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RESEARCH PAPER

A cholecystokinin-1 receptor agonist (CCK-8) mediates increased permeability of brain barriers to leptin

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Background and purpose: Leptin regulates energy expenditure and body weight by acting both on the hypothalamus and on peripheral targets. Central actions of leptin are enhanced by cholecystokinin (CCK). The interaction between leptin and CCK makes physiological sense, as rats lacking CCK_1 receptors are resistant to peripheral leptin but not to leptin directly infused into the brain. We have recently reported that CCK enhances leptin effects by increasing the entry of leptin into the CNS. The aim of this work was to further characterize the effect of CCK ($10\,\mu g\,kg^{-1}$) on leptin kinetics as well as the CCK receptor subtype involved in the interaction between CCK and leptin.
Experimental approach: Experiments were carried out both in free-feeding and in fasted rats receiving a single dose of leptin ($100\,\mu g\,kg^{-1}$; i.p.). Parameters analysed over the next 6 h were plasma and cerebrospinal fluid concentrations of leptin.
Key results: We observed that CCK-8 depressed the increase in plasma leptin that followed the i.p. injection and simultaneously increased leptin concentration in the cerebrospinal fluid from 92 ± 25 to $230 \pm 24\,pg\,mL^{-1}$ ($P < 0.05$). The effect of CCK-8 was totally prevented by the CCK_1 receptor antagonist, SR-27,897 ($0.3\,mg\,kg^{-1}$, s.c.), but not by the CCK_2 receptor antagonist, L-365,260 ($1\,mg\,kg^{-1}$).
Conclusions and implications: These results show that CCK plays a role in regulating the access of leptin to the brain and suggest that CCK analogues, acting on CCK_1 receptors, might be useful drugs in improving leptin actions within the brain.
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Keywords: cholecystokinin; leptin; hypothalamus; CCK_1 receptor; SR-27,897; leptin transport; choroid plexus; blood–brain barrier

Abbreviations: BBB, blood–brain barrier; CCK, cholecystokinin; CNS, central nervous system; CSF, cerebrospinal fluid; NTS, nucleus tractus solitarius; OLETF, Otsuka Long-Evans Tokushima Fatty

Introduction

Leptin is a hormone synthesized by adipocytes (Zhang *et al.*, 1994; Campfield *et al.*, 1995) and, to a minor extent, by other tissues or organs (Bado *et al.*, 1998). Leptin regulates energy balance by acting on hypothalamic sites (Schwartz *et al.*, 1996b), and binds at least two receptors in the central nervous system (CNS) encoded by the Ob-R gene. The Ob-Ra receptor, also called 'short form', is widely expressed in the choroid plexus (Tartaglia *et al.*, 1995; Devos *et al.*, 1996) and brain vessels (Golden *et al.*, 1997), and is involved in leptin transport from blood to the CNS. The Ob-Rb receptor, identified in several hypothalamic nuclei (Fei *et al.*, 1997; Håkansson *et al.*, 1998), is involved in the regulation of energy balance and autonomic functions (Elias *et al.*, 2000).

Obesity is a disorder of energy homeostasis related to a disruption of intrinsically interconnected central and peripheral systems (Lafontan, 2005) and linked, at least partially, to a deficit of leptin production (Pelleymounter *et al.*, 1995) or to resistance to the effect of leptin (Chen *et al.*, 1996; Schwartz *et al.*, 1996a; van Heek *et al.*, 1997; Niimi *et al.*, 1999). Leptin resistance and obesity have been associated with mutations of any of the many splice variants encoded by the Ob-R gene of the leptin receptor (Chua *et al.*, 1996). Overall, leptin resistance seems to be linked to impaired transport from the blood to the CNS (Schwartz *et al.*, 1996b).

The importance of the peripheral hormone cholecystokinin (CCK) in regulating energy expenditure and body weight has been stressed by many groups, suggesting that both CCK and leptin share common targets in the

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hypothalamus (Barrachina *et al*., 1997; Elias *et al*., 2000; Matson *et al*., 2000, 2002; Morton *et al*., 2005; Merino *et al*., 2008). CCK dramatically enhances the effect of leptin on c-Fos synthesis in the paraventricular nucleus of the hypothalamus (Barrachina *et al*., 1997) as well as in brain stem areas (Emond *et al*., 1999) involved in food intake regulation. This interaction has functional consequences, as chronically administered CCK facilitates the effect of leptin in decreasing body weight. We and others have recently reported that the synergy between leptin and CCK is not only linked to a decrease in food consumption, but also to the activation of metabolic pathways (Matson *et al*., 2000, 2002; Merino *et al*., 2008).

