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Review

Leptin-induced signal transduction pathways

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Abstract

Leptin is a multifunctional cytokine and hormone that primarily acts in the hypothalamus and plays a key role in the regulation of food intake and energy expenditure. In addition, it has direct effects on many cell types on the periphery. Leptin acts through its receptor, the product of the *db* gene, which has six isoforms. Only one of them (OB-Rb) has full signalling capabilities and is able to activate the Jak/STAT pathway, the major pathway used by leptin to exert its effects. However, some signalling events can be initiated by the short isoforms. Besides Jak/STAT, other pathways, such as MAPK and the 5'-AMP-activated protein kinase (AMPK) pathway, are also involved in leptin signalling. Leptin also interacts with insulin signalling. In this paper, we give an overview of the signal transduction mechanisms that are related to the actions of leptin.

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Keywords: Leptin; Receptor; Signal transduction

1. Introduction

The word leptin comes from the Greek *leptos*, meaning thin, referring to the anti-obesity effect of the molecule, which was believed to be the primary physiological function of the hormone. Leptin is the product of the *ob* gene, discovered by Zhang et al. (1994) using the positional cloning technique. The gene is located on chromosome 6 in the mouse and chromosome 7 in humans, and encodes a protein that shows a high degree of homology between species. Mutations in this *ob* gene revealed the pivotal role of leptin in energy balance (Zhang et al., 1994). *Ob/ob* mutant mice show early onset obesity, hyperphagia, hypothermia, hyperinsulinemia, hyperglycaemia and other metabolic and neuroendocrine abnormalities (Zhang et al., 1994). In humans, *ob* gene mutations cause morbid obesity, hyperphagia and hypothalamic hypogonadism, but unlike mutant mice, hypothermia, hyperinsulinemia and hyperglycaemia were not found (Barr et al., 1997). However, human *ob* gene mutations are infrequent, with few cases reported to date (Ahima and Flier, 2000).

Leptin is a 16 kDa non-glycosylated molecule that circulates in the blood, and can be considered a hormone. Its molecular structure shows similarities with members of the IL-6 cytokine family, including interleukin-11 (IL-11), interleukin-12 (IL-12), leukaemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), oncostatin-M (OSM), cardiotrophin-1 (CT-1), granulocyte colony-stimulating factor (G-CSF) and interleukin-6 (IL-6) (Madej et al., 1995; Prolo et al., 1998).

Leptin is secreted mainly by white adipocytes (an adipocytokine). Circulating levels show correlation with body-mass index and the amount of total body fat stores. However, white adipose tissue is not the only source of leptin, since other cell types—gastric mucosa, skeletal muscle, mammary epithelium, placenta, bone marrow and pituitary—produce substantial amounts (Ahima and Flier, 2000; Wauters et al., 2000), as also primary cultures of osteoblasts (to promote bone mineralisation; Reseland et al., 2001).

Leptin is secreted into the blood stream and becomes partially bound to plasma proteins (Ahima and Flier, 2000). Thus, it usually exerts its hormonal effects on cell types that possess specific receptors. Nevertheless, leptin as a cytokine also acts locally in a paracrine or autocrine way (Reseland et al., 2001).

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Abbreviations

84	
85	OBR leptin receptor
86	Jak Janus tyrosine kinase
87	STAT signal transducer and activator of transcription
88	MAPK mitogen-activated protein kinase
89	AMPK 5'-AMP-activated protein kinase
90	IL- interleukin-
91	LIF leukaemia inhibitory factor
92	CNTF ciliary neurotrophic factor
93	OSM oncostatin-M
94	CT-1 cardiotrophin-1
95	G-CSF granulocyte colony-stimulating factor
96	NPY neuropeptide Y
97	AGRP agouti-related peptide
98	POMC proopiomelanocortin
99	CART cocaine inducible element
100	APRE acute-phase-response-element
101	GAS γ -interferon activated sequence
102	PIAS3 protein inhibitor of activated STAT3
103	SOCS3 suppressor of cytokine signalling-3
104	PIAS protein inhibitor of activated STAT
105	SHP-2 SH2-domain containing protein tyrosine phosphatase
106	NF κ B nuclear factor kappa b
107	IRS insulin receptor substrate
108	PIP ₃ inositol-trisphosphate- and amphetamine-regulated transcript
109	GHR growth hormone receptor
110	SIE sis-
111	PDK PIP ₃ dependent serine/threonine kinase
112	GSK3 glycogen synthase kinase-3
113	GLUT4 glucose transporter4
114	ACC acetyl-coenzyme A-carboxylase
115	PPAR peroxisome proliferator activated receptor
116	CTP carnitine palmitoyl transferase
117	ACO acyl CoA oxidase.

67 Initial reports have called leptin an anti-obesity
68 hormone because of its primary physiological function
69 to prevent obesity by regulating food intake and energy
70 balance. However, the picture has been refined and it is
71 now viewed as an anti-steatotic peptide, with a major role
72 in the regulation of lipid metabolism (Unger, 2000).
73 Unger referred to it as an antilipogenic hormone, since it
74 prevents excessive non-oxidative fatty acid metabolism
75 and thus protects against lipotoxicity (Unger, 2000). It
76 impairs the loss of function and viability of cells due to
77 fat overload (Unger, 2002).

