Localization of leptin receptor splice variants in mouse peripheral tissues by immunohistochemistry

R. De Matteis[1], K. Dashtipour[1], A. Ognibene[2] and S. Cinti[1]*

[1]*Institute of Normal Human Morphology-Anatomy, University of Ancona, Ancona, Italy*
[2]*Laboratory of Cellular Biology and Electron Microscopy, IOR, Bologna, Italy*

The recently discovered leptin hormone is produced by adipocytes and acts on the hypothalamus inducing satiety and an increase in energy expenditure (for review, see Friedman, 1997), as well as on a number of organs involved in reproduction, metabolism and glucose homeostasis (Ahima *et al.* 1996; Chehab *et al.* 1996; Levin *et al.* 1996). These findings suggest that leptin also acts directly on peripheral tissues. Even though several studies have investigated the localization of the various spliced isoforms of the leptin receptor (Lee *et al.* 1996; Wang *et al.* 1996) in peripheral tissues (Ghilardi *et al.* 1996; Fei *et al.* 1997; Hoggard *et al.* 1997), the techniques used in most of them do not allow the exploration of their precise cytological localization, which is a precondition to gaining a better understanding of the functional role of the receptor in these tissues.

The present immunohistochemical study was thus performed to obtain cytological details of the localization of the receptor splice variants in a number of peripheral tissues of lean and obese mice.

Materials and methods

Animals were twelve adult (10–14 weeks old) obese C57BL/6-ob (*ob/ob*) and C57BL/ks-db (*db/db*) mice and lean mice of the same strains, three in each group (Harlan Nossan, Correzzana-MI, Italy). They were caged under standard laboratory conditions with tap water and regular chow available *ad libitum* in a 12 h–12 h light–dark cycle. Care and handling were in accordance with institution guidelines.

The mice were weighed and anaesthetized with ketamine (Ketavet; Farmaceutici Gellini, Aprilia-LT, Italy; 100 mg/ kg, intraperitoneally) in combination with xylazine (Rompum; Bayer AG, Leverkusen, Germany; 19 mg/kg, intraperitoneally). Transcardiac perfusion was performed using paraformaldehyde in 0·1 M-phosphate buffer (PB; pH 7·4; 40 mg/l). After perfusion, the animals were dissected under a surgical microscope (Zeiss OPM 19; Carl Zeiss, Oberkochen, Germany) and the samples were fixed by immersion in the same fixative overnight at 4°.

The tissues used for this investigation were: epididymal, periovarian, subcutaneous and retroperitoneal white adipose tissue, interscapular brown adipose tissue, testis, ovary, pancreas, liver, kidney, adrenal gland, heart, lung, skeletal muscle, small intestine and lymph nodes.

The samples were washed rapidly with 0·1 M-PB at pH 7·4, dehydrated in ethanol and embedded in paraffin blocks. Sections (3 μm thick) were used for light microscopy and immunohistochemistry.

Immunohistochemistry

Antibodies. Affinity-purified goat polyclonal antibodies directed to the peptides corresponding with amino acids 877-894 (M-18) and 32-51 (K-20) of leptin receptor OB-Ra of mouse origin (Santa Cruz Biotec, Santa Cruz, CA, USA) and an affinity-purified rabbit polyclonal antibody against an eighteen amino acid peptide near the C terminus of mouse leptin receptor OB-Rb (OBR-13; Alpha Diagnostic International, San Antonio, TX, USA) were used (Fig. 1).

Procedure. Immunohistochemical demonstration of leptin receptors was performed with the avidin–biotin–peroxidase (*EC* 1.11.1.7; ABC) method. Dewaxed sections (3 μm) were processed through the following incubation steps: (1) H_2O_2 (3 ml/l methanol) for 30 min to block endogenous peroxidase, (2) normal rabbit serum 1 : 75 (v/v; K-20 and M-18 protocol) and normal goat serum 1 : 75 (v/v; OBR-13 protocol) for 20 min to reduce non-specific background staining; (3) incubation with primary antibody against leptin receptor (K-20; M-18 and OBR-13) diluted 1 : 100 (v/v) to 1 : 300 (v/v) in PBS according to the type of tissue, overnight at 4°; (4) biotinylated secondary antibody: rabbit anti-goat immunoglobulin G 1 : 200 (v/v; K-20 and M-18 protocol) and goat anti-rabbit immunoglobulin G 1 : 200 (v/v; OBR-13 protocol) for 30 min (Vector Labs, Burlingame, CA, USA); (5) ABC complex for 1 h (Vectastain ABC kit, Vector Labs); (6) histochemical visualization of peroxidase using 0·75 ml 3,3′-diaminobenzidine hydrochloride as chromogen (Sigma, St Louis, MO, USA) and 0·2 ml H_2O_2/l 0·05 M-Tris buffer, pH 7·6, for 5 min in a dark room. Sections were then rinsed in tap water, counterstained with haematoxylin, dehydrated and mounted in Entellan (Merck, Darmstadt, Germany).

Method-specificity tests were performed by omitting the primary antibodies in the staining and by incubation of

Abbreviations: ABC, avidin–biotin–peroxidase; R, extracellular part of the leptin receptor common to all splice forms; R_S, intracytoplasmic part common to all short transmembrane splice forms of the leptin receptor; R_L, long form of the leptin receptor.

