Diurnal rhythm of cerebrospinal fluid and plasma leptin levels related to feeding in non-lactating and lactating rats

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Abstract

Leptin suppresses food intake and increases energy expenditure in the hypothalamus. Rats consume most of their daily food intake during the dark phase of the diurnal cycle. Lactating rats have increased food intake, but the involvement of leptin in the regulation of food intake in this physiological condition is not well understood. The present experiment was carried out to determine the circadian pattern of leptin concentrations in plasma and cerebrospinal fluid (CSF) in relation to the feeding behavior of non-lactating and lactating rats.

Female rats were maintained on a controlled lighting schedule (lights on between 0600 and 1800 h) and the food intake of lactating rats was two- or threefold higher than that of non-lactating rats. In both groups, food intake

was three times greater in the dark phase ($P < 0.01$) compared with the light phase. The plasma concentrations of leptin were lower ($P < 0.01$) in lactating rats than non-lactating rats in both light and dark phases, but there were no differences in plasma leptin levels between light and dark phases. In contrast, and in both groups, the leptin concentrations in CSF were lower ($P < 0.01$) in the dark phase than in the light phase. Leptin levels in CSF were lower ($P < 0.01$) in lactating rats than in non-lactating rats. We conclude that a diurnal pattern of leptin levels within the brain (but not in plasma) reflects characteristics of feeding behavior in lactating and non-lactating rats.

Journal of Endocrinology (2004) **180**, 283–286

Introduction

Lactation markedly increases the nutrient requirements of the mother in most mammals. Lactating animals increase their food intake in order to meet the increased nutrient demands. Food consumption in the rat usually occurs in the dark phase. Lactating rats increase their food intake several-fold mainly during the dark phase, as is the case for non-lactating rats (Barber *et al.* 1997). From the various observations, lactation might be expected to be a hypo-leptinemic state, which drives or at least facilitates the hyperphagia (Brogan *et al.* 1999, Woodside *et al.* 2000). Nevertheless, the relationship between circulating leptin levels and feeding behavior remains unclear.

Leptin is a hormone, mainly secreted by white adipose tissue, which acts on the hypothalamus to decrease appetite (Friedman & Halaas 1998). Leptin acts within the hypothalamus to inhibit the synthesis and secretion of neuropeptide Y (NPY), which is a powerful orexigenic peptide (Frankish *et al.* 1995). The hypothalamic content of NPY and NPY mRNA expression increase during lactation; this may be a consequence of an overriding effect of reduced inhibition of NPY neurons by leptin (Smith 1993). However, diurnal variation of the leptin content in the brain during lactation has yet to be examined.

It has also been shown that there is a marked rise in serum leptin levels during the night in non-lactating rats, and this nocturnal increase in leptin secretion was not observed in lactating rats. Thus, it is proposed that the link between nocturnal food intake and increased serum leptin levels is broken during lactation and hypoleptinemia may be an important factor in promoting the hyperphagia of lactation (Pickavance *et al.* 1998). Peripherally produced leptin may enter the brain by binding to the choroid plexus (Devos *et al.* 1996) before being transported into the cerebrospinal fluid (CSF) by a saturable mechanism (Banks *et al.* 1996, Caro *et al.* 1996), to act in the regulation of feed intake in the hypothalamus. Accordingly, the leptin levels in CSF may directly reflect the feeding behavior of non-lactating and lactating rats. The extent to which central levels of leptin relate to diurnal patterns of feeding behavior is not known in lactating rats. The present study was conducted to determine plasma and CSF leptin concentrations in lactating rats and non-lactating rats.

Materials and Methods

Experimental animals and diets

This study used 13- to 14-week-old female Wistar rats (JAPAN CLEA, Tokyo, Japan) with an initial average

body weight of approximately 230 g. The animals were housed under controlled lighting (12 h light:12 h darkness, lights on at 0600 h) and temperature ($22 \pm 2^\circ\text{C}$) conditions with free access to food and water.

The control animals used in the experiment were non-lactating; they were virgin and in the diestrous stage of the estrous cycle. Lactating rats, of the same age as the controls, were housed singly with their litters. The litter size was adjusted to ten pups at birth. All animals had been adapted to standard powder meal (CE-2; 352 kcal/100 g; JAPAN CLEA, Tokyo, Japan) for more than 2 weeks.

All procedures used in this study were performed in accordance with institutional guidelines for animal care at Kitasato University.