78 In summary, leptin plays a pivotal role in energy
79 homeostasis by decreasing food intake and increasing
80 energy expenditure via hypothalamic centres, affecting
81 feeding behaviour and activating the sympathetic
82 nervous system. Leptin-sensitive neurons have been
83 revealed in the arcuate, dorsomedial, ventromedial and

ventral pre-mammillary nuclei of the hypothalamus that
express neuropeptides such as NPY, AGRP, POMC and
CART involved in the regulation of energy balance
(Bjorbaek et al., 2001).

Leptin is also a signal for adaptation to fasting.
Fasting triggers complex actions to promote survival,
decreasing leptin levels which then increases adrenal
glucocorticoid activity and appetite, along with
decreases in thyroid and gonadal hormones, and sup-
pression of the immune system (Ahima et al., 1996; Lord
et al., 1998).

Besides mediating energy homeostasis, body weight,
appetite, fat stores or glucose metabolism and the action
of insulin, leptin also interacts with the hypothalamic–
pituitary–adrenal axis and influences sexual maturation,
thereby playing a key role in reproduction and develop-
ment. It may have a role in cardiovascular and renal

function, and it effects bone formation, liver functions, stimulates haematopoiesis, and phagocytic activity of macrophages (Ducy et al., 2000; Wauters et al., 2000).

Taken together, leptin is more than just an adipocyte-derived body weight controlling peptide; it affects a whole complex of activity throughout the body. We also need to take a look at the signal transduction mechanisms that leptin uses to exert its diverse effects on different tissues.

2. The leptin receptor

Leptin acts through its receptor (OBR), which is encoded by the *db* gene. Tartaglia et al. identified it from mouse choroid plexus using an expression cloning strategy (Tartaglia et al., 1995). OBR is a member of the class I cytokine receptor family (Tartaglia et al., 1995), which includes the receptors of IL-2, -3, -4, -6, -7, LIF, G-CSF, GRH, prolactin, and erythropoietin (Bazan, 1989). Members of this family have characteristic extracellular motifs of four cysteine residues and WSXWS (Bazan, 1990), and they contain a different number of fibronectin type III domains (Heim, 1996; Kishimoto et al., 1995). The extracellular region of OBR consists of four fibronectin type III domains and two cytokine receptor domains (Heshka and Jones, 2001). It forms homodimers even in the absence of leptin and is activated via ligand-induced conformational changes (Devos et al., 1997).

Intracellularly, the full-length receptor contains several sequence elements that are required for subsequent signalling events. The OBR does not have an intrinsic tyrosine kinase domain, therefore binds cytoplasmic kinases, mainly Janus tyrosine kinase 2 (Jak2), a member of the Jak family (Ghilardi and Skoda, 1997). Like other cytokine receptors, OBR contains a highly conserved, proline-rich box1 (present at intracellular amino acid 6–17) (Bjorbaek et al., 1997; White et al., 1997a) and two putative less conserved box2 motifs (intracellular amino acids 49–60 and 202–213) (Chua et al., 1997; Ghilardi and Skoda, 1997; Kloek et al., 2002). Box1 and box2 motifs are thought to recruit and bind Jaks (Jiang et al., 1996; Murakami et al., 1991). However, it was demonstrated that only box1 and the immediate surrounding amino acids are essential for Jak activation (Bahrenberg et al., 2002; Kloek et al., 2002). Box1 and intracellular amino acids 31–36 are indispensable for this interaction, whereas amino acids 37–48 appear to increase the signal, but can be substituted by other elements (Kloek et al., 2002).

Within these regions, two amino acids were identified as being crucial for signalling (Leu896, Phe897) and were fully conserved in different vertebrate species (Bahrenberg et al., 2002). Although an intact box2 motif is not required to activate Jak kinase (Bahrenberg et al.,