***Corresponding author:** Professor S. Cinti, fax +39 71 220 60 87, email cinti@popcsi.unian.it

https://doi.org/10.1079/PNS19980063 Published online by Cambridge University Press

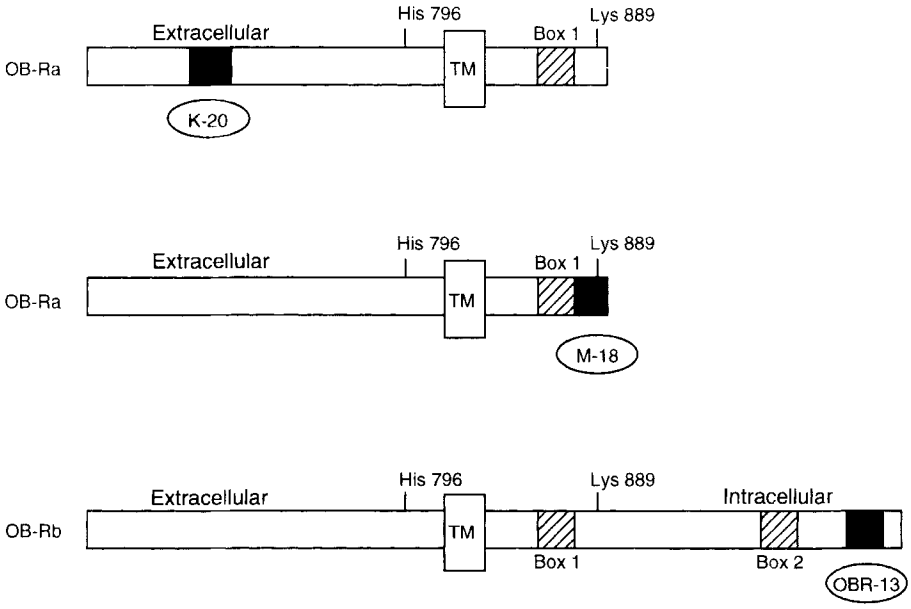

Fig. 1. Scheme illustrating the position of the peptides used to raise the antibodies recognizing the extracellular part of the leptin receptor common to all splice forms (R; K-20), the intracytoplasmic part common to all short transmembrane forms (R_S; M-18) and the long form of the receptor (R_L; OBR-18), in leptin receptors OB-Ra and OB-Rb of mouse origin. TM, transmembrane domain.

sections with an antiserum saturated with the homologous antigen.

For the neutralization of the antibodies, they were incubated with a tenfold excess of homologous peptide (from 20 to 70 μg/ml, according to the antibody dilution) for 48 h (adsorption test).

Cell culture

Mouse C2C12 myoblasts from American Type Culture Collection (Rockville, MD, USA) were grown in Dulbecco's modified Eagle's medium supplemented with heat-inactivated fetal calf serum (100 ml/l) containing 100 U penicillin/ml and 0·1 mg streptomycin/ml.

Preparation of total cell homogenate

Subconfluent C2C12 cells were washed once in PBS and resuspended in lysis buffer (10 mM-Tris hydrochloride, pH 7·8, Nonidet P-40 (10 ml/l), 10 mM-mercaptoethanol, 1 mM-phenylmethylsulphonylfluoride, 10 g leupeptin and aprotinin/ml, 10 g soyabean trypsin inhibitor/ml, 15 g calpain inhibitor I/ml and 7 g calpain inhibitor II/ml). After 10 min swelling on ice, cells were lysed by forty passages through a 25-gauge hypodermic needle. Protein content was determined using the Bio-Rad protein assay kit (Bio-Rad, Milano, Italy).

SDS-PAGE and Western blotting

Total cell homogenate (30 μg) in loading buffer was run on a 7·5 % SDS-PAGE using a Bio-Rad Minigel apparatus and transferred to a nitrocellulose membrane using a Bio-Rad wet blotting system overnight at 0·25 A (Towbin *et al.* 1979). The nitrocellulose was blocked in PBS containing (/l) 1 ml Tween 20, 30 g bovine serum albumin and 50 g non-fat dried milk for 1 h at room temperature. The blot was incubated with primary antibody (1 : 750, Ob-R (K-20); Santa Cruz Biotec) appropriately washed, incubated with secondary antibody conjugated to horseradish peroxidase (anti-goat 1 : 2000; Dako, Italy) and visualized using the ECL kit (Amersham, Milano, Italy).

Results

Results are summarized in Table 1.

In the adrenal gland (Fig. 2(a)), medullary cells were intensely positive only with the antibody recognizing the common part of the receptor (R). *Zona fasciculata* cells of the cortex stained for all three antibodies. In the pancreas, the exocrine portion was not stained while most of the cells in the Langerhans islets (Fig. 2(c and d)) were labelled, though more weakly for the long-splice variant (R_L)

In the gonads, Leydig cells (Fig. 2(b)) in the testis and luteal and interstitial cells in the ovary (Fig. 3) were positive for all splice forms. Leydig cells were weakly positive for R_L.

The heart (Fig. 4(d and e)) displayed diffuse positivity of myocardial fibres for all three antibodies which was more intense in atrial cells. Also, the striated cells of myocardial origin (Ludatscher, 1968; Hebel & Stromberg, 1986) in the wall of the main trunks of the pulmonary veins were intensely positive for the different splice forms of the receptor (Fig. 4(c)). The striated muscle cells of *soleus* (Fig. 5(c)) exhibited a focal staining pattern. The consistent positivity of different types of striated muscle cells prompted us to study by immunoblotting the expression of the receptor also in cultured cells. The present study (performed only with the antibody for R) revealed the presence of R in myoblasts of the C2C12 cell line (Fig. 6).

https://doi.org/10.1079/PNS19980063 Published online by Cambridge University Press

