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NUTRITIONAL STATUS MODULATES BEHAVIOURAL AND OLFACTORY BULB Fos RESPONSES TO ISOAMYL ACETATE OR FOOD ODOUR IN RATS: ROLES OF OREXINS AND LEPTIN

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Abstract—Food odours are major determinants for food choice, and their detection depends on nutritional status. The effects of different odour stimuli on both behavioural responses (locomotor activity and sniffing) and Fos induction in olfactory bulbs (OB) were studied in satiated or 48-h fasted rats. We focused on two odour stimuli: isoamyl acetate (ISO), as a neutral stimulus either unknown or familiar, and food pellet odour, that were presented to quiet rats during the light phase of the day. We found significant effects of nutritional status and odour stimulus on both behavioural and OB responses. The locomotor activity induced by odour stimuli was always more marked in fasted than in satiated rats, and food odour induced increased sniffing activity only in fasted rats. Fos expression was quantified in periglomerular, mitral and granular OB cell layers. As a new odour, ISO induced a significant increase in Fos expression in all OB layers, similar in fasted and satiated rats. Significant OB responses to familiar odours were only observed in fasted rats. Among the numerous peptides shown to vary after 48 h of fasting, we focused on orexins (for which immunoreactive fibres are present in the OB) and leptin, as a peripheral hormone linked to adiposity, and tested their effects of food odour. The administration of orexin A in satiated animals partially mimicked fasting, since food odour increased OB Fos responses, but did not induce sniffing. The treatment of fasted animals with either an orexin receptors antagonist (ACT-078573) or leptin significantly decreased both locomotor activity, time spent sniffing food odour and OB Fos induction in all cell layers, thus mimicking a satiated status. We conclude that orexins and leptin are some of the factors that can modify behavioural and OB Fos responses to a familiar food odour.

Key words: olfaction, active wake, orexin receptors antagonist, leptin receptors, orexin A, immunohistochemistry.

Most animals depend on olfaction when searching and choosing food, and the odours associated with food are essential to food intake control (Le Magnen, 1959). Various studies have demonstrated that the nutritional status of individuals influences odour detection: in primates and humans, fasting results in an increased perception of some food-related odours (Mulligan et al., 2002). In rodents, behavioural studies have demonstrated that olfactory sensitivity to a neutral odour increases in fasted rats (Aime et al., 2007). Indeed, odour signals are processed during multiple steps that start in the olfactory mucosa, where olfactory sensory neurons (OSNs) project to the first relay in the brain (mitral cells of the olfactory bulb) before being integrated in different areas of the CNS. The electrical activity of mitral cells varies as a function of nutritional status: fasting has been found to selectively increase mitral cell multiunit responses to food odour (Pager et al., 1972); in more recent studies, mitral cell single-unit responses were increased whatever the odorant (food or neutral) (Apelbaum and Chaput, 2003).

Numerous peripheral and hypothalamic peptides involved in food intake control vary according to nutritional status and may be responsible for modulating olfactory sensitivity. These include orexins, which are synthesised by neurons of the lateral hypothalamic area and are a stimulator of food intake (Sakurai et al., 1998; Edwards et al., 1999); the level of hypothalamic prepro-orexin mRNAs is maximal after 48 h of fasting (Cai et al., 1999). Orexin neurons project centrifugal fibres into the rat olfactory bulb (Nambu et al., 1999; Shibata et al., 2008). In narcoleptic human patients with decreased levels of orexin A (OxA), olfactory performance is impaired, and intranasal OxA treatment restores the olfactory function (Baier et al., 2008). When injected in the lateral cerebral ventricle of rats, OxA increases olfactory sensitivity (Julliard et al., 2007); orexin receptors are present in neurons of the bulb (Caillol et al., 2003; Hardy et al., 2005). Inversely, leptin is a potent satiety hormone, synthesised peripherally by adipocytes and acting on hypothalamic feeding networks to halt food intake (Shiraishi et al., 2000). In rats, i.c.v. leptin administration reduces food intake (Flynn et al., 1998) and olfactory sensitivity (Julliard et al., 2007); in mice, leptin modulates olfactory-mediated pre-ingestive behaviour (Getchell et al., 2006). We thus hypothesize that leptin may act on the olfactory bulb in a manner contrary to that of orexins, which may partly explain the modulation of olfactory performance by nutritional status.