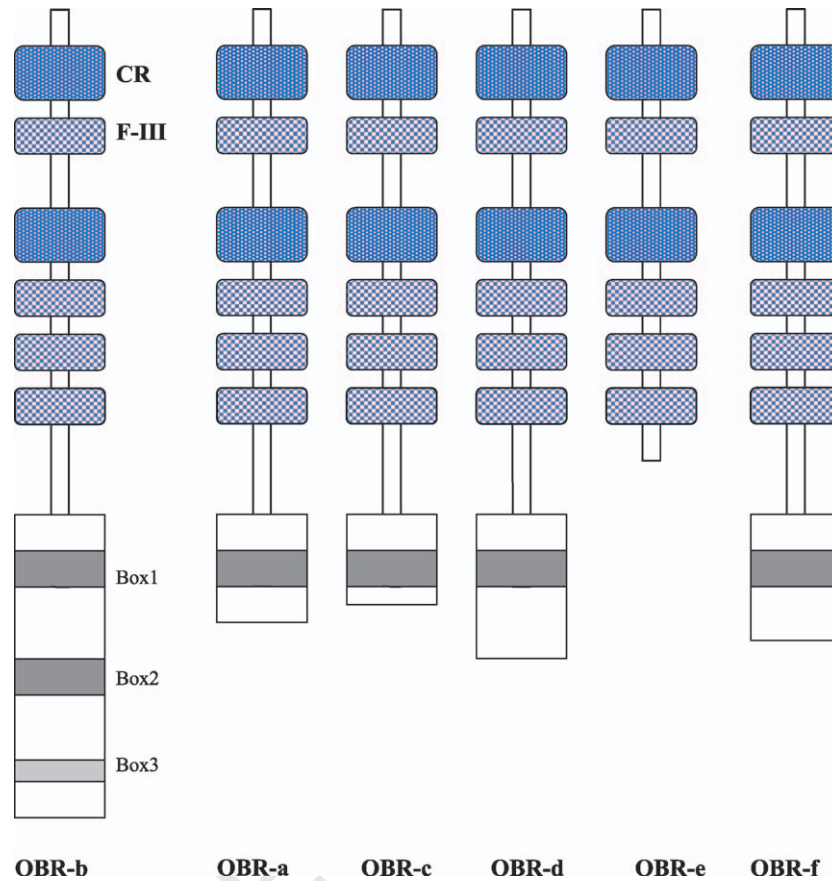
2002; Kloek et al., 2002), the pivotal STAT (signal transducer and activator of transcription) signalling pathway cannot be induced without box2 (Murakami et al., 1997). Forming homodimers and showing signalling capabilities with mutated box2 motifs, OBR can be classified as a member of the GHR subfamily (Heldin, 1995). For downstream signalling events, tyrosine residues at positions 985 and 1138 are needed to provide docking sites for subsequent signalling molecules (Banks et al., 2000).

OBR has at least six isoforms generated primarily by alternative splicing of the RNA transcript of the *db* gene (Chua et al., 1996) (Fig. 1). They all share an identical extracellular ligand-binding domain and have the characteristic motif of four cysteine residues and WSXWS (Gainsford et al., 1996). Five of them also possess transmembrane and cytoplasmic regions. The transmembrane and proximal 29 intracellular amino acid residues, including the box1 motif, are the same in all forms; the additional cytoplasmic region is different in length. OBRb contains 301 intracellular amino acids, whereas the short forms, OBRa, OBRc and OBRd, have 34, 32 and 40, respectively. The sixth one (OBRe), lacking the transmembrane and cytoplasmic parts, serves as a soluble receptor (Bjorbaek et al., 1997; Lee et al., 1996; Wang et al., 1996). As only the long form has the box2 motif and the specific residues, this seems to be the functional signalling one.

Although the membrane-bound short forms containing only the box1 motif are also able to recruit Jaks and activate certain signalling cascades (Murakami et al., 1991), their major function is probably related to leptin internalisation and degradation (Uotani et al., 1999). Another possibility is that they are involved in leptin clearance or receptor-mediated transport into the brain (Hileman et al., 2000). However, leptin transport through the blood–brain barrier is independent of receptors in Koletsky rats (Banks et al., 2002).

The soluble form of the leptin receptor is not produced by alternative splicing, since an OBRe transcript has not been discovered in humans (Chua et al., 1996). Indeed, it can be generated by ectodomain shedding of membrane-spanned receptors (Ge et al., 2002). The soluble leptin receptor circulates in the blood and can bind leptin with a high affinity (Lammert et al., 2001; Li et al., 1998). Thus, it plays a role in regulating the plasma levels of free leptin, the biologically active form (Chan et al., 2002). In obese individuals, soluble receptor levels are reduced (Ogier et al., 2002), whereas the receptor is upregulated in emaciation (Kratzsch et al., 2002).

The long and fully functional isoform is expressed mainly in the hypothalamus (Bjorbaek et al., 1997), and represented on many other cell types as well, such as lung, kidney, adipocytes, endothelial cells, mononuclear blood cells, stomach, muscle, liver, pancreatic islets,



269 Fig. 1. Leptin receptor isoforms. CR=cytokine receptor domain, F-III=fibronectin type III domain, Box 1, 2, 3=consensus intracellular motifs.

244 keratinocytes located at wound margins, osteoblasts,
 245 endometrium, placenta and umbilical cord (Akerman
 246 et al., 2002; Buyse et al., 2001; Ebenbichler et al., 2002;
 247 Frank et al., 2000; Goiot et al., 2001; Kim et al., 2000;
 248 Lee et al., 2002; Morton et al., 1999). OBR found in
 249 mouse testicular germ cells show an age- and stage-
 250 dependent distribution (El-Hefnawy et al., 2000),
 251 whereas levels in the ovary change with the menstrual
 252 cycle (Koshiba et al., 2001). In late-onset obese rats,
 253 diminished hypothalamic OBR is observed as they age
 254 (Scarpace et al., 2001).