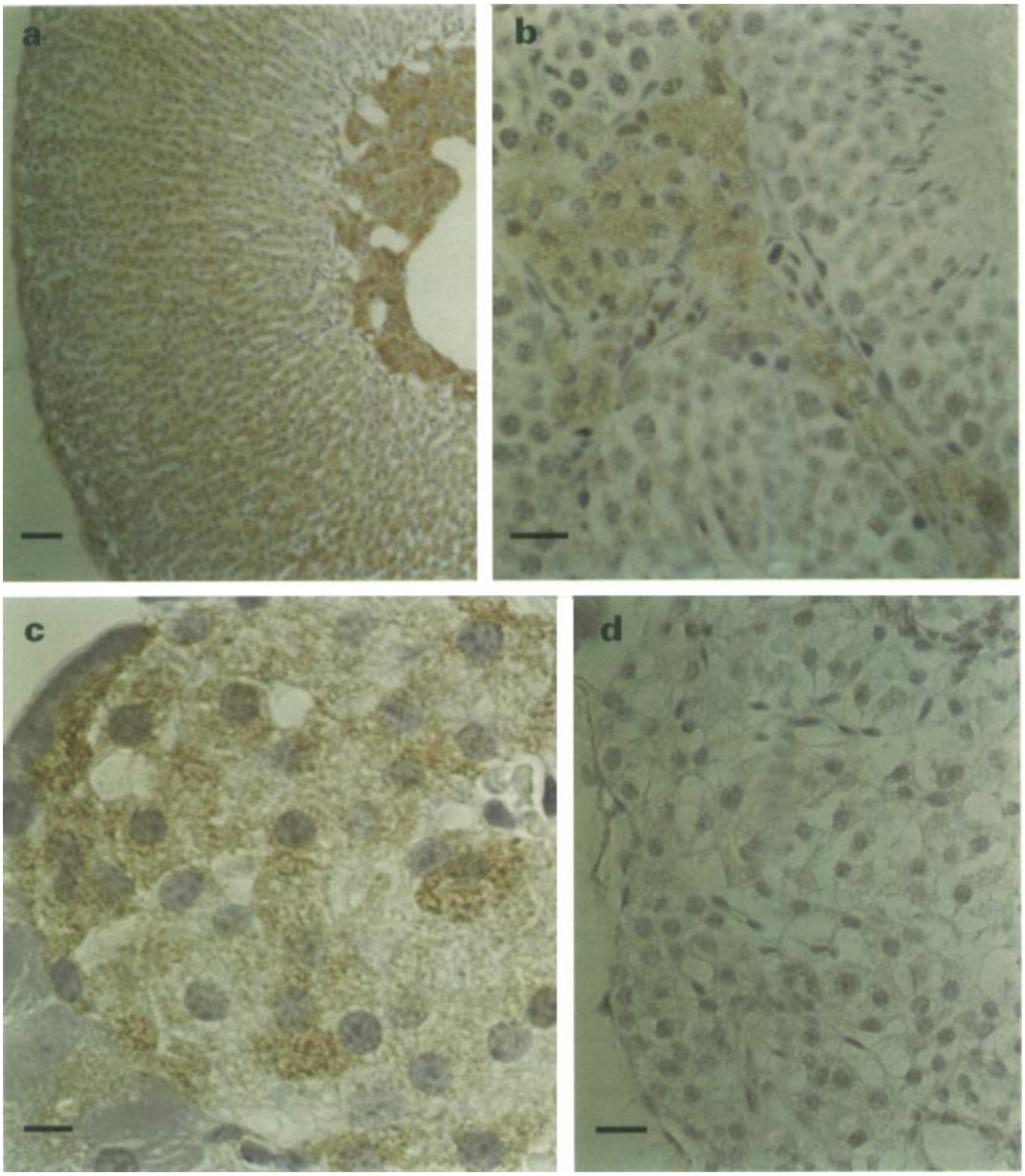

Fig. 2. **(a)** Adrenal gland of lean mouse showing intense labelling of *zona fasciculata* of cortical and medullary cells by the anti-R (intracellular part of leptin receptor common to all splice forms) antibody. Scale bar = 40 μm. **(b)** Testis of lean mouse showing Leydig cells labelled by the anti-R_S (the intracytoplasmic part of the leptin receptor common to all transmembrane forms) antibody. Scale bar = 20 μm. **(c)** Langerhans isle of the pancreas of lean mouse showing R_L-positive cells; negative exocrine pancreas is visible on the left of the figures. Scale bar = 10 μm. **(d)** Langerhans isle of the pancreas of obese *db/db* mouse negative for the anti-R_L antibody. Scale bar = 20 μm. For details of procedures, see pp.441–442. R_L, long form of the leptin receptor; R_S, the intracytoplasmic part of the leptin receptor common to all transmembrane forms.

In the lung, in addition to the myocardial cells in the walls of the pulmonary veins, there were positive cells in the bronchiolar epithelium (Fig. 4(a and b)). These cells displayed a dome-like surface devoid of cilia and were identified as Clara cells.

In adipose tissues (Fig. 5(b)), the thin peripheral rim of white adipocytes stained for all three antibodies. In interscapular brown adipose tissue only the cells in the peripheral portion of the organ were positive (Fig. 5(a)). They differed from the majority of the cells of the organ in their few and large lipid droplets, sometimes gathered in a single vacuole mimicking the morphology of white adipocytes.

In the liver, the hepatocytes in the central part of the lobule were diffusely positive (Fig. 5(d)). Staining was more intense with the antibodies for R and the short splice variant (R_S). Small intestine and lymph nodes were negative.

In the tissues of *ob/ob* mice we obtained the same results as in lean mice, but Leydig cells and the endocrine cells of the pancreas stained intensely with all three antibodies.

