the rat. Neuroendocrinology 7, 193-197.

Downing J.R., Margolis B.L., Zilberstein A., Ashmun R.A., Ullrich A., Sherr C.J. and Schlessinger J. (1989). Phospholipase C-gamma, a substrate for PDGF receptor kinase, is not phosphorylated on tyrosine during the mitogenic response to CSF-1. EMBO J. 11, 3345-50.

Elmquist J.K., Ahima R.S., Maratos-Flier E., Flier J.S. and Saper C.B. (1997). Leptin activates neurons in the ventrobasal hypothalamus and brainstem. Endocrinology 839-842.

Folli F., Saad M.J.A., Backer J.M. and Kahn C.R. (1992). Insulin stimulation of phosphatidylinositol 3-kinase activity and association with insulin receptor substrate-1 in liver and muscle of the intact rat. J. Biol. Chem. 267, 22171-22177.

Folli F., Bonfanti L., Renard E., Kahn C.R. and Merighi A. (1994). Insulin receptor substrate-1 (IRS-1) distribution in the rat central nervous system. J. Neurosci. 14, 6412-6422.

Folli F., Saad M.J.A. and Kahn C.R. (1996). Insulin receptor/IRS-1/PI 3-kinase signaling system in corticosteroid-induced insulin resistance. Acta Diabetol. 33, 185-192.

Grunstein H.S., James D.E., Storlien L.H., Smythe G.A. and Kraegen E.W. (1985). Hyperinsulinemia suppresses glucose utilization in specific brain regions: *In vivo* studies using the euglycemic clamp in the rat. Endocrinology 116, 604-610.

Hadari Y.R., Tzahar E., Nadiv O., Rothenberg P., Roberts C.T.R. Jr., LeRoith D., Yarden Y. and Zick Y. (1992). Insulin and insulinomimetic agents induce activation of phosphatidylinositol 3'-kinase upon its association with pp185 (IRS-1) in intact rat livers. J. Biol. Chem. 267, 17483-17486.

Häring H.U. (1991). The insulin receptor: signalling mechanism and contribution to the pathogenesis of insulin resistance. Diabetologica 34, 848-861.

Hom F.C., Goodner C.J. and Berrie M.A. (1984). A (^{3}H)2-deoxyglucose method for comparing rates of glucose metabolism and insulin responses among rat tissues in vivo. Validations of the model and absence of an insulin effect on brain. Diabetes 33, 141-152.

Johnson R.D. (1995). Opioid involvement in feeding behaviour and the pathogenesis of certain eating disorders. Med. Hypotheses 45, 491-497.

Kanematsu T., Irahara M., Miyake T., Shitsukawa K. and Aono T. (1991). Effect of insulin-like growth factor-I on gonadotropin release from the hypothalamus-pituitary axis in vitro. Acta Endocrinol. 125, 227-233.

Keller S.R. and Lienhard G.E. (1994). Insulin signalling: the role of insulin receptor substrate-1. Trends Cell Biol. 4, 115-119.

Kennedy G.C. (1953). The role of depot fat in the hypothalamic control of food intake in the rat. Proc. R. Soc. Lond. (Biol.) 140, 579-592.

King G.L. and Johnson S.M. (1985). Receptor-mediated transport of insulin across endothelial cells. Science 227, 1583-1586.

Koch A.C., Anderson D., Moran M.F., Ellis C. and Pawson T. (1991). SH2 and SH3 domains: Elements that control interactions of cytoplasmic signaling proteins. Science 252, 668-674.

Kotz C.M., Grace M.K., Briggs J., Levine A.S. and Billington C.J. (1995). Effects of opioid antagonists naloxone and naltrexone on neuropeptide Y-induced feeding and brown fat thermogenesis in the rat J. Clin. Invest. 96, 163-170.