The Otsuka Long-Evans Tokushima Fatty (OLETF) rat, a naturally obese strain lacking CCK_1 receptors, is a useful model to characterize the interaction between CCK and leptin by means of a genetic approach. In spite of other proteomic alterations that might eventually account for the metabolic alterations observed in these animals, OLETF rats exhibit type II diabetes together with leptin resistance (Moran and McHugh, 1988; Takiguchi *et al*., 1998). Interestingly, exogenous leptin only increases c-Fos expression in the hypothalamus and inhibits food intake in OLETF rats when administered i.c.v., but not when administered peripherally (Niimi *et al*., 1999). All these data suggest that both CCK and leptin might act coordinately and also support a role for CCK receptors in the accessibility of leptin to its targets in the CNS. In this regard, we have reported that CCK receptor antagonists, administered during the onset of the dark period, increase plasma leptin concentration (Cano *et al*., 2003). The aim of this study was to further characterize the effect of CCK on the entry of leptin into the CNS, as well as the CCK receptor subtype eventually involved in such an effect.

Materials and methods

Animals

All animal procedures and experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC) for the care and use of laboratory animals.

Seven-week-old male Sprague–Dawley rats (CRIFA, Barcelona, Spain) were individually housed in hanging cages, under a 12-h light/dark cycle in a temperature-controlled room (22 °C), with standard chow and water available *ad libitum*. Animals were handled daily, for at least 1 week, to avoid stress by manipulation on the day of the experiment.

Treatment and experimental design

To measure the kinetics of plasma leptin, four groups of 6–8 animals received (at 0900 hours) either CCK-8 or saline. After 30 min, rats received either leptin or vehicle. Rationale for timing of these injections is based on a study showing that previous administration of CCK-8 enhances pSTAT3 (phosphorylated signal transducer and activator of transcription-3) immunostaining within the arcuate nucleus of the hypothalamus of rats treated with a tracer dose of human leptin (Merino *et al*., 2008). Because CCK reduces meal size but not overall food intake over long periods (West *et al*., 1984), we reasoned that a delay of 30 min between CCK and leptin administration would minimize the eventual influence of a reduced caloric intake on leptin kinetics. For plasma leptin monitoring, blood samples were obtained from the same rat at different times (0–6 h) after leptin administration. Animals were killed after the last sampling.

These assays were carried out both in free-feeding and overnight-fasted (16 h) animals. In the latter group, rats were fasted from 1700 hours on the previous day and they remained without food during the assay. To analyse the effect of CCK-8 on leptin concentration within the cerebrospinal fluid (CSF), two groups (5–6 animals per group) of rats were used. In this case, all animals received an administration of CCK-8 or saline (0930 hours) followed by an administration of leptin (1000 hours). The effect of CCK receptor antagonists on both plasma and CSF leptin concentration was analysed following a similar protocol. Briefly, CCK receptor antagonists or vehicle were administered at 0900 hours, CCK-8 or saline were given at 0930 hours and leptin was finally administered to all animals at 1000 hours. In this case, blood and CSF samples were obtained from the same animal. Six to eight animals per group were used in this assay.

Sampling for leptin kinetic studies

Blood samples were obtained at 0, 1, 2, 3, 4 and 6 h after leptin or vehicle administration. Samples at time 0 were obtained by a transversal cut in the apical segment of the tail and blood collected in chilled heparinized tubes (Microvette, Sarstedt, Germany). For further samples, the wound was re-opened by means of a gentle massage. Samples were then centrifuged for 15 min at 2500 *g* and plasma stored at −80 °C until leptin, glucose and insulin assay. For CSF sampling, rats were anaesthetized with ketamine/xylazine 2 h after rat leptin administration and CSF obtained from the cisterna magna. Briefly, the head was positioned at a 50° angle to the vertebral column, and a microsyringe was placed between the external occipital bone and the atlas. The atlanto-occipital membrane was then perforated and CSF (~100 μL) extracted. Blood contaminated samples were discarded and the remaining samples centrifuged for 10 min at 2500 *g* and stored at –80 °C until assay.