With adsorption test (see pp. 441–442) the tissues were negative (not shown).

https://doi.org/10.1079/PNS19980063 Published online by Cambridge University Press

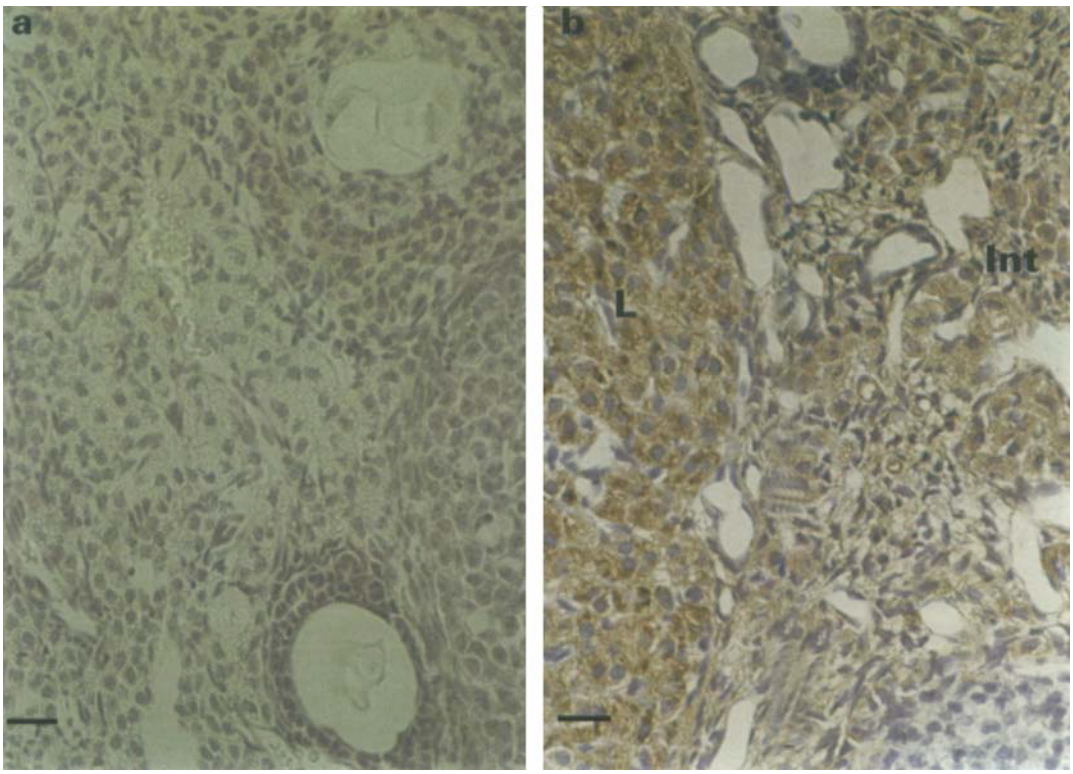

Fig. 3. **(a)** Ovary of *db/db* mouse. All tissues resulted negative for the anti-R_L (the long form of the leptin receptor) antibody. **(b)** The same organ in a lean mouse shows luteal (L) and interstitial (Int) cells stained for the same antibody. For details of procedures, see pp. 441–442. Scale bar = 20 μm.

The same tissues, tested with R_L in *db/db* mice, were negative (Figs. 2–3), except the kidney, where there were some positive cells in the medullary elements of the nephron. Since this finding requires further investigation, kidney results were not included.

Discussion

The presence of leptin-receptor splice variants in peripheral tissues, described by several authors, suggests that leptin plays a direct role in peripheral organs (Tartaglia *et al.* 1995; Ghilardi *et al.* 1996; Fei *et al.* 1997; Hoggard *et al.* 1997; Lollmann *et al.* 1997). Thus, their cytological localization in these tissues must be known precisely if the role of the leptin receptor is to be fully understood. Since most published studies have been performed using techniques that do not achieve a precise cytological localization of the protein, the present work used immunohistochemistry to localize the splice variants in a number of peripheral tissues.

Our results indicate that most of the organs expressing R_S (adrenal gland, adipose tissues, heart, liver, lung, ovary, endocrine pancreas, skeletal muscle and testis) also express R_L in the same cell type, although in some of them (adrenal gland, liver, endocrine pancreas and testis) staining for R_L was weaker.

In the adrenal gland the medullary portion was intensely positive only with the antibodies for R, while the cortex (only the cells of *zona fasciculata*) was intensely positive also for R_S. Some authors (Cao *et al.* 1997) reported staining of the medulla and negative staining of the cortex by autoradiography and immunohistochemistry using an antibody recognizing both R_S and R_L in rats. Others (Hoggard *et al.* 1997), by *in situ* hybridization, showed a detectable signal in the medulla of mice that was stronger with the probe for R_S, and a weak signal also in the cortex.

In white and brown adipose tissue, our results confirm the recent data (Emilsson *et al.* 1997; Fruhbeck *et al.* 1997; Siegrist-Kaiser *et al.* 1997; Tanizawa *et al.* 1997; Kutoh *et al.* 1998), showing the presence of all isoforms, but cytological localization allows us to specify that only the unilocular and mainly unilocular cells at the periphery of interscapular brown adipose tissue were labelled. Interestingly, we obtained the same type of results for leptin expression in a previous study (Cinti *et al.* 1997). This suggests that only when brown adipocytes contain large lipid vacuoles, or are unilocular, do they express both leptin and its receptor.