255 Mutation in OBR causes early onset obesity in
 256 rodents (Ahima and Flier, 2000). In diabetic *db/db* mice,
 257 a truncated form of the receptor is produced, which
 258 lacks functional activity and actually leads to obesity
 259 and diabetes. These mice show highly elevated leptin
 260 levels and are not able to respond to leptin. Zucker
 261 diabetic fatty rats (ZDF, *fa/fa*) are homozygous for a
 262 Glu→Pro substitution in the extracellular domain of
 263 their OBR (Carpenter et al., 1998; Unger, 2000), while
 264 Koletsky rats have a point mutation, which results in
 265 failure of expression of OBR (Ahima and Flier, 2000). In
 266 humans, mutations in the OBR gene are extremely rare.
 267 Both humans and rodents lacking functional leptin
 268 receptors show early onset obesity, hyperphagia and

hypothalamic hypogonadism. In the mouse, mutations 270
 are also associated with hyperglycaemia, hypercorticism 271
 and hypothermia (Ahima and Flier, 2000). 272

**3. The primary signal transduction pathway of leptin: 273
 Jak/STAT signalling 274**

The Jak/STAT pathway mainly transmits leptin sig- 275
 nalling. Cytoplasmic tyrosine kinases, members of the 276
 Jak family, recognise and associate with a specific 277
 membrane-proximal domain of the receptor upon ligand 278
 binding (Heim, 1996). OBR has been shown to recruit 279
 Jak2 and Jak1 (Bjorbaek et al., 1997), however, a recent 280
 study indicates that, under physiological conditions, 281
 only Jak2 is activated during OBR signalling (Kloek 282
 et al., 2002). Activated Jaks transphosphorylate each 283
 other, as well as certain tyrosine residues of the receptor. 284
 In the case of OBR, these residues are Tyr985 and 285
 Tyr1138 (Banks et al., 2000; Eyckerman et al., 1999), 286
 thus providing a docking site for downstream molecules. 287

Phosphorylated Tyr1138 serves as a binding site for 288
 STAT proteins. Replacement of this tyrosine residue 289
 with serine specifically disrupts STAT signalling (Bates 290
 et al., 2003). OBR signalling predominantly results in 291

292 STAT3 binding (Baumann et al., 1996; Bendinelli et al.,
293 2000; Briscoe et al., 2001a,b; Ghilardi et al., 1996; Goiot
294 et al., 2001; Sanchez-Margalet and Martin-Romero,
295 2001; Tsumanuma et al., 2000). STAT1 (Baumann et al.,
296 1996; Bendinelli et al., 2000), STAT5 (Baumann et al.,
297 1996; Bendinelli et al., 2000; Briscoe et al., 2001b) and
298 STAT6 (Bendinelli et al., 2000) were activated by leptin.
299 STAT protein is activated by leptin in vivo, dependent
300 on the target tissue, and may differ considerably in
301 different cell types.

302 It has recently been discovered that these molecules
303 are not recruited from a cytosolic monomeric pool, but
304 from scaffolding/chaperone complexes, stasosomes, of
305 which even their inhibitors appear to be a part
306 (Ndubuisi et al., 1999; Sehgal, 2000). As a next step,
307 recruited STATs become tyrosine-phosphorylated by
308 Jaks (Heim, 1996), which leads to dissociation from the
309 receptor and forming of homo- or heterodimers. STAT
310 dimers then translocate into the nucleus and act as
311 transcription factors by binding specific response
312 elements in the promoter of their target genes, such
313 as sis-inducible-element (SIE), acute-phase-response-
314 element (APRE) and other GAS-like elements
315 (Baumann et al., 1996; Bendinelli et al., 2000; Heim,
316 1996).

317 This signalling pathway can be inhibited by a recently
318 described specific molecule that is a suppressor of
319 cytokine signalling-3 (SOCS-3; Endo et al., 1997; Starr
320 et al., 1997). This molecule is a member of a small SH2
321 domain-containing protein family, which appears to
322 operate by binding to the phosphorylated tyrosine resi-
323 dues of signalling molecules and mediates either their
324 degradation or inhibition (Hansen et al., 1999). SOCS-3
325 binds to Jak2 in a leptin-dependent fashion, inhibiting
326 Jak-induced autophosphorylation and phosphorylation
327 of the receptor. As a potent inhibitor of leptin signalling,
328 it has been implicated in the leptin resistance seen in
329 obesity (Bjorbaek et al., 1999). Another molecule has
330 recently been implicated as a negative regulator of
331 leptin's effect through Jak/STAT signalling; protein
332 tyrosine phosphatase 1B (PTP1B) (Cheng et al., 2002;
333 Kaszubska et al., 2002; Zabolotny et al., 2002). Jak2 has
334 a consensus recognition motif and it was dephos-
335 phosphorylated by PTP1B in transfection studies (Zabolotny
336 et al., 2002). PTP1B knockout mice show increased
337 leptin sensitivity, enhanced STAT3 phosphorylation,
338 and weight loss (Cheng et al., 2002; Kaszubska et al.,
339 2002). PIAS3 (protein inhibitor of activated STAT3)
340 was also characterised as an inhibitor of the Jak/STAT
341 pathway. However, it must block STAT3 DNA binding
342 activity specifically, as no such effect was observed with
343 STAT1 (Chung et al., 1997).