Biochemical determinations

Leptin was determined in plasma and CSF by using specific EIA (enzyme immunoassay) kits (Assay Designs Inc., Ann Arbor, Michigan, USA). Intra- and interassay variations were 7.3–7.7 and 1.2–5.8%. Insulin was determined by using a specific EIA kit (1.8% intraassay variation and 3.8% interassay variation) for rat insulin (Mercodia, Uppsala, Sweden). Glucose was measured by a spectrophotometric method (Glucose Trinder Method; Roche, Barcelona, Spain).

Effect of CCK-8 and leptin on food intake

The effect CCK-8 (10 μg kg^{-1}, i.p.), leptin (100 μg kg^{-1}, i.p.) or their combination was only tested in free-feeding animals.

Pre-weighed pelleted chow was placed in the feeding box at 1000 hours and weighed, together with spilled food, at 1200 hours and 1600 hours.

Statistical analysis

Individual group comparisons were made using a two-way ANOVA. The factors of variation were treatment and time. Individual dose effects within a given group were analysed by using a one-way ANOVA, followed by Newman–Keuls' *post hoc* test. Statistical significance was set at $P<0.05$.

Drugs and doses

Rat recombinant leptin and CCK-8 were from Sigma (St Louis, Missouri, USA). The CCK_1 receptor antagonist, SR-27,897, and the CCK_2 receptor antagonist, L-365,260, were a generous gift of Sanofi-Synthélabo (Paris, France) and MSD (Treton, New Jersey, USA), respectively. Other chemicals and solvents were from Sigma. SR-27,897 (CCK_1 receptor antagonist; $0.3\,mg\,kg^{-1}$) and L-365,260 (CCK_2 receptor antagonist; $1\,mg\,kg^{-1}$) were dissolved in 4% carboxymethylcellulose and administered s.c. The doses of respective antagonists have been shown to bind selectively to respective CCK receptors (Ruiz-Gayo *et al.*, 2000). CCK-8 was dissolved in saline and administered i.p. at the dose of $10\,\mu g\,kg^{-1}$, to maintain its plasma concentration ranging from 20 to 40 pM, which is fairly close to physiological postprandial concentrations (Linden and Södersten, 1990). Leptin was dissolved in saline buffer (pH = 7) and administered i.p. at $100\,\mu g\,kg^{-1}$.

Results

CCK-8 decreased plasma leptin concentration

Figure 1a illustrates the effect of CCK-8 (i.p., $10\,\mu g\,kg^{-1}$) on plasma leptin concentration in free-feeding rats treated with exogenous leptin (i.p., $100\,\mu g\,kg^{-1}$) or vehicle. Two-way ANOVA revealed that plasma leptin concentration was dependent on pharmacological treatment ($F_{(3,139)}=112.8$; $P<0.001$) and time after leptin administration ($F_{(5,139)}=27\,453$; $P<0.001$). The interaction between both treatment and time was also significant ($F_{(15,139)}=16\,335$; $P<0.001$). In animals treated with exogenous leptin, plasma concentration reached a maximal value of $40\,ng\,mL^{-1}$ approximately 2 h after leptin administration. CCK-8 significantly suppressed the leptin surge, which was only significant 1 h after leptin administration. One-way ANOVA showed a significant effect of CCK-8, at 1 ($F_{(3,23)}=88\,154$; $P<0.01$), 2 ($F_{(3,25)}=33\,043$; $P<0.01$), three ($F_{(3,27)}=72\,359$; $P<0.01$) and 4 h ($F_{(3,27)}=19\,952$; $P<0.01$) after leptin administration.

A similar assay was performed in overnight-fasted animals (Figure 1b). Under these conditions, plasma leptin concentration reached a maximal value of $22\,ng\,mL^{-1}$, at 1 h after leptin administration. As in the case of free-feeding animals, here also CCK-8 depressed the increase in plasma leptin over time (two-way ANOVA, $F_{(3,68)}=25\,525$, $P<0.01$ for treatment; $F_{(5,68)}=7662$, $P<0.01$ for time; $F_{(15,68)}=2823$, $P<0.01$ for the interaction) (Figure 1b).