Kuhné M.R., Pawson T., Lienhard G.E. and Feng G.S. (1993). The insulin receptor substrate-1 associates with the SH2-containing phosphotyrosine phosphatase Syp. J. Biol. Chem. 268, 11479-11481.

Lamphere L. and Lienhard G.E. (1992). Components of signalling pathways for insulin and insulin-like growth factor-I in muscle myoblasts and myotubes. Endocrinology 131, 2196-2202.

Lamphere L., Carpenter C.L., Sheng Z.F., Kallen R.G. and Lienhard G.E. (1994). Activation of PI3-kinase in 3T3-L1 adipocytes by association with insulin receptor substrate-1. Am. J. Physiol. 266, E486-E494.

Lavan B.E., Kuhné M.R., Garner C.W., Anderson D., Reedijk M., Pawson T. and Lienhard G.E. (1992). The association of insulin-elicited phosphotyrosine-proteins with src homology 2 domains. J. Biol. Chem. 267, 11631-11636.

Lee J. and Pilch P.F. (1994). The insulin receptor: structure, function, and signaling. Am. J. Physiol. 266, C319-C334.

Leibowitz S.F. (1992). Neurochemical-neuroendocrine systems in the brain controlling macronutrient intake and metabolism. TINS 15, 491-497.

Manin M., Broer Y., Balage M., Rostene W. and Grizard J. (1990). Metabolic clearance of insulin from the cerebrospinal fluid in the anesthetized rat. Peptides 11, 5-12.

McElduff A., Poronnik P., Baxter R.C. and Williams P. (1988). A comparison of the insulin and insulin-like growth factor I receptors from rat brain and liver. Endocrinology 122, 1933-1939.

Michels K.M., Lee W.H., Seltzer A., Saavedra J.M. and Bondy C.A. (1993). Up-regulation of pituitary [125I]insulin-like growth factor-I (IGF-I) binding and IGF binding protein-2 and IGF-I gene expression by estrogen. Endocrinology 132, 23-29.

Moss A.M., Unger J.W., Moxley III R.T. and Livingston J.N. (1990). Location of phosphotyrosine containing proteins in the rat forebrain corresponds to the distribution of the insulin receptor. Proc. Natl. Acad. Sci. USA 87, 4453-4457.

Myers M.G., Sun X.J. and White M.F. (1994). The IRS-1 signaling system. TIBS 19, 289-293.

Nishiyama M., Inazawa J., Ariyama T., Nakamura Y., Matsufuji S., Furusaka A., Tanaka T., Hayashi S. and Wands J.R. (1993). The human insulin receptor substrate-1 gene (IRS1) is localized on 2q36. Genomics 20, 139-141.

Pardridge W.M. (1986) Receptor-mediated transport through the blood-

brain-barrier. Endocr. Rev. 7, 314-330.

Pawson T. and Gish G.D. (1992). SH2 and SH3 domains: From structure to function. Cell 71, 359-362.

Plata-Salamán C.R. (1990). Insulin in the cerebrospinal fluid. Neurosci. Biobehav. Rev. 15, 243-258.

Rordorf-Nikolic T., VanHorn D.J., Chen D., Whites M.F. and Backer J.M. (1995). Regulation of phosphatidylinositol 3'-kinase by tyrosyl phosphoproteins. J. Biol. Chem. 270, 3662-3666.

Rothenberg P.L., Lane W.S., Karasik A., Backer J., White M. and Kahn C.R. (1991). Purification and partial sequence analysis of pp185, the major cellular substrate of the insulin receptor tyrosine kinase. J. Biol. Chem. 266, 8302-8311.

Rottapel R., Reedijk M., Williams D.E., Lyman S.D., Anderson D.M., Pawson T. and Bernstein A. (1991) The Steel/W transduction pathway: kit autophosphorylation and its association with a unique subset of cytoplasmic signaling proteins is induced by the Steel factor. Mol. Cell Biol. 11, 3043-3051.

