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Leptin in adipose tissue - morphological aspects

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Summary

Leptin is a peptide hormone encoded by the *ob*-gene and released by adipocytes. Over the past few years, the synthesis, regulation and effects of leptin have been extensively investigated in view of its pleiotropic role in human (patho)physiology. This work aimed at reviewing current morphological knowledge on leptin synthesis and secretion in adipocytes. It is concluded that the intracellular trafficking of leptin and the structural basis of leptin secretion remain to be defined. Further morphological work, especially at the ultrastructural level, is needed to firmly establish the intracellular distribution and the release pathways of the hormone.

Key words: Adipocyte – Morphology – Confocal microscopy – Ultrastructure – Secretion

Introduction

In 1953, Kennedy proposed a mechanism for the regulation of body weight based on presumptive circulating signals, which would have been generated in proportion to body fat stores in order to affect appetite and energy expenditure. In 1958, Hervey suggested the existence of a circulating satiety factor acting as a negative feedback signal to control food intake, and in 1973 Coleman provided substantial evidence that such circulating signals exist. Convincing evidence is now available to believe that the adipose-derived hormone leptin, a member of the 1st class cytokine super family, is one of these factors (for a review, see e.g. Frühbeck et al., 1998; Houseknecht et al., 1998; Ahima and Flier, 2000; Bjorbaek and Kahn, 2004). Leptin is a peptide hormone (167 aminoacids) encoded by the *ob* gene (Zhang et al., 1994), which has been demonstrated to play an important role in diverse physiological functions beyond the regulation of adiposity such as energy homeostasis, endocrine regulation, reproduction and pregnancy, and haematopoiesis. Furthermore, the relationships between leptin and physical exercise have been the object of extensive investigation (Hickey et al., 1997; Dirlewanger et al., 1999; Halle et al., 1999; Okazaki et al., 1999; Houmard et al., 2000; Noland et al., 2001).

White adipose tissue is the major source of leptin in non-pregnant subjects and leptin mRNA is particularly abundant in the subcutaneous, omental, retroperitoneal, perilymphatic and mesenteric fat deposits (Masuzaki et al., 1995). While the biochemical, functional and molecular biology aspects of leptin physiology have been extensively investigated, the morphological counterparts of leptin synthesis, storage and secretion in adipocytes have received less attention. Quite recently, it has been suggested that “leptin may represent a cell-specific cargo of a widely distributed regulated secretory compartment of yet unknown biochemical composition and regulation” (Roh et al., 2001). In light of this new, exciting hypothesis, we have reviewed the available literature dealing with the morphology of leptin expression and intracellular distribution.

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Overall, leptin distribution in subcellular compartments has been studied in the rat (Tsuruo et al., 1996; Barr et al., 1997; Roh et al., 2000), mouse (Cinti et al., 1997) and the human (Borstein et al., 2000; Zhang et al., 2000) adipose cell. Different methods of detection have been used, but in most studies the immunohistochemical approach was used; conventional light microscopy (Barr et al., 1997; Cinti et al., 1997; Borstein et al., 2000), confocal laser scanning microscopy (Barr et al., 1997; Zhang et al., 2000; Roh et al., 2001) and electron microscopy (Borstein et al., 2000) have also been employed. In the studies of Parker et al. (1996) and Roh et al. (2000) immunocytochemistry was applied after cell fractioning and centrifugation.

HISTOLOGY

Some basic morphological findings on leptin distribution in adipose tissue have been presented in studies aimed at a more general investigation of the adipocyte structure, such as comparisons of white and brown adipose cells, the adipose tissues of fasting and obese animals, or the stages of adipocyte differentiation.