344 Leptin exerts its effects mainly through the hypo-
345 thalamus, primarily by utilising the Jak/STAT pathway.
346 It seems that the only STAT stimulated by leptin in the
347 hypothalamus is STAT3. STAT3 immunoreactivity has

348 been demonstrated in the paraventricular nucleus, peri-
349 ventricular neurons, arcuate nucleus and the lateral
350 hypothalamic area of the rat hypothalamus (Hakansson
351 and Meister, 1998; Hubschle et al., 2001). Hakansson
352 et al. (1999) showed STAT3 immunoreactivity in
353 hypocretin/orexin neurones of the lateral hypothalamus,
354 which stimulate food intake, as well as in galanin
355 neurones that also have an effect on feeding. NPY,
356 POMC and AGRP neuropeptide-containing neurons
357 respond directly to leptin, most likely via the Jak/STAT
358 pathway (Brown et al., 2001). STAT3 activation
359 together with OBR was also detected in vagal afferent
360 neurons located in nodose ganglia, as well as in the
361 nucleus tractus solitarius and the dorsal motor nucleus
362 of the vagus nerve (Buyse et al., 2001).

363 Leptin has several other effects in different cell types
364 mediated by the activation of STAT proteins. Increased
365 STAT3 activity due to leptin treatment has been found
366 in the antral mucosa, followed by a decrease in gastric
367 secretion and release of gastrin and somatostatin (Goiot
368 et al., 2001). Leptin has an impact through STAT3 on
369 oocytes, testicular germ cells and the endometrium
370 (Devos et al., 1997; El-Hefnawy et al., 2000; Matsuoka
371 et al., 1999). In blood mononuclear cells, leptin increases
372 Jak2/3 and STAT3 phosphorylation, and both phos-
373 phosphorylation and STAT3 association of the RNA binding
374 protein Sam68. This results in proliferation and acti-
375 vation of T lymphocytes when stimulated by PHA or
376 ConA, whereas it promotes proliferation and cytokine
377 production in monocytes (Sanchez-Margalet and
378 Martin-Romero, 2001). STAT3 activation as a result of
379 leptin treatment has also been reported in keratinocytes,
380 suggesting a role for leptin in skin repair (Goren et al.,
381 2003).

382 In *db/db* mice, which lack the long form of the leptin
383 receptor, impaired STAT signalling has been demon-
384 strated (Ghilardi et al., 1996). The short form of the
385 leptin receptor is also unable to activate STAT proteins
386 (Ghilardi et al., 1996), suggesting that *db* phenotypes,
387 especially morbid obesity, are caused by the failure of
388 STAT signalling, and STAT-induced events play a key
389 role in controlling energy homeostasis. In leptin-
390 deficient *ob/ob* mice, a significantly lower STAT3
391 immunoreactivity was observed in the hypothalamic
392 arcuate nucleus compared to the wild type (Hakansson-
393 Ovesjo et al., 2000). This again supports the idea that
394 STAT signalling has a pivotal role in controlling body
395 weight.

396 Aged obese rats also show impairment of STAT3
397 activation. In a rodent model of late-onset obesity,
398 leptin-induced maximal phosphorylation and binding of
399 STAT3 in the hypothalamus was greater in young rats
400 than old ones (Scarpace et al., 2001). In another study,
401 diminished phospho-STAT3 binding to its responsive
402 element was observed after leptin administration in
403 aged rats (Scarpace et al., 2000). Ageing often causes

404 abnormalities in lipid metabolism and obesity-related
405 complications. To determine whether these problems
406 are due to leptin resistance, Wang et al. (2001) studied
407 Zucker diabetic fatty rats that were hyperleptinemic
408 after gene transfer. In young rats, expression of proteins
409 involved in the reduction of body fat, such as acyl-
410 CoA-oxidase (ACO), carnitine-palmitoyl-transferase-1
411 (CTP-1) and peroxisome proliferator activated
412 receptor- α (PPAR α), increased, but decreased in old
413 rats. After induced hyperleptinemia, expression of an
414 inhibitor of the Jak/STAT pathway, SOCS-3, was higher
415 in the white adipose tissue of older rats. This suggests
416 that the decline of leptin-induced anorexic actions with
417 age is due to increased inhibition of the Jak/STAT signal
418 transduction pathway, which is primarily used to exert
419 leptin's effect on body weight and energy balance (Wang
420 et al., 2001).

421 However, it seems that some of leptin's major effects
422 do not require STAT3 signals. Disrupting STAT3 sig-
423 nalling by replacing tyrosine with serine at position 1138
424 of the leptin receptor results in obesity, but reproduc-
425 tion, linear growth and control of NPY expression is not
426 impaired, and glucose levels do not increase as much as
427 in *db/db* mice (Bates et al., 2003). This indicates the
428 importance of other, STAT-independent, signalling
429 pathways for leptin.

430 4. The MAPK pathway in leptin signalling

431 The Ser/Thre MAPK (Erk1/Erk2) pathway can be
432 stimulated by either the long or the short isoform, but to
433 a lesser extent by the latter (Banks et al., 2000; Bjorbaek
434 et al., 1997). This observation supports the idea that the
435 distal portion of the OBR is not essential to activate
436 MAPK signalling, although to achieve maximal acti-
437 vation, an intact form of the long receptor is needed.
438 This further supports the idea that leptin stimulates the
439 MAPK pathway in two different ways.