Some of the cell types expressing the receptor isoforms were steroid-secreting cells: cells of *zona fasciculata* of the adrenal-gland cortex, luteal and interstitial (Blandau, 1977) cells of the ovary and Leydig cells of the testes. These results are consistent with the hypothesized interaction of leptin with the glucocorticoid system (Ahima *et al.* 1996; Campfield *et al.* 1997) and with its trophic role in the gonads of obese *ob/ob* mice (Mounzih *et al.* 1997; Zachow & Magoffin, 1997). On the other hand, they are only partially in line with those of other researchers, mainly because in our samples the receptor was not expressed in the spermatogenic epithelium of the testis (Hoggard *et al.* 1997) nor in the follicular cells of the ovary (Cioffi *et al.* 1997; Karlsson *et al.*

https://doi.org/10.1079/PNS19980063 Published online by Cambridge University Press

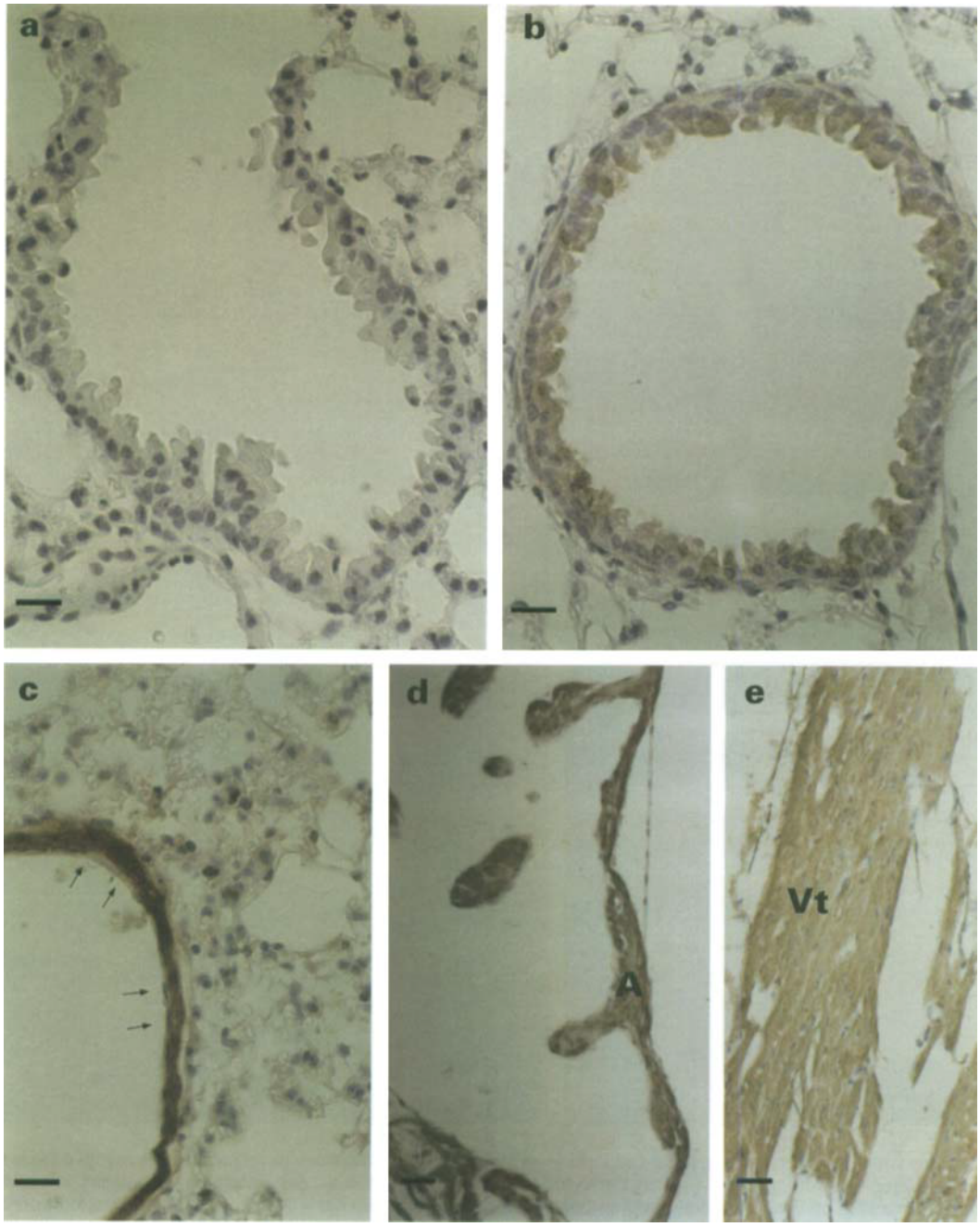

Fig. 4. **(a)** Pulmonary parenchyma and bronchiolar cells were completely negative in *db/db* mice with the anti-R_L antibody. Scale bar = 20 μm. **(b)** Bronchiolar epithelial cells strongly labelled by the same antibody in lean mouse. Scale bar = 20 μm. **(c)** Pulmonary vein showing striated muscular fibres in the media R_S-positive (→). Scale bar = 20 μm. **(d–e)** Heart of lean mouse; atrial (A) and ventricular (Vt) fibres were R_L-positive. Scale bar = 40 μm. For details of procedures, see pp. 441–442. R_L, long form of the leptin receptor; R_S, the intracytoplasmic part of the leptin receptor common to all transmembrane forms.

1997), although the latter localization has been found in human specimens. Our findings confirm the presence of R_S and R_L in Langerhans islets, in agreement with the results obtained with different techniques by other authors (Kieffer *et al.* 1996; Leclercq-Meyer *et al.* 1996; Emilsson *et al.* 1997; Fei *et al.* 1997; Tanizawa *et al.* 1997).

