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Choroid plexus epithelial cells co-express the long and short form of the leptin receptor

Beatriz Merino, Carmen Díez-Fernández, Mariano Ruiz-Gayo *, Beatriz Somoza

Departamento de Farmacología, Tecnología y Desarrollo Farmacéutico, Facultad de Farmacia, Universidad San Pablo-CEU, Madrid, Spain

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Abstract

Two types of leptin receptors (Ob-R) are structurally and functionally characterized. The Ob-Ra, also called short form of leptin receptor, and the Ob-Rb, which is functionally coupled to intracellular signalling pathways. In the CNS, the Ob-Ra is mainly present in the choroid plexus and in the blood–brain barrier and it is involved in leptin transport from the periphery to the brain. The Ob-Rb is widely distributed in the hypothalamus and in many peripheral tissues and is coupled to the JAK/STAT signalling pathway. In this work, we describe the presence of both Ob-Ra and Ob-Rb in a cell line of choroid plexus epithelium. In addition, we demonstrate that Ob-Rb in the choroid plexus is functionally coupled to intracellular signalling pathways as leptin induced the phosphorylation of the signal transducer and activator of transduction 3 (STAT3).
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Keywords: Leptin; Ob-Ra; Ob-Rb; STAT3; pSTAT3; Desensitization

Leptin, the product of the *ob* gene, is an adipocyte-derived hormone involved in body weight and thermogenesis regulation [20,22]. Alternative splicing of Ob-R mRNA yields at least four isoforms of transmembrane leptin receptors (Ob-Ra, Ob-Rb, Ob-Rc, and Ob-Rd). All these receptors contain identical extracellular and transmembrane domains and differ in the length of the intracellular aminoacid sequence [15,21]. Ob-Rb are widely distributed in the hypothalamus [9,18] as well as in many peripheral tissues [6,10,12]. Binding of leptin to Ob-Rb results on the activation of JAK and a subsequent phosphorylation of Tyr^{985} and Tyr^{1138} of the intracellular domain of the receptor. Phosphorylated Ob-Rb activate signalling pathways involving STAT proteins [3]. In contrast, Ob-Ra are mainly restricted to the choroid plexus [7,20] and the blood–brain barrier [23] and are involved in the transport of leptin from the periphery to the brain [2,14]. These receptors contain a short intracellular domain but, at present, no transduction mechanism coupled to them has been properly characterized. Nevertheless, the physiopathological relevance of these receptors is important as it has been proposed that obesity might be linked to impaired transport of leptin from the periphery to the brain [19,21]. Although kinetic characteristics of leptin internalization in the choroid plexus are well characterized [11,13,17,23], the mechanism responsible for leptin transport disruption remains unknown.

The aim of this study was to evaluate (i) the ability of a tumoral cell line from choroid epithelial cells (SCP-89101302) to preserve the expression of leptin receptors, and (ii) the eventual functionality of these receptors.

Epithelial ovine choroid plexus cells (SCP-89101302, ECACC, UK) were grown on six-well plates in Eagle Minimum Essential Medium (EMEM), supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml) and streptomycin (100 μg/ml), until 80–85% confluency, then washed with calcium-free PBS and incubated for four days in EMEM supplemented with 1% FBS and 1% insulin–transferrine–selenium (see Fig. 1). For pSTAT3 determination, recombinant ovine leptin (Prospect, Israel) was added at a final concentration of either 10 ng/ml or 30 ng/ml. After 15–30 min, cells were washed and collected in calcium-free PBS. After centrifugation, samples were homogenized in ice-cold 20 mM Tris–HCl (pH 7.6) containing 100 mM potassium chloride, 5 mM sodium chloride, 2 mM EDTA, 1 mM EGTA, 5 μg/ml aprotinin, 10 μg/ml leupeptin, 5 mM sodium fluoride, 0.5% Nonidet P-40, 1 mM trisodium orthovanadate, 10 mM sodium molibdate, 1 mM phenylmethylsulfonyl fluoride and 1 mM sodium pyrophosphate. After centrifugation (5 min, 1000 rpm), the supernatant

Abbreviations: JAK, Janus kinases; Ob-Ra, short form of leptin receptor; Ob-Rb, long form of leptin receptor; STAT3, signal transducer and activator of transduction 3; pSTAT3, phosphorylated STAT3

* Corresponding author. Tel.: +34 913 724 700; fax: +34 913 510 475.
E-mail address: ruigayo@ceu.es (M. Ruiz-Gayo).

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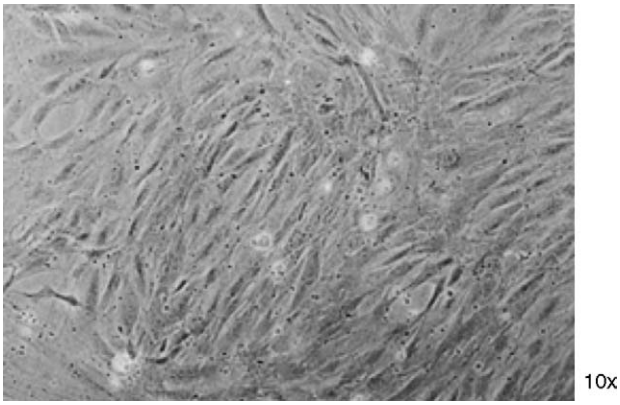

Fig. 1. Microphotography of a confluent monolayer of ovine choroids plexus epithelial cells with the typical cobble-stone morphology.