440 It has previously been reported that Tyr985 of the
441 long leptin receptor isoform plays an important role in
442 leptin-induced full Erk activation (Banks et al., 2000).
443 Bjorbaek et al. revealed several steps of this signalling
444 cascade in an elegant study of the leptin receptor
445 (Bjorbaek et al., 2001). As a result of leptin admin-
446 istration, Tyr985 becomes phosphorylated by the
447 recruited Jaks, mainly Jak2 and Jak1, and provides a
448 docking site for the SH2-domain containing protein
449 tyrosine phosphatase, SHP-2. After binding to that
450 specific tyrosine residue, SHP-2 is phosphorylated at the
451 C-terminus. This phosphorylated form, together with its
452 adapter molecule Grb-2, activates downstream signal-
453 ling effects (Banks et al., 2000). OBR lacking Tyr985 is
454 less able to induce Erk signalling, but is not completely
455 inhibited (Banks et al., 2000).

456 Based upon this observation, it seems that there is
457 another way of inducing the Erk pathway through

458 SHP-2 that is completely independent of the presence of
459 Tyr985, and can also be stimulated by the short isoform
460 of the leptin receptor (Banks et al., 2000). In this case,
461 Jak2 enhances Erk activation via OBR independently of
462 receptor phosphorylation (Bjorbaek et al., 1997). Jak2
463 associates with the SH2 domain-containing adapter pro-
464 tein, Grb-2 and SHP-2 (Banks et al., 2000; Stofega et al.,
465 2000), and this complex activates further signalling
466 steps. SHC, another SH-2 containing protein that is able
467 to associate with Grb-2, has also been shown to phos-
468 phorylate tyrosine after leptin treatment (Gualillo et al.,
469 2002).

470 Downstream signalling in both pathways requires an
471 intact catalytic domain of SHP-2. A lack of phosphatase
472 activity causes a failure of Erk phosphorylation
473 (Bjorbaek et al., 2001). However, it is not clear which
474 molecules are involved in transmitting the leptin signal.
475 In many systems, subsequent steps lead to the activation
476 of ras and raf molecules, followed by the activation of
477 MEK1 (Blenis, 1993). Alternatively, SHP-2 signalling
478 may require stimulation by integrins to induce MEK1
479 activation (Fujioka et al., 1996). Activated MEK1 phos-
480 phorylates Erk1/2, and finally specific target genes are
481 expressed, such as c-fos or egr-1, a zinc-finger transcrip-
482 tion factor that influences the initiation of growth and
483 differentiation (Ahima and Flier, 2000; Bjorbaek et al.,
484 2001).

485 It seems that SHP-2 does not affect leptin-induced
486 STAT3 tyrosine phosphorylation or STAT-mediated
487 gene transcription, although SHP-2 was thought to be
488 a negative regulator of STAT3 gene induction (Car-
489 penter et al., 1998). Nevertheless, Bjorbaek et al. did
490 not detect such an effect using SHP-2 mutant proteins,
491 and thus concluded that SHP-2 does not directly affect
492 the STAT pathway (Bjorbaek et al., 2001). Instead,
493 they suggest that SOCS-3 may be the one responsible
494 for this negative regulatory effect, because it also
495 recognises and binds Tyr985 to exert its inhibitory
496 effect. Therefore, SHP-2 and SOCS-3 are competitors
497 and SHP-2 acts as an indirect positive regulator for
498 STAT signalling.

499 Activation of the MAPK signalling cascade has been
500 demonstrated both in vitro (Banks et al., 2000; White
501 et al., 1997b) and in vivo in the hypothalamus, liver and
502 adipose tissue (Bjorbaek et al., 2001; Figenschau et al.,
503 2001; Machinal-Quelin et al., 2002; Yamashita et al.,
504 1998). In the human pancreatic beta cell line MIN6,
505 leptin-induced MAPK activation has also been detected.
506 Leptin induced proliferation via this cascade. In
507 addition, specific MAPK inhibitors blocked DNA syn-
508 thesis and cell viability caused by leptin. This suggests
509 that, at least in part, this mechanism is involved in
510 obesity-induced pancreatic islet hypertrophy (Tanabe
511 et al., 1997). In monocytes, leptin induces expression
512 and secretion of the interleukin-1 receptor antagonist
513 (IL-1Ra), utilising the MAPK pathway that activates

the NF κ B binding site of the promoter by an as yet uncharacterised factor (Dreyer et al., 2003).

Leptin has also been shown to induce apoptosis through the MAPK pathway in precursor cells of the osteoblastic lineage. In this case, Erk1/2 activates cytosolic phospholipase A2 (cPLA2) that leads to cytochrome c release and finally caspase-3 and caspase-9 activation, which co-ordinate the execution of the cell (Kim et al., 2003).

5. Cross-talk of leptin signalling with insulin-induced pathways

Leptin can also act through some of the components of the insulin-signalling cascade, although reports disagree about its importance in modifying insulin-induced gene expression. Insulin itself acts through its receptor by recruiting different insulin receptor substrates (IRSs) that are tyrosine phosphorylated by the intrinsic kinase activity of the receptor. Phosphorylation of IRSs increases the affinity by which they bind other signalling molecules, and initiates further steps on the pathway. An important target of IRS molecules is phosphatidylinositol 3-kinase (PI 3-kinase) that generates inositol-trisphosphate (PIP₃). IRSs exert PI 3-kinase activation through association with its regulatory subunit (p85), thus increasing the activity of the catalytic domain. Increased PIP₃ levels lead to activation of PIP₃-dependent serine/threonine kinases, such as PDK-1,2, which can activate Akt, another serine/threonine kinase that has several targets, such as glycogen synthase kinase-3 (GSK3). GSK3 is a serine kinase and plays a role in several actions, such as phosphorylation of glycogen synthase, C/EBP α (Kido et al., 2001; Szanto and Kahn, 2000). Insulin decreases GSK3 activity by serine phosphorylation (Szanto and Kahn, 2000).