The aim of the present study was thus to analyze possible modulations by nutritional status of both global behavioural responses and defined olfactory bulb cellular modifications induced by different olfactory stimuli. We chose isoamyl acetate as a neutral odorant capable of

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Abbreviations: BSA, bovine serum albumin; b.w., body weight; ICC, immunocytochemistry; i.c.v., intra-cerebro-ventricular; i.p., intra-peritoneal; IR, immunoreactive; OSN, olfactory sensory neuron; OxA, orexin A; PB, phosphate buffer; PBS, phosphate-buffered saline; PEG, polyethylene glycol; TX, Triton X-100.

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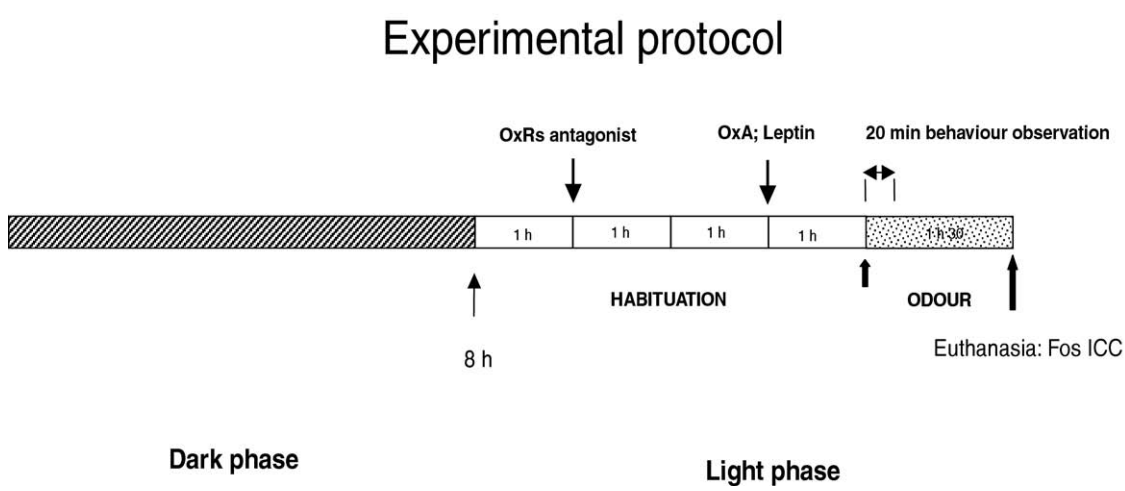

Fig. 1. Diagram representing the experimental procedures: after a 4-h habituation period (clean cage, no food pellets, under a fume hood), the odour stimulus was presented for 1 h 30 to 48-h fasted or satiated rats. Orexin receptor antagonist (ACT-078573) or vehicle was orally administered 3 h before the initiation of odour presentation; OxA, leptin or vehicle was administered i.c.v. 1 h before the initiation of odour presentation. Behavioural observations were performed during the first 20 min of odour presentation and rats were euthanized 90 min later.

stimulating large populations of sensory neurons in the mucosa, and food odour as a familiar biologically relevant stimulus. In terms of behavioural responses, we recorded locomotor activity and sniffing time. To measure the activity of cells in the different layers of the olfactory bulb, we chose immunohistochemical detection of the nuclear Fos protein. This product of the immediate-early gene c-fos, viewed as a third messenger, is widely employed as a functional anatomical marker of activated neurons and its expression is induced in the OB by the presentation of an odour (Sallaz and Jourdan, 1993; Guthrie and Gall, 1995).