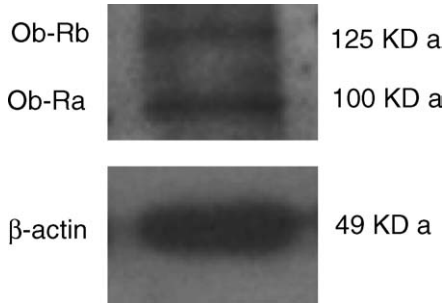

Fig. 2. Western blot analysis of SCP-89101302 cells reveals the presence of two bands at 100 and 125 kDa which are compatible with the molecular size of the OB-Ra and the Ob-Rb, respectively.

was combined with Laemli buffer (50 mM Tris pH 6.8 containing 10% SDS, 10% glycerol, 5% mercaptoethanol and 2 mg/ml blue bromophenol) to reach a final protein concentration of 1 mg/ml protein. Equivalent amounts of proteins were size-separated in 7% SDS–polyacrylamide gel electrophoresis. Proteins were transferred to PVDF membranes (Amersham Pharmacia, Spain) by using a transblot apparatus (BioRad, Spain). For immunoblotting, membranes were blocked with 5% non-fat dried milk in Tween–PBS for 1 h and incubated with primary antibodies against STAT3 (Santa Cruz Biotechnology, USA; anti-rabbit; 1:500 final dilution), pSTAT3 (Santa Cruz Biotechnology, USA; anti-mouse; 1:100 final dilution) or leptin receptor (Affinity Bioreagents, USA; anti-rabbit; final dilution 1:2000) overnight at 4 °C. After washing, appropriate IgG-peroxidase conjugated antibodies (Amersham Bioscience, UK; 1:10,000) were applied for 1 h. Blots were washed, incubated in commercial enhanced chemiluminescence reagents (ECL, Amersham Bioscience, UK) and exposed to autoradiographic films (Amersham Bioscience, UK). To prove equal loading of samples, blots were re-incubated with β-actin antibody (Sigma, USA). Films were scanned using a GS-800 Calibrated Densitometer (Bio-Rad, Spain) and blots were quantified using Quantity One software (Bio-Rad, Spain). The values for pSTAT3 were normalized with STAT3 to account for variations in gel loading. The antiserum against leptin receptor used in this study was raised to an epitope corresponding to amino acids 577–594 belonging to the extracellular domain of the receptor, which is conserved in all spliced forms of the rat leptin receptor. Specificity of primary antibodies was assessed by using the appropriated blocking peptides.

As illustrated in Fig. 2, Western blot analysis revealed the presence of a dense band compatible with the molecular weight of the Ob-Ra (100 kDa) and a less intense spot at 125 kDa, which is compatible with the Ob-Rb isoform. Both 100 and 125 kDa

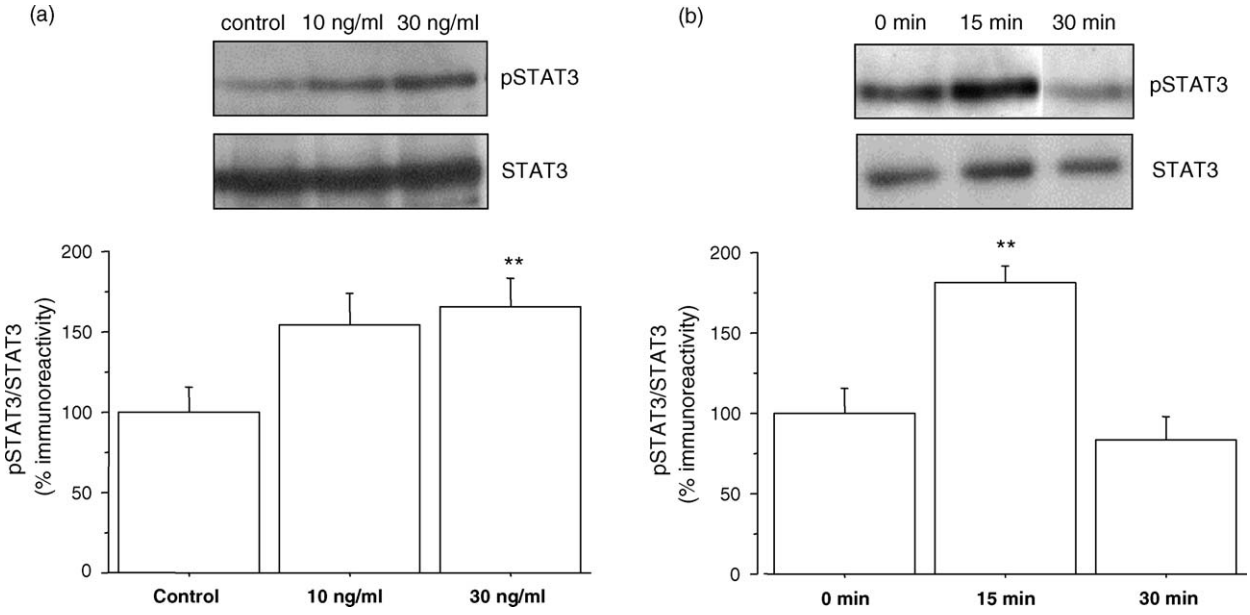

Fig. 3. (a) Western blots of SCP-89101302 cells incubated during 15 min either with 10 or 30 ng/ml of ovine leptin. Immunoreactive bands for STAT3 (bottom) and pSTAT3 (top) are representative of five independent experiments. Values were first nomalized with STAT3 and then expressed as percentage of the control group. pSTAT3 immunoreactivity was significantly increased in cells exposed to 30 ng/ml leptin ($^{*}P < 0.05$, Newman-Keul's test). (b) Idem in cells incubated with 30 ng/ml leptin for 15 and 30 min. pSTAT3 immunoreactivity increased after 15 min incubation but was similar to that found in controls in cells exposed to leptin during 30 min. Values are means ± S.E.M. of five independent experiments ($^{***}P < 0.001$, Newman-Keuls' test).

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