Catheterization of the jugular vein

Lactating rats underwent surgery at 3 days postpartum. Rats were anesthetized during the surgery by i.p. injection of pentobarbital sodium (40 mg/kg body weight). The right jugular vein was exposed as for i.v. injection. A catheter was prepared by cutting squarely across it with a scalpel blade to produce a blunt end. If the catheter was to be used only for administering substances then only a few millimeters needed to be within the blood vessel. After flushing with a small amount of 20 U heparin-saline solution ensuring that no air entered the circulation, the catheter was finally filled with 300 U heparin-saline solution and a suitable plastic cap was attached to a 23-gauge needle on the end of the catheter.

Food intake and blood sampling

Food intake in lactating and non-lactating rats ($n=6$) implanted with jugular venous catheters was measured at 6 h intervals for 24 h, beginning at 0600 h. Blood collections in lactating rats were performed on day 9 of lactation. Blood samples of 0.5 ml were taken from the jugular vein at 0900, 1500, 2100 and 0300 h. Plasma samples were stored at -80°C until assay.

Cannulation of the cisterna magna

The surgery for CSF sampling was performed as previously described (Sarna *et al.* 1983, Huang *et al.* 1996). Lactating rats underwent surgery 3 days postpartum; cannulation of the cisterna magna was performed in addition to catheterization of the jugular vein. Rats were anesthetized with pentobarbital sodium (40 mg/kg body weight, i.p.). The anesthetized rat was positioned in a stereotaxic frame and the calvarium exposed via a midline incision. Briefly, a hole (2 mm diameter) was drilled in the midline 3 mm caudal to lambda under constant irrigation with sterile, buffered saline. A polyethylene tube (internal diameter 0.28 mm, outer diameter 0.61 mm; intracranial length 6–7 mm; PE-10, Becton Dickinson and Co., MD, USA) was inserted through the hole in the calvarium and gently

forced epidurally along the internal surface of the occipital bone into the cisterna magna and fixed to the bone with dental cement (GC Corporation, Tokyo, Japan).

CSF sampling

The rats were freely moving during the sampling of CSF on day 9 of lactation and non-lactation. The CSF samples were taken at 0900 h (minimum consumption phase of food intake) and 2100 h (maximum consumption phase of food intake); samples were frozen immediately and kept at -80°C until analyzed.

Sample analyses

Leptin concentrations in plasma (10 μl) and CSF (50 μl) were determined using the Rat Leptin ELISA kit (Yanaihara Institute Inc., Shizuoka, Japan). The assay sensitivity was 78.1 pg/ml. The inter- and intra-assay coefficients of variation were 3.2–5.6 and 3.5–5.9% respectively.

Statistical analyses

Results were analysed by two-way ANOVA with two physiological states (lactating and non-lactating) and two photoperiods (light and dark). Significant differences between each value were tested using Fisher's PLSD. Data are reported as means $\pm$ S.E.M.

Results

Food intake

Food intakes in lactating rats were greater ($P<0.01$) than in non-lactating rats (Fig. 1). Food consumption of both lactating and non-lactating rats was greater ($P<0.01$) in the dark phase than in the light phase. Lactating rats ate two or three times more ($P<0.01$) than non-lactating rats in each of the four periods sampled, as shown in Fig. 1.

Plasma leptin concentrations

Plasma leptin levels were lower ($P<0.01$) in lactating rats than in non-lactating rats (Fig. 2). Significant differences ($P<0.01$) in leptin levels between lactating and non-lactating were observed at 0300 and 0900 h. Plasma leptin concentrations in both physiological states remained relatively constant during the light and dark phases

CSF leptin concentrations

Overall CSF leptin concentrations in lactating rats were significantly decreased ($P<0.01$) compared with those in non-lactating rats (Fig. 3). CSF leptin levels were lower ($P<0.01$) during the dark phase than in the light phase in

Figure 1 Food intake in non-lactating rats (open bar, $n=6$) and lactating rats (solid bar, $n=6$). Mean values are shown and vertical lines represent the s.e. The effect of physiological state on food intake was significant ($P<0.01$). Means with different superscripts are significantly different within a physiological state (a, b and c, $P<0.01$; A and B, $P<0.01$). The effect of photoperiod on food intake was significant ($P<0.01$). **Significant differences between physiological states in each period, $P<0.01$.

both groups. In the light phase, CSF leptin concentrations in lactating rats were significantly lower ($P<0.01$) than in non-lactating rats.