Effect of CCK-8 and leptin on the plasma concentration of glucose and insulin

The effect of CCK-8 (i.p., $10\,\mu g\,kg^{-1}$) and leptin (i.p., $100\,\mu g\,kg^{-1}$) on plasma levels of glucose and insulin was analysed 2 and 4 h after leptin administration, both in free-feeding and in fasted rats (Table 1). Neither glucose nor insulin values were modified by pharmacological treatment.

Table 1 Effect of CCK-8 and leptin on insulin and glucose plasma concentrations both in free-feeding and fasted rats

Treatment	*Insulin ($\mu g\,L^{-1}$)*			*Glucose (mM)*		
	Basal	*2 h*	*4 h*	*Basal*	*2 h*	*4 h*
Free-feeding rats						
Control	4.8 ± 0.6	4.9 ± 0.8	6.3 ± 0.6	8.2 ± 0.4	7.7 ± 0.3	8.0 ± 0.4
Leptin	5.6 ± 0.9	4.1 ± 1.1	4.4 ± 1.3	8.1 ± 0.3	7.6 ± 0.3	7.3 ± 0.3
CCK-8	5.9 ± 0.9	4.4 ± 0.6	3.9 ± 0.8	8.2 ± 0.4	7.5 ± 0.3	7.2 ± 0.4
Leptin + CCK-8	5.0 ± 0.9	5.7 ± 0.6	4.9 ± 1.0	8.1 ± 0.4	8.1 ± 0.2	7.7 ± 0.5
Fasted rats						
Control	1.4 ± 0.1	2.2 ± 0.6	1.6 ± 0.1	5.9 ± 0.8	4.2 ± 0.2	3.8 ± 0.5
Leptin	1.4 ± 0.2	1.3 ± 0.8	1.3 ± 0.1	4.5 ± 0.6	4.0 ± 0.4	4.2 ± 0.3
CCK-8	1.3 ± 0.2	1.9 ± 0.4	1.8 ± 0.1	5.4 ± 0.4	5.0 ± 0.4	4.6 ± 0.4
Leptin + CCK-8	1.6 ± 0.3	1.2 ± 0.1	1.1 ± 0.1	5.4 ± 0.4	4.3 ± 0.3	4.3 ± 0.3

Abbreviation: CCK, cholecystokinin.
Data are means ± s.e.mean of six determinations.

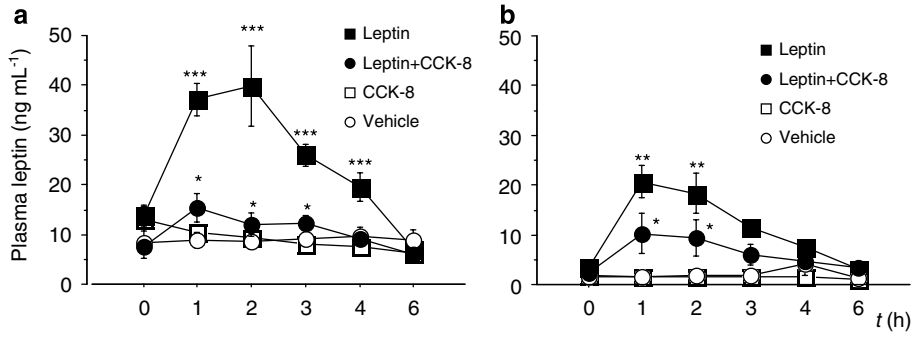

Figure 1 (**a**) Time course of plasma leptin concentration, determined during 6 h after leptin or saline administration to free-feeding rats. Rats were given leptin ($100\,\mu g\,kg^{-1}$) i.p., 30 min before CCK-8 ($10\,\mu g\,kg^{-1}$) also i.p. (**b**) The same experiments carried out in rats fasted for 16 h overnight. Data are means ± s.e.mean of 6–8 determinations. *$P<0.05$, **$P<0.01$; ***$P<0.001$, compared with the other three groups (Newman–Keuls' test). CCK, cholecystokinin.

CCK-8 increased leptin concentration in cerebrospinal fluid

This experiment was carried out in free-feeding animals that all received 100 μg kg^{-1} of recombinant rat leptin. The effect of CCK-8 (10 μg kg^{-1}) on leptin concentration in CSF was determined 2 h after leptin administration. One-way ANOVA ($F_{(1,10)} = 15\,991$, $P < 0.01$) revealed that leptin concentration was higher in CSF from rats treated with CCK-8. These data are summarized in Table 2.