By means of in situ hybridization, Maffei et al. (1995) showed that leptin is contained in any of the adipocytes in mouse epididymal white fat: the signal was in a thin rim at the periphery of the adipocytes; it was absent in non-adipose cells and in intracellular vacuoles representing lipid droplets: however, the signals from the cytoplasm and the plasma membrane were indistinguishable, due to insufficient resolution. In cultured 3T3-adipocytes (Parker et al., 1996) and rat tissue white (Tsuruo et al., 1996) adipocytes, leptin immunofluorescence was found in the peripheral cytoplasmic rim surrounding the triglyceride deposit, but not in the nucleus or the

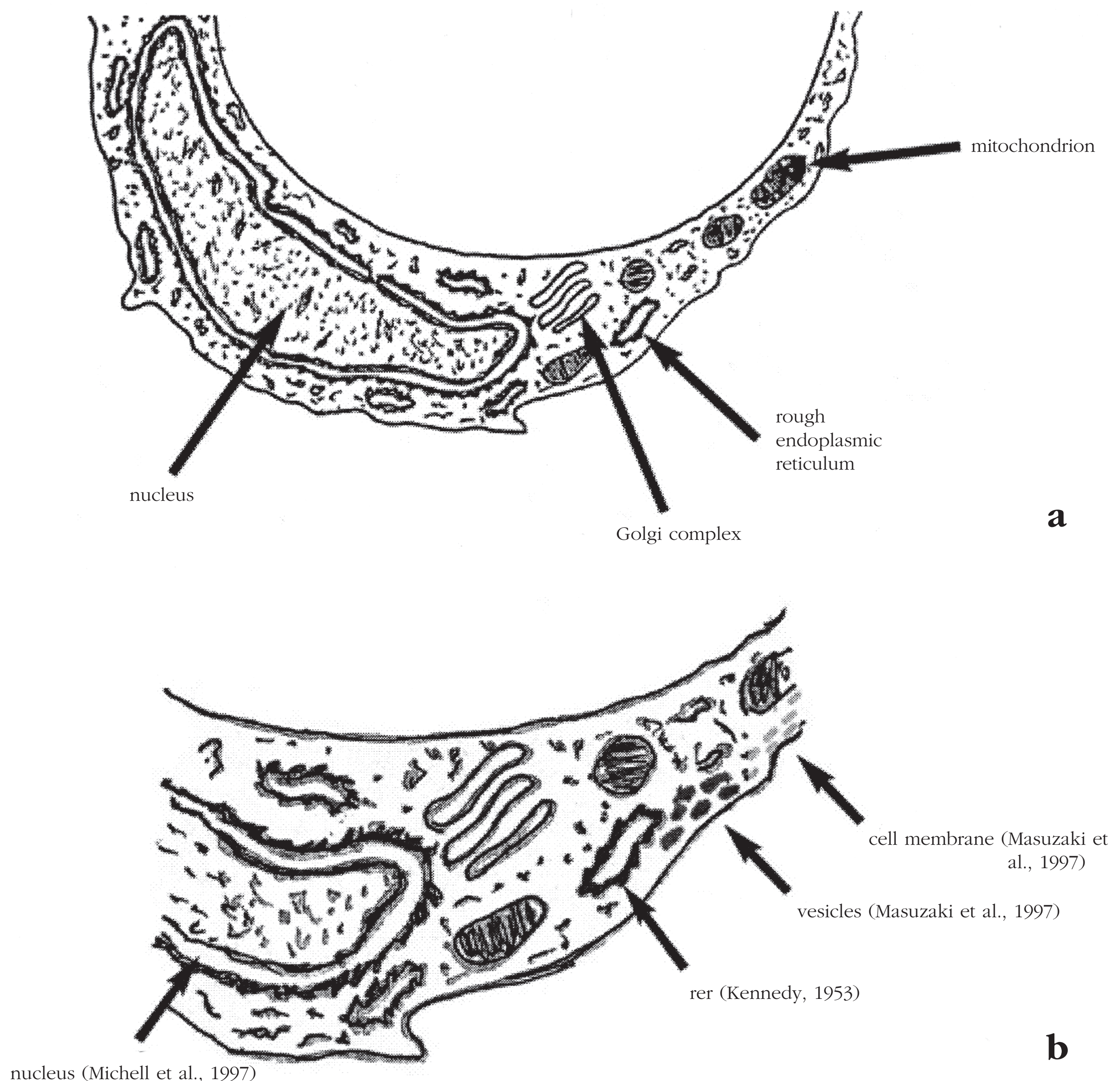

Fig. 1.- **a)** schematic drawing of white adipocyte ultrastructure; **b)** selected hypotheses concerning leptin intracellular localisation; the corresponding reference is quoted in parenthesis.