Schick R.R., Samsami S., Zimmermann J.P., Eberl Th., Endres C., Schusdziarra V. and Classen M. (1993). Effect of galanin on food intake in rats: involvement of lateral and ventromedial hypothalamic sites. Am. J. Physiol. 264, R355-R361.

Schlessinger J. and Ullrich A. (1992). Growth factor signaling by receptor tyrosine kinases. Neuron 9, 383-391.

Schwartz M.W., Figlewicz D.P., Baskin D.G., Woods S.C. and Porte D. Jr. (1992a). Insulin in the brain: A hormonal regulator of energy balance. Endocr. Rev. 13, 387-414.

Schwartz M.W., Sipols A.J., Marks J.L., Sanacora G., White J.D., Scheurink A., Kahn S.E., Baskin D.G., Woods S.C., Figlewicz D.P. and Porte D. Jr. (1992b). Inhibition of hypothalamic neuropeptide Y gene expression by insulin. Endocrinology 130, 3608-3616.

Shemer J., Adamo M., Wilson G.L., Heffez D., Zick Y. and LeRoith D. (1987). Insulin and insulin-like growth factor-I stimulate a common endogenous phosphoprotein substrate (pp185) in intact neuroblastoma cells. J. Biol. Chem. 262, 15476-15482.

Shoelson S.E., Chatterjee S., Chaudhuri M. and White M.F. (1992). YMXM motifs of IRS-1 define substrate specificity of the insulin receptor kinase. Proc. Natl. Acad. Sci. USA 89, 2027-2031.

Soldani R., Cagnacci A. and Yen S.S. (1994). Insulin, insulin-like growth factor I (IGF-I) and IGF-II enhance basal and gonadotrophin-releasing hormone-stimulated luteinizing hormone release from rat anterior pituitary cells in vitro. Eur. J. Endocrinol. 131, 641-645.

Stanley B.G., Kyrkouli S.E., Lampert S. and Leibowitz S.F. (1986). Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7, 1189-1192.

Stoffel M., Espinosa III R., Keller S.R., Lienhard G.E., LeBeau M.M. and Bell G.I. (1993). Human insulin receptor substrate-1 gene (IRS1): chromosomal localization to 2q35-q36.1 and identification of a simple tandem repeat polymorphysm. Diabetologia 36, 335-337.

Sun X.J., Rothenberg P., Kahn C.R., Backer J.M., Araki E., Wilden P.A., Cahill D.A., Goldstein B.J. and White M.F. (1991). Structure of the insulin receptor substrate IRS-1 defines a unique signal transduction protein. Nature 352, 73-77.

Sun X.J., Crimmins D.L., Myers M.G. Jr, Miralpeix M. and White M.F. (1993). Pleiotropic insulin signals are engaged by multisite phosphorylation of IRS-1. Mol. Cell. Biol. 13, 7418-7428.

Tobe K., Tamemoto H., Yamauchi T., Aizawa S., Yazaki Y. and Kadowaki T. (1995). Identification of a 190-kDa protein as a novel substrate for the insulin receptor kinase functionally similar to insulin receptor substrate-1. J. Biol. Chem. 270, 5698-5701.

Ullrich A., Bell J.R., Chen E., Herrera R., Petruzzelli L.M., Dull T.J., Gray A., Coussens L., Liao Y.-C., Tsubokawa M., Mason A., Seeburg P.H., Greenfeld C., Rosen O.M. and Ramachandran J. (1985). Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313, 756-761.

Ullrich A., Gray A., Tam A.W., Yang-Zeng T., Tsubokawa M., Collina C., Henzel W., LeBon T., Kathuria S., Chen E., Jacobs S., Frabcke U., Ramachandran J. and Fujita-Yamaguchi Y. (1986). Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinates that define functional specificity. EMBO J. 5, 2503-2512.

Unger J., McNeill TH., Moxley III. R.T., White M., Moss A.M. and Livingston J.N. (1989). Distribution of insulin receptor-like immunoreactivity in the rat forebrain. Neuroscience 31, 143-157.