Blockade of CCK$_1$ receptors antagonized the effect of CCK-8 on leptin distribution

The CCK receptor subtype involved in the response elicited by CCK-8 was investigated by using selective CCK_1 (SR-27,897) and CCK_2 receptor (L-365,260) antagonists. All assays were carried out in rats treated with exogenous leptin. Plasma samples were obtained immediately after leptin administration as well as 1 and 3 h later. After the last sampling, rats were anaesthetized and CSF obtained by means of intracisternal puncture. As illustrated in Figure 2a, SR-27,897 (0.3 mg kg^{-1}) completely blocked the effect of CCK-8 on plasma leptin 3 h after leptin administration (1-ANOVA, $F_{(3,24)} = 5934$; $P < 0.01$). Figure 2b shows that the effect of CCK-8 on leptin concentration in the CSF was also antagonized by SR-27,897 (1-ANOVA, $F_{(3,25)} = 6254$; $P < 0.01$). In contrast, L-365,260 (1 mg kg^{-1}) did not modify the effect of CCK-8 either in plasma (Figure 2c) or in CSF leptin concentration (Figure 2d).

Table 2 Effect of CCK-8 (10 μg kg^{-1}) on leptin concentration in CSF in rats receiving 100 μg kg^{-1} recombinant leptin (**$P < 0.01$; Newman–Keuls' test).

Treatment	*Leptin CSF concentration (pg mL^{-1})*
Leptin	91.8 ± 24.8
Leptin + CCK-8	229.5 ± 23.9**

Abbreviations: CCK, cholecystokinin; CSF, cerebrospinal fluid.

Effect of leptin and CCK-8 on food intake

To evaluate the relevance of an eventual satiating effect of CCK-8 (10 μg kg^{-1}) on leptin kinetics, we determined the effect of CCK-8 on food intake 2 and 6 h after leptin administration. Under our conditions, all treatments were devoid of effect on food intake (Table 3). Because food intake, determined 30 and 60 min after CCK-8 administration, exhibited large interindividual variations within the same group (data not shown), only measurements made 2 and 6 h after CCK administration are shown.