lipid droplet. A few spindle-shaped or ovoid immunoreactive cells were visible among mature adipocytes (Tsuruo et al., 1996), suggesting the expression of leptin in differentiating adipocytes. The leptin staining pattern was similar in control, diabetic, and dexamethasone-treated non-diabetic rats (Tsuruo et al., 1996). The above-reported intracellular distribution of leptin immunoreactivity in tissue unilocular white adipocytes was confirmed by Cinti et al. (1997), who investigated lean, lean fasting and obese mice. In accordance with the presumably lower need for leptin in fasting, the staining intensity was higher in lean fed than lean fasting animals. Differentiating, multilocular white adipocytes were found to express leptin. The expression of leptin during the early stages of adipose differentiation was confirmed in human embryos by Atanassova and Popova (2000) using light microscopy immunohistochemistry. A leptin signal of different intensities was detected exclusively in the cytoplasm of subcutaneous, multilocular preadipocytes also expressing lipoprotein lipase (LPL) during the 8th embryonic week. However, scattered leptin immunoreactive cells were found in the subcutaneous mesenchyme during the 6th embryonic week. These findings support the view that leptin is produced/secreted in utero by developing adipose tissue, thereby contributing to explaining the detection of leptin in the circulation of newborn babies and at mid-term (Schubring et al., 1997; Geary et al., 1999), possibly in association with placenta-derived leptin (Masuzaki et al., 1997).

Morphological investigation of the presence and distribution of leptin in brown adipose tissue, the other type of fat found in mammals, was performed by Tsuruo et al. (1996) and Cinti et al. (1997). In frozen sections of rat brown fat, Tsuruo et al. (1996) found leptin immunolabelling in cytoplasmic areas of the typical multilocular brown adipocytes that were not occupied by lipid droplets. The labelling intensity was higher in the region bordering the lipid droplets. In mouse brown adipose tissue, Cinti et al. (1997) found that typical multilocular uncoupling protein (UCP)-positive adipocytes were not stained with the leptin antibody in either fed on fasted lean animals; instead, small (UCP-positive) and large (UCP-negative) unilocular fat cells in the brown fat of obese mice showed leptin immunoreactivity in the peripheral cytoplasm (Cinti et al., 1997). Taken together, these finding suggest that the expression of leptin in brown adipocytes could be differently regulated among species and/or the developmental/differentiation stage of brown adipocytes.

Confocal laser microscopy

Confocal microscopy would be expected to afford a more precise localization of leptin in adipocytes. Using this technique in isolated rat white adipocytes, Barr et al. (1997) found that leptin immunofluorescence in the peripheral cytoplasm rim showed a honeycomb pattern compatible with a localization of the hormone in cisternae of the endoplasmic reticulum (ER); this was confirmed by the co-localization of leptin with calnexin, an integral membrane protein that is an established marker of the ER (Hochstenbach et al., 1992; Rajagopalan and Brenner, 1994). According to Barr et al. (1997), leptin immunofluorescence is never seen in a punctate pattern, which would imply storage in secretory vesicles. Interestingly, acute (15 min) insulin administration induced partial dissociation of leptin and calnexin staining, together with a reduction in leptin labeling intensity in many cells. Confocal laser scanning microscopy was also used by Zhang et al. (2000) to evaluate a modified ceiling culture method (Sugihara et al., 1986) of mature human adipocytes. These authors found leptin immunofluorescence in the region of the plasma membrane, cytoplasm, nuclear periphery and the nucleus, although the cytoplasmic and nuclear localization of leptin was found in some, but not all, the adipocytes. The nuclear localization of leptin in some human but not rat adipocytes (Tsuruo et al., 1996; Barr et al., 1997) was not explained. Zhang et al. (2000) suggested that nuclear leptin could act as a nuclear receptor or nuclear factor affecting the genome of target cells, taking into account previous findings of secreted cytokines in the nucleus (Rifkin et al., 1994; Burysek and Houstek, 1996; Michell et al., 1997).