The present work is the first to show the presence of R_S and R_L in the epithelial cells of the bronchioles and in striated muscle cells (Ludatscher, 1968; Hebel & Stromberg, 1986) of the pulmonary veins. The positive cells in the bronchioles had a dome-like surface and were devoid of cilia, which suggested their identification as Clara cells. Interestingly, the striated muscle cells of the wall of the pulmonary veins are of cardiac origin (Ludatscher, 1968; Hebel & Stromberg, 1986) and the striated cardiac cells of the heart were positive, more intensely in atrial cells. Previous studies have shown the presence of only R_S in lung and heart (Tartaglia *et al.* 1995; Fei *et al.* 1997; Hoggard *et al.* 1997) but, in agreement with

https://doi.org/10.1079/PNS19980063 Published online by Cambridge University Press

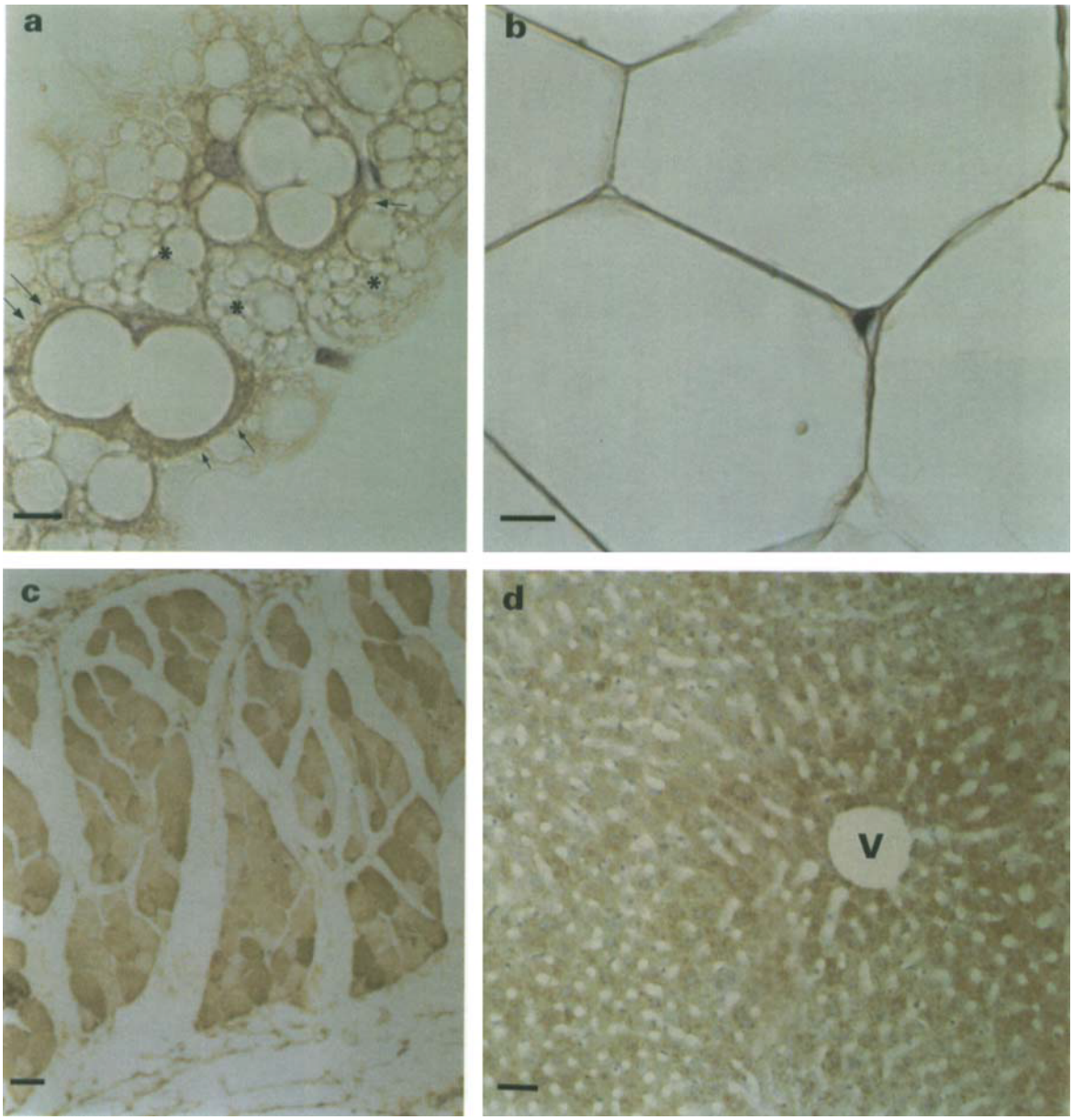

Fig. 5. **(a)** R_L-positive interscapular brown adipose tissue of lean mouse. Classic multilocular adipocytes (*) resulted negative. Only adipocytes with a few lipid droplets were positive (→) in the cytoplasm. Scale bar = 10 μm. **(b)** Unilocular adipocytes in periepididymal white adipose tissue of lean mouse. The thin peripheral rim of cytoplasm was positive. Scale bar = 10 μm. **(c)** Soleus muscle in *ob/ob* mice R_S-positive. Scale bar = 40 μm. **(d)** R_S-positive liver in lean mice. The hepatocytes around the centrolubular vein (V) were more intensely positive. Scale bar = 10 μm. For details of procedures, see pp. 441–442. R_L, long form of the leptin receptor; R_S, the intracytoplasmic part of the leptin receptor common to all short transmembrane forms.

our results, Lollmann *et al.* (1997) reported high levels of R_L in the lung. The presence of the receptor in cultured C2C12 myoblasts suggests their precocious appearance during development and confirms the presence of R_S in this cell line, providing evidence that this form could mediate the functional activities observed in these cells after leptin administration (Berti *et al.* 1997; Kellerer *et al.* 1997; Muoio *et al.* 1997). In the *soleus* muscle, the receptor expression was in agreement with the studies showing important effects of glucose intake and glycogen synthesis in this muscle after leptin administration (Liu *et al.* 1997; Muoio *et al.* 1997).