Unger J.W. and Lange W. (1997). Insulin receptors in the pituitary gland: morphological evidence for influence on opioide peptide-synthesizing cells. Cell Tissue Res. 288, 471-483.

Unger J.W., Livingston J.N. and Moss A.M (1991). Insulin receptors in the central nervous system: localization, signalling mechanisms and functional aspects. Progr. Neurobiol. 36, 343-362.

Unger J.W., Moss A.M. and Livingston J.N. (1993). The pars tuberalis is enriched with distinct phosphotyrosine-containing proteins not detected in other areas of the brain and pituitary. Cell Tissue Res. 272, 499-507.

Van Houten M., Posner B.I., Popriwa B.M. and Brawer J.R. (1979). Insulin binding sites in the rat brain: *In vivo* localization to the circumventricular organs by quantitative autoradiography. Endocrinology 105, 666-673.

Van Houten M., Nance D.M., Gauthier S. and Posner B.I. (1983). Origin of insulin-receptive nerve terminals in rat median eminence. Endocrinology 113, 1393-1399.

Waters S.B. and Pessin J.E. (1996). Insulin receptor substrate 1 and 2 (IRS1 and IRS2): what a tangled web we weave. Trends Cell. Biol. 6, 1-4.

Waters S.B., Yamauchi K. and Pessin J.E. (1993). Functional expression of insulin receptor substrate-1 is required for insulin-simulated mitogenic signaling. J. Biol. Chem. 268, 22231-22234.

Werther G.A., Hogg A., Oldfield B.J., McKinley M.J., Figdor R., Allen A.M. and Mendelsohn F.A.O. (1987). Localization and characterization of insulin receptors in the rat brain and pituitary gland using in vitro autoradiography and computerized densitometry. Endocrinology 121, 1562-1570.

Werther G.A., Hogg A., Oldfield B.J., McKinley M.J., Figdor R. and Mendelsohn F.A.O. (1989). Localization and characterization of IGF-I receptors in rat brain and pituitary gland using in vitro autoradiography and computerized densitometry: A distinct distribution from insulin receptors. J. Neuroendocrinol. 1, 369-377.

White M.F., Maron R. and Kahn R. (1985). Insulin rapidly stimulates tyrosine phosphorylation of a Mr-185,000 protein in intact cells. Nature 318, 183-186.

White M.F. and Kahn C.R. (1989). Cascade of autophosphorylation in the beta-subunit of the insulin receptor. J. Cell. Biochem. 39, 429-441.

White M.F. and Kahn C.R. (1994). The insulin signaling system. J. Biol. Chem. 269, 1-4.

White J.D. (1993). Neuropeptide Y: a central regulator of energy homeostasis. Regul.Peptides 49, 93-107.

Williams G., Jaswinder S.G., Lee Y.C., Cardoso H.M., Okpere B.E. and

Bloom S.R. (1989). Increased neuropeptide Y concentrations in specific hypothalamic regions of streptozocin-induced diabetic rats. Diabetes 38, 321-327.

Woods S.C., Porte D. Jr., Bobbioni E., Ionescu E., Sauter J.-F., Rohner-Jeanrenaud F. and Jeanrenaud B. (1985). Insulin: its relationship to the central nervous system and control of food intake and body weight. Am. J. Clin. Nutr. 42, 1063-1071.

Young W.S. (1986). Periventricular hypothalamic cells in the rat brain contain insulin mRNA. Neuropeptides 8, 93-97.

Zhang-Sun G., Yang C.-R., Viallet J., Feng G.-S., Bergeron J.J.M and Posner B.I. (1996). A 60-Kilodalton protein in rat hepatoma cells overexpressing insulin receptor was tyrosine phosphorylated and associated with Syp, phosphatidylinositol 3-kinase, and Grb2 in an insulin-dependent manner. Endocrinology 137, 2649-2658.