Quite recently, Roh et al. (2001) investigated the intracellular distribution of leptin, LPL, and calreticulin (a marker of the endoplasmic reticulum) in isolated rat adipocytes by means of double immunofluorescence staining. Confocal microscopy showed that the major intracellular pools of leptin and LPL do not overlap. LPL was mainly found in the endoplasmic reticulum, as also suggested by co-localization with calreticulin. Instead, the pattern of leptin immunofluorescence pointed towards the localization of the hormone in homogeneous secretory vesicles. It is concluded from these light microscopy works (Sugihara et al., 1986; Hochstenbach et al., 1992; Rifkin et al., 1994; Rajagopalan and Brenner, 1994; Tsuruo et al., 1996; Burysek and Houstek, 1996; Barr et al., 1997; Masuzaki et al., 1997; Michell et al., 1997; Roh et al., 2001) that leptin staining may be heterogeneous in adipocytes.

Electron microscopy

It is reasonable envisage that electron microscopy immunocytochemistry would allow a precise localization of leptin in cell compartments. This was tackled by Bornstein et al. (2000) in acrylic resin-embedded human white adipose tissue and differentiating human adipose cells in primary culture. Besides confirming the cyto-

plasmic localization of leptin in mature tissue adipocytes at the light microscopy level, Bornstein et al. (2000) found individual and clustered gold particles representing leptin along cell membranes and in putative vesicles (40-80 nm in diameter) associated with the cell membrane. According to those authors, no large storage organelle for leptin was detected in adipocytes. A leptin signal was also found in cultured preadipocytes in both light- and electron immunohistochemistry (Bornstein et al., 2000): the signal was present in the cytoplasm and was associated with internal membranes and in putative vesicles along the cell membrane. From these data the authors suggested that a regulated exocytotic mechanism of leptin secretion would exist in adipocytes. This is in accordance with the functional data from Russell et al. (2001) and the morphological evidence reported by Roh et al. (2000). They showed that leptin is localized in a type of secretory compartment that is different from the classic peptide-containing secretory granules detectable in endocrine cells. These compartments may be present as a distinct pathway of insulin-dependent secretion, different from the well-known ubiquitous insulin-dependent pathway for the transport of the glucose transport channel GLUT-4 (Barr et al., 1997; Roh et al., 2000; Mora and Pessin, 2002). A possible reason for the absence of a detectable leptin storage organelle is that leptin is not glycosylated and might pass through the Golgi complex rapidly without becoming concentrated there. Alternatively, this novel secretory pathway could completely bypass the Golgi complex, such that leptin would be secreted without being stored first. However, this would be the only reported example of such a secretion mechanism (Barr et al., 1997). Interestingly, the immunoelectron microscopic work of Bornstein et al. (2000) revealed the presence of leptin immunoreactivity outside adipocytes in the interstitial space between neighboring cells, the outer membrane of endothelial cells, and on collagen fibers and the outside of macrophages. This is consistent with a leptin-mediated paracrine regulation of cellular components different from adipocytes in adipose tissue and the finding of a possible angiogenic (Park et al., 2001) and cytokine-like (Waelput et al., 2002) effect for leptin.

In summary, the available morphological evidence consistently indicates that leptin is produced in developing and mature white adipose tissue. The pattern of leptin expression in brown adipose tissue is less firmly established. Regarding intracellular leptin distribution, it is generally agreed that leptin is not found in the cell lipid vacuole(s). The prevailing evidence is that there is no large secretory compartment for leptin, but the final definition of the intracellular steps leading to its secretion is still lacking; a scheme of current hypotheses is shown in Fig. 1. One reason for this could be the insufficient resolution of confocal microscopy and the use of less than optimal embedding techniques for immunoelectron microscopy. Further specific morphological work is required to establish the precise intracellular distribution of leptin and its secretion mechanisms.

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