Interaction with these signalling molecules has been studied both in vitro and in vivo (Szanto and Kahn, 2000; Wang et al., 1998), supporting the idea that leptin and insulin pathways may be connected. However, results are inconsistent in different cell lines and the complete mechanism remains unclear. Studying a well-differentiated hepatoma cell line, Fao, shows that leptin itself has no direct effect on the insulin pathway, but leptin pre-treatment transiently enhances insulin-induced IRS-1 phosphorylation and its association with p85, while decreasing IRS-2 activation. Leptin administration results in the elevation of phosphorylated Akt, but does not modify insulin-induced phosphorylation. Leptin alone affects GSK3 serine-phosphorylation to a lesser extent than insulin, but does not enhance insulin's effect any further (Szanto and Kahn, 2000).

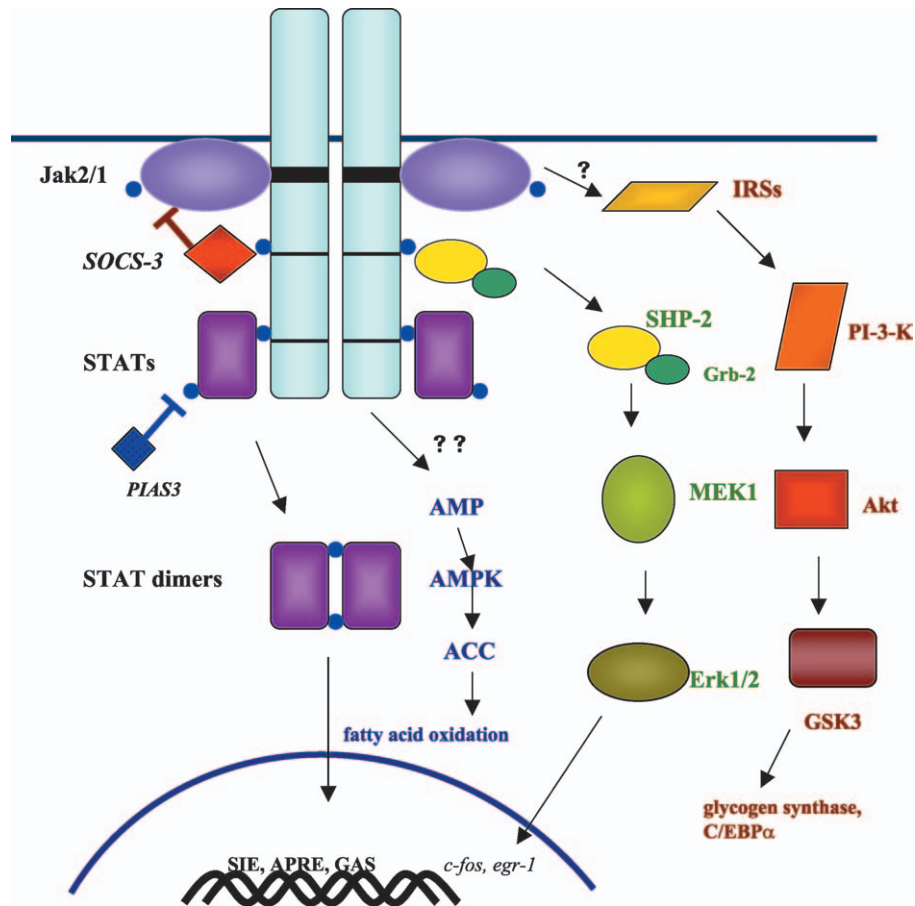
Wang et al. (1997) used hepatoma cell lines over-expressing the long form of OBR. They found no significant changes in IRS-1 or IRS-2 phosphorylation,

although they detected recruitment of PI3-kinase to IRS-2. No modulation of the immediate cell response to insulin was seen, however. In C2C12 muscle cells, leptin stimulates glucose transport by recruiting GLUT4 to the cell surface, and it is blocked by wortmannin, which can inhibit both PI3-kinase and MAPK (Berti and Gammeltoft, 1999). Leptin increases PI3 kinase activity and affects hormone-sensitive lipase that could be blocked by PI3 kinase inhibitors (O'Rourke et al., 2001). In in vivo experiments, leptin stimulated IRS-1-associated PI3-kinase activity shortly after administration in several tissues, whereas this effect was not detected after longer treatment, although leptin did enhance insulin's effect (Kim et al., 2000).