In the liver, a major concentration of the receptor (mainly R_S) was observed near the centrolobular vein. Other authors (Hoggard *et al.* 1997) described the presence of R_S adjacent to the larger hepatic blood vessels, although some authors also found R_L (Emilsson *et al.* 1997).

The absence of immunoreactivity in the exocrine pancreas is in agreement with the results of other authors (Emilsson *et al.* 1997; Fei *et al.* 1997), but our negative results in small intestine and lymph nodes contrast with some previous reports (Cioffi *et al.* 1996; Ghilardi *et al.* 1996; Lollmann *et al.* 1997).

https://doi.org/10.1079/PNS19980063 Published online by Cambridge University Press

Table 1. Immunohistochemical localization of leptin receptor OB-R splice variants*

Organ		R	R_S	R_L
Adrenal gland		Medulla and *zona fasciculata* of cortex	*Zona fasciculata* of cortex and a few medullary cells (*weak*)	*Zona fasciculata* of cortex and a few medullary cells (*weak*)
Adipose tissues:	BAT	Only unilocular cells at the periphery	Only unilocular cells at the periphery	Only unilocular cells at the periphery
	WAT	Unilocular cells	Unilocular cells	Unilocular cells
Heart		Atrial and ventricular (*weak*) striated cells	Atrial and ventricular (*weak*) striated cells	Atrial and ventricular (*weak*) striated cells
Liver		Centrolobular region	Centrolobular region	Centrolobular region (*weak*)
Lung		Bronchiolar Clara cells and striated muscle cells of pulmonary veins	Bronchiolar Clara cells and striated muscle cells of pulmonary veins	Bronchiolar Clara cells and striated muscle cells of pulmonary veins
Lymph nodes		Negative	Negative	Negative
Ovary		Luteal and interstitial cells	Luteal and interstitial cells	Luteal and interstitial cells
Pancreas:	Endocrine	Insular cells	Insular cells	Insular cells (*weak*)
	Exocrine	Negative	Negative	Negative
Skeletal muscle (*soleus*)		Most fibres	Most fibres	Most fibres
Small intestine		Negative	Negative	Negative
Testis		Leydig cells	Leydig cells	Leydig cells (*weak*)

R, the extracellular part of the receptor common to all known splice variants; R_S, intracytoplasmic part common to all short transmembrane forms, short-splice variants; R_L, long form of the receptor, i.e. long-splice variant; BAT, brown adipose tissue; WAT, white adipose tissue.
*For details of procedures, see pp. 441–442.

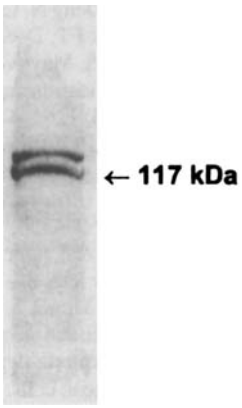

Fig. 6. Immunochemical detection of the extracellular part of the leptin receptor common to all splice forms (R) in total cell homogenate of C2C12 myocytes. Total cell homogenate (30 μg) was subjected to SDS-PAGE using 7·5 % acrylamide gels and transferred to nitrocellulose membrane. Anti-R antibody dilution was 1 : 750. For details of procedures, see pp.441–442.

Further work is in progress in our laboratory to study other organs and provide further details on the cellular localization of the leptin-receptor splice variants.

Acknowledgements

This work was supported by MURST, Grant 40 % 1996 (to SC), European Community Grant CHRX-CT94-0490 (to SC) and Ancona University Grant 1998 (to SC).

References

Ahima RS, Prabakaran D, Mantzoros C, Qu D, Lowell B, Maratos-Flier E & Flier JS (1996) Role of leptin in the neuroendocrine response to fasting. *Nature* **382**, 250–252.

Berti L, Kellerer M, Capp E & Haring HU (1997) Leptin stimulates glucose transport and glycogen synthesis in C2C12 myotubes: evidence for a P13-kinase mediated effect. *Diabetologia* **40**, 606–609.

Blandau RJ (1977) The female reproductive system. In *Histology*, pp. 900–902 [L Weise and RO Greep, editors]. New York: McGraw-Hill.

Campfield LA, Smith FJ, Kochan JP & Burn P (1997) Central nervous system mediation of the biologic activity of obese protein (leptin): Potential role in states of altered energy balance. In *Leptin – the Voice of Adipose Tissue*, pp. 118–125 [WF Blum, W Kiess and W Rascher, editors]. Leipzig, Germany: J. A. Barth Verlag.

Cao GW, Considine RV & Lynn RB (1997) Leptin receptor in the adrenal medulla of the rat. *American Journal of Physiology* **273**, E448–E452.

Chehab FF, Lim ME & Lu R (1996) Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nature Genetics* **12**, 318–320.

Cinti S, Fredrich RC, Zingaretti MC, De Matteis R, Flier JS & Lowell BB (1997) Immunohistochemical localisation of leptin and uncoupling protein in white and brown adipose tissue. *Endocrinology* **138**, 797–804.

Cioffi JA, Shafer AW, Zupancic TJ, Smith-Gbur J, Mikhail A, Platika D & Snodgrass HR (1996) Novel B219/OB receptor isoforms: Possible role of leptin in hematopoiesis and reproduction. *Nature Medicine* **2**, 585–589.

Cioffi JA, Van Blerkom J, Antczak M, Shafer A, Wittmer S & Snodgrass HR (1997) The expression of leptin and its receptors in pre-ovulatory human follicles. *Molecular Human Reproduction* **3**, 467–472.