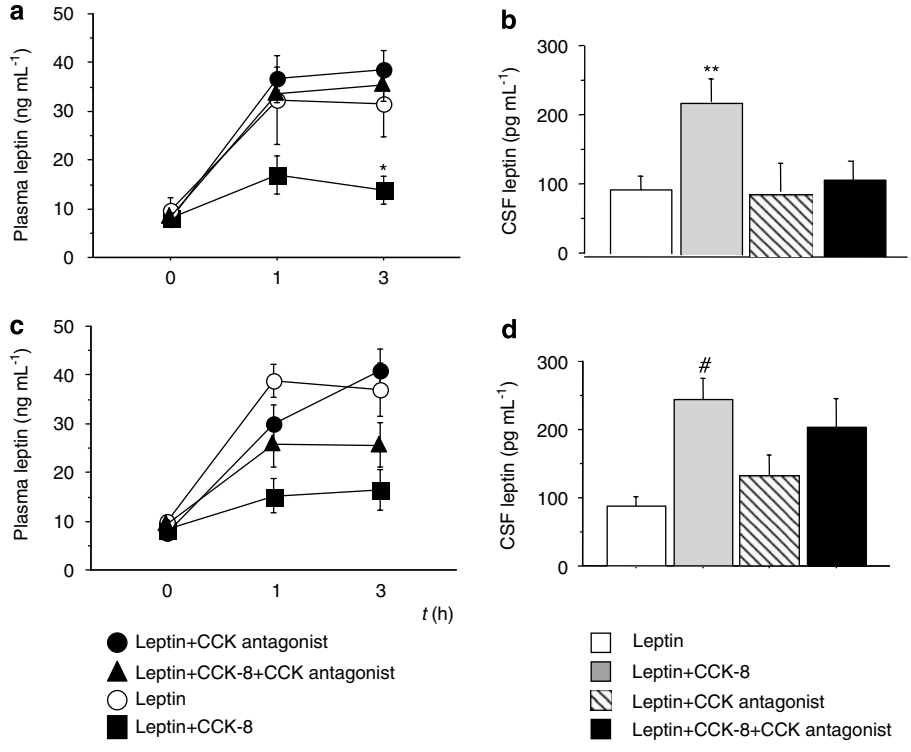

Figure 2 (**a**) Effect of SR-27,897 on plasma leptin concentration of animals treated with exogenous leptin (100 μg kg^{-1}) and CCK-8 (10 μg kg^{-1}). Samples were taken at 0, 1 and 3 h after leptin administration. (**b**) Effect of SR-27,897 on cerebrospinal fluid (CSF) leptin concentration in animals treated with leptin (100 μg kg^{-1}) and CCK-8 (10 μg kg^{-1}), 3 h after leptin administration. (**c**) Effect of L-365,260 on plasma leptin concentration of animals treated with exogenous leptin (100 μg kg^{-1}) and CCK-8 (10 μg kg^{-1}). Samples were taken at 0, 1 and 3 h after leptin administration. (**d**) Effect of L-365,260 on CSF leptin concentration of animals treated with leptin (100 μg kg^{-1}) and CCK-8 (10 μg kg^{-1}), 3 h after leptin administration. Data are means ± s.e.mean of 6–8 determinations. *$P < 0.05$, **$P < 0.01$ compared with the other three groups. $^{\#}P > 0.01$, compared with the control group. (Newman–Keuls' test). CCK, cholecystokinin.

Table 3 Effect of CCK-8 and/or leptin on food intake, measured 2 and 6 h after CCK-8 administration in free-feeding rats

	Food intake (g)	
Treatment	*2 h*	*6 h*
Control	1.6 ± 0.4	5.1 ± 0.7
Leptin	1.8 ± 0.4	4.5 ± 0.4
CCK-8	1.2 ± 0.2	4.6 ± 0.5
Leptin + CCK-8	1.8 ± 0.3	4.2 ± 0.3

Abbreviation: CCK, cholecystokinin.
Data are means ± s.e.mean of 6–8 determinations.

Discussion

Abundant evidence supports the idea that leptin enters the brain by means of Ob-Ra receptors located in the blood–brain barrier (BBB) and in the choroid plexus (Banks *et al*., 1996; Zlokovic *et al*., 2000; Hileman *et al*., 2002). However, little is known about the modulation of this transport mechanism by other hormones involved in food intake and energy balance regulation. Recently, we have reported that CCK evokes metabolic responses by activating leptin-mediated hypothalamic pathways and we have suggested that the effect of CCK is linked to the increase of the permeability of brain barriers to leptin (Cano *et al*., 2003; Merino *et al*., 2008). Our goal was to characterize the CCK receptor subtype involved in modulating leptin access to the CSF. We report here that CCK-8, by acting on CCK_1 receptors, decreases plasma leptin concentration in rats loaded with exogenous leptin and simultaneously increases leptin concentration in the CSF (Table 2). The effect of CCK was also detected in fasted rats, suggesting that the effect of CCK is independent of both the nutritional status and insulin concentration (Table 1) (Banks *et al*., 1996).

The effect of CCK-8 on plasma leptin concentration (Figure 1) is in agreement with the increase of plasma leptin elicited by CCK receptor antagonists both in fed and fasted rats (Cano *et al*., 2003). In contrast to our data, Bado *et al*. (1998) have reported that CCK induces leptin release from adipose tissue and leads to a subsequent increase of plasma leptin concentration. This discrepancy may be due to methodological differences linked to the treatment schedule followed by these authors, namely the dose (100 instead $10\,\mu g\,kg^{-1}$) as well as the time of administration of CCK-8 (15 min before sampling). In any case, our data point to an effect of CCK-8 on leptin distribution, which is compatible with the role of CCK in regulating the release of leptin.

The effect of CCK-8 on plasma leptin concentration might be also linked to the inhibition of leptin absorption from the intraperitoneal cavity elicited by CCK-8. However, this is unexpected, as CCK-8 elicits vasodilatation in the rat mesenteric vascular bed (Sánchez-Fernández *et al*., 2004). CCK-8 might eventually decrease leptin bioavailability by increasing the hepatic first-pass effect. This possibility also seems unlikely as renal elimination appears to be the main mechanism responsible for leptin clearance (Zeng *et al*., 1997).