PI3-kinase can further activate a signalling pathway through the activation of cyclic nucleotide phosphodiesterase-3B (PDE3B), a cAMP-degrading enzyme (Zhao et al., 1998). In pancreatic beta cells, leptin diminishes cAMP levels and inhibits glucagon-like peptide-1-stimulated insulin secretion (Zhao et al., 1998). Leptin-mediated PDE3B induction and subsequent cAMP degradation has also been demonstrated in rat hepatocytes (Zhao et al., 2000). Thus, in hepatocytes, leptin-like insulin antagonises glucagon actions. Furthermore, this PDE3B pathway seems to interact with Jak/STAT signalling in the hypothalamus, as clostamide, a PDE3 inhibitor, blocked the tyrosine phosphorylation of STAT3 and reversed the effects of leptin on food intake and body weight (Zhao et al., 2002).

A signalling pathway divergent from activated PI3-kinase results in the induction of K(ATP) channels, which leads to hyperpolarisation of the cell (Harvey and Ashford, 1998). This effect was described in the CRI-G1 rat insulinoma cell line (Harvey et al., 1997), isolated human pancreatic islets (Lupi et al., 1999) and glucose-receptive hypothalamic neurons (Spanswick et al., 1997). Downstream signalling cascades stimulated by insulin, such as p70s6k, Akt or MAPK, do not seem to be utilised by leptin in this process, since blockade of them does not occlude leptin activation of K(ATP) channels (Harvey et al., 2000a). Phosphatidylinositol(3,4,5)-trisphosphate (PtdIn(3,4,5)P₃) appears to be an attractive candidate downstream from PI3-kinase, as it mimics leptin's effects. Surprisingly, leptin does not increase the total cellular PtdIn(3,4,5)P₃ content, but it is possible that there is only a localised increase in it (Harvey et al., 2000a). It is likely that PtdIn(3,4,5)P₃ leads to disruption of actin filaments, the final known step in enhancing the activity of K(ATP) channels by leptin (Harvey et al., 2000b). This is supported by the observation that phalloidin (an actin filament stabiliser) prevents activation of K⁺ channels, both by leptin and PtdIn(3,4,5)P₃ (Harvey et al., 2000b).

These observations show how leptin is implicated in insulin signalling, but data are conflicting. However, all tissue types respond differently to signals, and cross talk



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Fig. 2. Signalling pathways affected by leptin. Blue dots represent phosphate groups. Abbreviations are shown in the text.

623 between signalling pathways is also highly dependent on
624 the local environment.

625 6. Leptin-induced signalling pathways in lipid 626 metabolism

627 Leptin has a protective effect against lipotoxicity in
628 nonadipose tissues (Unger et al., 1999), however, the
629 pathway used to exert this effect is not fully understood.
630 Nonadipose tissues have low levels of triacylglycerol
631 (TG), which markedly increase with a lack of functional
632 OBR (Lee et al., 1994). Leptin stimulates the oxidation
633 of fatty acids and thereby prevents cells from lipo-
634 apoptosis and the accumulation of lipid droplets in
635 nonadipose tissues (Unger et al., 1999). Recently,
636 Minokoshi et al. (2002) have suggested a novel pathway
637 that could be involved in leptin's action in metabolism.
638 Leptin has been shown to activate the $\alpha 2$ subunit of
639 5'-AMP-activated protein kinase (AMPK), which
640 stimulates fatty-acid oxidation by blocking the effect of
641 acetyl coenzyme A-carboxylase (ACC) in skeletal
642 muscle. AMPK phosphorylates ACC- β , the muscle
643 isoform, which leads to its inhibition and increased

fatty-acid oxidation by disinhibiting carnitine palmitoyl
transferase (CTP) (Kahn and Flier, 2000). Elevated
AMP levels activate AMPK 15 min after leptin treat-
ment. This effect has been shown to be due to a direct
effect of leptin on muscle. Leptin also acts through the
 α -adrenergic system, causing similar but delayed effects,
as a result of its hypothalamic actions (Lee et al., 1994).
It is not yet clear how leptin elevates AMP levels and
activates AMPK when it directly targets muscle cells.

Nevertheless, this is not the only way that leptin
increases fatty acid oxidation. In normal non-
adipocytes, e.g. pancreatic islets, PPAR α is activated by
STAT3 induction after leptin administration. Then,
binding to its response element, it induces expression of
acyl CoA oxidase and CTP-1, which leads to increased
oxidation of fatty acids (Unger et al., 1999). In cells
lacking functional OBR, this system does not function.
Instead, high levels of fatty acids result in increased
expression of PPAR γ and lipogenic enzymes, such as
ACC or fatty acid synthase (Unger et al., 1999).

In summary, leptin acts as a multifunctional cytokine
in different tissues, and is involved in many cellular
functions throughout the whole body. To perform its
widespread effects, it interacts with many intracellular

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669 signalling molecules, cross talking with different signal
 670 transduction pathways through its receptor, which
 671 seems to be the only receptor that leptin binds to
 672 (Fig. 2). Several physiological effects of leptin have been
 673 elucidated, but the signalling steps for many of them are
 674 still unclear or not detailed. To have a complete view of
 675 leptin's actions and to be able to modify its effects in the
 676 right way therapeutically, these gaps need to be filled.
 677 Having a complete signalling network map for leptin
 678 could help us find effective ways to influence the func-
 679 tion we need to, and thus leptin signalling could be
 680 inserted in the complex signalling pattern that charac-
 681 terises a cell challenged by different agents at one time.

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