Emilsson V, Liu YL, Cawthorne MA, Morton NM & Davenport M (1997) Expression of the functional leptin receptor mRNA in pancreatic islets and direct inhibitory action of leptin on insulin secretion. *Diabetes* **46**, 313–316.

Fei H, Okano HJ, Li C, Lee GH, Zhao C, Darnell R & Friedman JM (1997) Anatomic localisation of alternatively spliced leptin receptors (Ob-R) in mouse brain and other tissues. *Proceedings of the National Academy of Sciences, USA* **94**, 7001–7005.

Friedman JM (1997) Role of leptin and its receptors in the control of body weight. In *Leptin – the Voice of Adipose Tissue*, pp. 3–22

[WF Blum, W Kiess and W Rascher, editors]. Leipzig, Germany: J. A. Barth Verlag.

Fruhbeck G, Aguado M & Martinez JA (1997) In vitro lipolytic effect of leptin on mouse adipocytes: evidence for a possible autocrine/paracrine role of leptin. *Biochemical and Biophysical Research Communications* **240**, 590–594.

Ghilardi N, Ziegler S, Wiestner A, Stoffel R & Heim MH (1996) Defective STAT signaling by the leptin receptor in diabetic mice. *Proceedings of the National Academy of Sciences, USA* **93**, 6231–6235.

Hebel R & Stromberg MW (1986) Circulatory system. In *Anatomy and Embryology of the Laboratory Rat*, p. 113 [R Hebel and MW Stromberg, editors]. Wörthsee, Germany: BioMed Verlag.

Hoggard N, Mercer JG, Rayner DV, Moar K, Trayhurn P & Williams LM (1997) Localisation of leptin receptor mRNA splice variants in murine peripheral tissues by RT-PCR and in situ hybridisation. *Biochemical and Biophysical Research Communications* **232**, 383–387.

Karlsson C, Lindell K, Svensson E, Bergh C, Lind P, Billing H, Carlsson LM & Carlsson B (1997) Expression of functional leptin receptors in the human ovary. *Journal of Clinical Endocrinology and Metabolism* **82**, 4144–4148.

Kellerer M, Koch M, Metzinger E, Mushack J, Capp E & Haring HU (1997) Leptin activates PI-3 kinase in C2C12 myotubes via janus kinase-2 (JAK-2) and insulin receptor substrate-2 (IRS-2) dependent pathways. *Diabetologia* **40**, 1358–1362.

Kieffer TJ, Heller RS & Habener JF (1996) Leptin receptors expressed on pancreatic β-cells. *Biochemical and Biophysical Research Communications* **224**, 522–527.

Kutoh E, Boss O, Levasseur F & Giacobino JP (1998) Quantification of the full length leptin receptor (OB-R) in human brown and white adipose tissue. *Life Science* **62**, 445–451.

Leclercq-Meyer V, Considine RV, Sener A & Malaisse WJ (1996) Do leptin receptors play a functional role in the endocrine pancreas? *Biochemical and Biophysical Research Communications* **229**, 794–798.

Lee GH, Proenca R, Montez JM, Carroll KM, Darvishzadeh JG, Lee JI & Friedman JM (1996) Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **379**, 632–635.

Levin N, Nelson C, Gurney A, Vandlen R & Sauvage F (1996) Decreased food intake does not completely account for adiposity reduction after ob protein infusion. *Proceedings of the National Academy of Science, USA* **93**, 1726–1730.

Liu YL, Emilsson V & Cawthorne MA (1997) Leptin inhibits glycogen synthesis in the isolated soleus muscle of obese (ob/ob) mice. *FEBS Letters* **411**, 351–355.

Lollmann B, Grüninger S, Stricker-Krongrad A & Chiesi M (1997) Detection and quantification of the leptin receptor splice variants Ob-Ra, b, and e in different mouse tissues. *Biochemical and Biophysical Research Communications* **238**, 648–652.

Ludatscher RM (1968) Fine structure of the muscular wall of rat pulmonary veins. *Journal of Anatomy* **103**, 345–357.

Mounzih K, Lu R & Chehab FF (1997) Leptin treatment rescues the sterility of genetically obese ob/ob males. *Endocrinology* **138**, 1190–1193.

Muoio DM, Lynis Dohn G, Fiedorek FT, Tapscott EB & Coleman RA (1997) Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* **46**, 1360–1363.

Siegrist-Kaiser CA, Pauli V, Juge-Aubry CE, Boss O, Pernin A, Chin WW, Cusin I, Rohner-Jeanrenaud F, Burger AG, Zapt J & Meier CA (1997) Direct effects of leptin on brown and white adipose tissue. *Journal of Clinical Investigation* **100**, 2858–2864.

Tanizawa Y, Okuya S, Ishihara H, Asano T, Yada T & Oka Y (1997) Direct stimulation of basal insulin secretion by physiological concentration of leptin in pancreatic β cells. *Endocrinology* **138**, 4513–4516.

Tartaglia LA, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moor KJ, Smutko JS, Mays GG, Woolf EA, Monroe CA & Tepper RI (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell* **83**, 1263–1271.

Towbin H, Staehelin T & Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences, USA* **76**, 4350–4354.

Wang MY, Zhou YT, Newgard CB & Unger RH (1996) A novel leptin isoform in rat. *FEBS Letters* **392**, 87–90.

Zachow RJ & Magoffin DA (1997) Direct intraovarian effects of leptin: Impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone-dependent estradiol-17 β production by rat ovarian granulosa cells. *Endocrinology* **138**, 847–850.

https://doi.org/10.1079/PNS19980063 Published online by Cambridge University Press