The effect of CCK-8 was observed only in rats loaded with exogenous leptin (plasma leptin concentration reached $40\,ng\,mL^{-1}$ in these animals, which is in the range of concentrations exhibited by leptin-transfected rats; Wang *et al*., 1999), suggesting that at physiological levels, CCK-8 does not modulate plasma leptin clearance. Nevertheless, the effect of CCK-8 characterized in this study might make physiological sense. Leptin transport is carried out by specific uptake mechanisms located both in the blood–CSF and in the BBB (Banks *et al*., 1996). Leptin transporters exhibit Michaelis constants in a wide range from 0.2 to $350\,ng\,mL^{-1}$. Physiological concentrations of leptin are high enough to saturate high-affinity transporters, those with K_m values from 0.2 to $1.1\,ng\,mL^{-1}$, but are probably too low to bind low-affinity uptake systems (Zlokovic *et al*., 2000). As a possibility, low-affinity systems might be relevant in leptin transport when plasma leptin concentrations are elevated and we speculate that kinetic properties of leptin transport might be regulated by other hormones or mediators, such as CCK. In addition, the choroid plexus seems to play a minor role, compared with the BBB, in regulating the entry of leptin into the brain (Banks *et al*., 1996; Banks, 2006). As a consequence, it might be difficult to detect the effect of CCK-8 on endogenous leptin concentration within the CSF. Quantitative differences between the effect of CCK-8 in free-feeding and fasted rats also support this hypothesis. In fasted animals, CCK-8 reduced plasma leptin concentration to values close to those detected in free-feeding animals treated with leptin + CCK-8. This supports the idea that CCK would rather modulate low-affinity mechanisms of leptin transport.

Finally, our data demonstrate that the effect of CCK on leptin kinetics is mediated by CCK_1 receptors. The involvement of CCK_1 receptors gives further relevance to the interaction between leptin and CCK in the context of feeding behaviour and energy expenditure. In fact, although amply investigated, the mechanism underlying the satiating effect of CCK remains in part unknown. Most studies point to the involvement of peripheral CCK_1 receptors (Crawley and Corwin, 1994) and it is widely assumed that CCK activates neurons of the NTS (nucleus tractus solitarius) via stimulation of CCK_1 receptors on vagal afferents (Moran and McHugh, 1988). Interestingly, NTS and hypothalamic sites involved in food intake regulation seem to be interconnected by leptin pathways (Morton *et al*., 2005), suggesting that integrity of leptin signalling within the hypothalamus is necessary for the effect of CCK to be observed. Our data suggest that leptin would also mediate endocrine effects of peripheral CCK by acting on CCK_1 receptors located in the choroid plexus and, eventually, in the BBB. Interestingly, CCK_1 receptors are expressed in the choroid epithelium (results not shown). This hypothesis is supported by results of Niimi *et al*. (1999) reporting that OLETF rats, which lack CCK_1 receptors, are resistant to the effect of peripheral leptin but keep complete responsiveness to centrally administered leptin in terms of food intake.

From our data, we cannot suggest a cellular mechanism to explain the effect of CCK and further studies will be required to assess this question. As a possibility, activation of CCK_1 receptors in the choroid plexus might modulate the affinity of Ob-Ra receptors for leptin, and, thus, affect their ability to transport leptin. Otherwise, it must be noted that the amount of leptin cleared from plasma in rats treated with

CCK-8 greatly exceeds the amount found in the CSF. We speculate that CCK-8 is not only involved in the entry of leptin into the CNS, but could also modulate the distribution of leptin in other tissues, and/or the metabolism and excretion of the hormone.

In summary, a concomitant effect of CCK-8 in decreasing plasma leptin content and increasing leptin in the CSF strongly suggests that CCK-8 regulates the distribution of leptin. This study shows that CCK can modulate the accessibility of leptin to the hypothalamus. The synergy between leptin and CCK can now be interpreted in a new context, and it can be hypothesized that both CCK and leptin are integrated in a homeostatic mechanism aimed at maintaining body weight between narrow margins by increasing the access of leptin to the brain. Finally, and in spite of the increase of plasma leptin content detected both in fasted and in free-feeding rats treated with CCK_1 receptor antagonists (Cano *et al*., 2003), our present data must be interpreted cautiously in physiological terms, as the effect of CCK-8 on CSF leptin was observed only in animals treated with exogenous leptin. In any case, these results indicate that an increase of plasma leptin may be ineffective, in terms of energy expenditure, if plasma CCK levels remain low, or, alternatively, if there is a loss of functionality of CCK_1 receptors. These results may be clinically relevant for the treatment of leptin-resistant obesity.

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Conflict of interest

The authors state no conflict of interest.

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