The first part of our study was designed to investigate the effect of nutritional status (satiated vs. 48-h fasted rats) on both behavioural and OB Fos responses after presentation of an odour. The possible roles of orexins and leptin were then studied.

EXPERIMENTAL PROCEDURES

Animals

The experiments were performed on adult male Wistar rats, weighing 300–350 g. The rats were housed individually at a constant temperature (22 °C) with free access to food and water, under a 12-h light/dark cycle. A total of 60 rats were included in these experiments. All animals were handled in compliance with the principles of laboratory animal care and French laws on the protection of animals (law 87-848, decret du 13 février 2001). Every effort was made to minimize the suffering of rats and the number of animals.

Drugs

OxA and leptin were purchased from Sigma-Aldrich (St. Louis, MO, USA) and diluted according to the manufacturer's guidelines. The rats received 3 nmol OxA in 3 μl saline (NaCl 0.9%) (Apelbaum et al., 2005), 5 μg leptin in 4 μl saline (Clark et al., 2006), or saline alone (vehicle) via i.c.v. cannulas (see below). The orexin receptor antagonist ACT-078573 (generous gift from Actelion Pharmaceuticals Ltd., Allschwil, CH) was formulated in polyethylene glycol 400 (PEG; Merck, Hohenbrunn, Germany) (vehicle). Rats received either the vehicle (1.75 ml) or ACT-078573 (100 mg/kg b.w. in 1.75 ml of vehicle) via gastric cannulae. Angiotensin II was purchased from Sigma-Aldrich and diluted in a saline vehicle.

Surgery

One week before OxA, leptin or saline infusion, the rats were anaesthetized (equitezine, a mixture of chloral hydrate and pentobarbital, 0.3 ml/100 g body weight i.p.) and cannulas (Plastics One, 22-gauge stainless steel guide, Phymep, Paris, France) were implanted in the left lateral cerebral ventricle (Bregma: A=−0.8; L=−1.6; H=4). The correct positioning of the cannula was verified by an intense drinking response to an i.c.v. administration of angiotensin II (100 ng in 3 μl).

Experimental protocol (Fig. 1)

For all experiments, at the beginning of the light phase, the rats were placed in clean cages under a laboratory fume hood as the standard ambient odour for a 4-h habituation period, with water but no food. The experiments were performed during the light phase since the Fos response to odours during the night (active period for rats) was significantly higher than during the day (Amir et al., 1999) and able to mask the modulation by nutritional status. At the end of the 4-h habituation period, different odour stimuli were presented in tea-balls: (i) control ambient odour (empty tea ball) (ii) unknown pure isoamyl acetate (150 μl on a filter paper; Montag-Sallaz and Buonviso, 2002; ISO unknown; Sigma-Aldrich) (iii) familiar isoamyl acetate (familiarisation for 20 min per day on 6 consecutive days in a cage containing litter odorized with 150 μl pure isoamyl acetate; Montag-Sallaz and Buonviso, 2002; ISO familiar) (iv) food odour (pellets in the tea ball). During odour exposure, the behaviour of rats was recorded for 20 min (time spent moving and sniffing the tea ball). After 90 min of odour exposure (peak of Fos protein induction after a stimulus; Kovacs, 1998), the rats were deeply anaesthetized and euthanized (Fig. 1).

The first experiment was designed to compare behavioural responses and Fos expression in the olfactory bulb after exposure to the different odour conditions (control, ISO unknown, ISO familiar, food odour) in rats either fasted for 48 h or fed *ad libitum* (satiated) (n=3 rats per group). This 48-h fasting period resulted in an 11% body weight decrease (318±5 vs. 281±5 g).

The second experiment was designed to test the role of orexins and leptin regarding behaviour and Fos responses to food odour. Satiated animals implanted with cannulas in the lateral