Discussion

Our results show that plasma leptin levels are reduced in lactating rats compared with non-lactating controls, though the diurnal change of circulating leptin did not reflect the pattern of feeding behavior. The reduced levels

Figure 2 The circadian rhythm of plasma leptin concentrations for one day in non-lactating (open bar, $n=6$) and lactating rats (solid bar, $n=6$) with *ad libitum* feeding. Mean values are shown and vertical lines represent the s.e. The effect of physiological state on plasma leptin levels was significant ($P<0.01$) but the effect of photoperiod on plasma leptin concentrations was not significant. **Significant differences between physiological states in each time period, $P<0.01$.

Figure 3 Effect of lactation on diurnal changes in leptin concentrations in CSF. CSF samples were collected from the cisterna magna of freely moving non-lactating (open bar, $n=7$) and lactating rats (solid bar, $n=7$) at 0900 h (light phase) and 2100 h (dark phase). Mean values are shown and vertical lines represent the s.e. The effect of physiological state on CSF leptin concentrations was significant ($P<0.01$) and the effect of photoperiod on CSF leptin levels was also significant ($P<0.01$). Means with different superscripts are significantly different (a, b and c, $P<0.01$).

of circulating leptin in lactating rats may contribute to the massive hyperphagia observed during lactation. In several studies, serum leptin decreased during early lactation to values lower than those found in virgin rats (Johnstone & Higuchi 2001). If peripheral leptin directly affects food intake, circulating levels of leptin should decrease during the dark phase. However, plasma leptin has been generally found to increase at night, the period when rats consume most of their food. Some studies have shown diurnal changes in the amount of leptin mRNA in adipose tissue in male rats fed *ad libitum*, with maximum levels at night and minimum levels during the daytime (Pickavance *et al.* 1998). Therefore, we tested the hypothesis that the variation in CSF leptin levels, rather than plasma leptin levels, would affect the characteristics of feeding behavior in rats. Overall CSF leptin concentrations in lactating rats were lower than those in non-lactating rats. CSF leptin levels in lactating and non-lactating rats were substantially lower during the dark phase compared with the light phase. Thus, the present results confirm that a diurnal pattern of change in CSF leptin concentrations reflects characteristics of feeding behavior in both lactating and non-lactating rats.

In the present study, CSF leptin levels in both groups of rats were significantly lower in the dark phase than in the light phase. In contrast, plasma leptin levels tended to increase during the dark phase in lactating and non-lactating rats. In other words, the variation of CSF leptin concentrations did not parallel changes in plasma leptin levels. The inconsistency between blood and CSF leptin concentrations may be due to the permeability of

leptin at the blood–brain barrier (BBB). In some cases of obesity, food intake is not inhibited even if blood leptin levels increase and this is thought to be due to the failure of leptin to enter the hypothalamus (Lynn *et al.* 1996, Kastin *et al.* 1999). For a hormone to reach most sites within the brain, it must cross the BBB. There is a unidirectional saturable transport system across the BBB for leptin (Maness *et al.* 1998, Zlokovic *et al.* 2000). A recent report has suggested that the expression of short isoforms of leptin receptors, especially Ob-Ra, play a role in transporting circulating leptin to the hypothalamus (Bjørbaek *et al.* 1998). Impairment of the transport of leptin across the BBB has been considered to be responsible for the obesity and the pregnancy referred to as ‘leptin resistance’ (Wu-Peng *et al.* 1997).

Overall CSF leptin levels, as well as plasma leptin levels, were lower in lactating rats compared with non-lactating controls. Degradation of blood leptin may cause a fall in CSF leptin levels in lactation, although there is no research on the resolution of plasma leptin. There was no significant difference in circulating leptin levels between light and dark phases in the present study, nevertheless the circadian rhythm of CSF leptin concentrations in lactating and non-lactating rats was also observed, with low concentrations in the dark phase and high concentrations in the light phase. Thus, the variation of CSF leptin suggests that Ob-Ra receptor expression, related to leptin transport at the BBB, might have circadian rhythm in both lactating and non-lactating rats. A recent study has shown that expression of the Ob-Ra gene in the hypothalamus is unaltered by lactation (Denis *et al.* 2003), however Ob-Ra receptor expression might have circadian rhythm in limited areas, such as the BBB in lactating and non-lactating rats.

In conclusion, we have demonstrated for the first time that, although there is no diurnal variation in plasma levels of leptin, there is a diurnal pattern in the concentrations in the CSF. This may relate to the pattern of feeding behavior in rats.

Acknowledgements

The authors are very grateful to Professor Iain Clark and Dr Javed Iqbal of Prince Henry’s Institute of Medical Research, Australia for their kind advice on the manuscript. We thank Yuki Ogawa, Yukiko Saito, Michiko Sasaki and Sachiko Watanabe for skilled technical assistance.

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Received 26 July 2003

Accepted 22 October 2003