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INFLUENCE OF PALATABILITY ON MOTIVATION TO OPERATE FOR CALORIC AND NON-CALORIC FOOD IN NON FOOD-DEPRIVED AND FOOD-DEPRIVED RATS

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Abstract—Palatability is the hedonic food component that is considered to override the homeostatic mechanisms that control food intake, and we compared how much effort non food-deprived and food-deprived rats were willing to spend in order to earn a palatable caloric (sucrose) or non-caloric (saccharin) snack. We first studied the dopaminergic response, in terms of dopamine levels and dopamine and cAMP-regulated phosphoprotein Mr 32,000 (DARPP-32) phosphorylation pattern, to two consecutive palatable caloric or non-caloric snacks in the nucleus accumbens shell (NAcS) of non food-deprived and fasted rats. We report that non food-deprived rats developed rapid habituation in the NAcS dopaminergic response to the second consumption of both caloric and non-caloric palatable food, while food-deprived rats developed rapid habituation only to saccharin. Next, we show that in self-administration experiments, non food-deprived rats spent a similar effort when operating for sucrose or saccharin. However, the same rats showed an increased response specifically for sucrose after 18-h fasting. After pre-feeding devaluation, rats reduced their response to sucrose but not for saccharin. These results strengthen the hypothesis that food intake is mainly controlled by palatability in non food-deprived rats and by caloric content in food-deprived rats. Moreover, they show that rapid habituation development was associated with a similar, basal working activity aimed at ingesting both caloric and non-caloric food, as observed in non food-deprived rats consuming sucrose or saccharin and in fasted rats consuming saccharin. Conversely, lack of habituation, as present in fasted rats consuming a caloric food, was associated with extra energy expenditure. © **2013 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: dopamine, dopamine and cAMP-regulated phosphoprotein of Mr 32,000 (DARPP-32), microdialysis, saccharin, self-administration.

INTRODUCTION

The regulation of body weight is controlled by a complex integration of peripheral signals and central effector systems to produce behavioral and physiological outputs that regulate food intake and energy expenditure (Benoit et al., 2010). Animals discriminate foods on the basis of flavor and taste and have the competence of detecting the post-ingestive consequences of a meal (Elizalde and Sclafani, 1990; Drucker et al., 1994). The introduction of food into the oral cavity produces subjective awareness (sensation) of the quality (the purely sensory aspect) and palatability (the hedonic aspect) of its taste. Moreover, when food reaches the gastrointestinal tract, it activates visceral post-ingestive sensations that are coded in a comprehensive sensory and hedonic gustatory representation (Yamamoto and Sawa, 2000). Animals can sense the negative internal signals produced by food deprivation in terms of hunger or the positive consequences of food ingestion in terms of satiety. Moreover, they learn to associate flavors and/or tastes with specific nutrients on the basis of their caloric content when the post-ingestive sensation makes them aware of the caloric content of a given nutrient (Friedman et al., 1983; Tordoff et al., 1987; Lucas and Sclafani, 1989; Drucker et al., 1994; Perez et al., 1999). The post-ingestive component is the added value of palatable caloric food that confers on it an incentive value higher than that of a palatable non-caloric food. Caloric content is the food primary value, and the taste–calorie association measures and codifies the incentive value of nutrients and will influence subsequent food choice decisions.

Through a process known as energy homeostasis, food intake is adjusted over time so as to promote stability in the amount of body fuel stored as fat. The regulation of energy homeostasis includes endocrine and peripheral cues that indicate either long-term

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Abbreviations: ANOVA, analysis of variance; AUC, area under the curve; BP, breaking point; DARPP-32, dopamine and cAMP-regulated phosphoprotein of Mr 32,000; FR, fixed-ratio; NAcS, nucleus accumbens shell; PR, progressive ratio; SDS, sodium dodecyl sulfate; Thr, threonine; VS, vanilla sugar.

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availability of energy stores or short-term, meal-related changes in metabolic state. One consequence of this integration is that the drive to eat decreases as food is ingested up to satiation. The motivation to eat or stop eating is however more complex than a simple homeostatic system that responds to metabolic and satiety signals from the periphery. In fact, the brain reward systems when stimulated by the sight, smell and taste of food (or cues that predict food) may override the homeostatic system, which evolved under conditions in which food was never chronically abundant, and promote an excess of food consumption (Palmiter, 2007). In other words, palatability is unquestionably a key factor in guiding choice and amount of food consumed, and this concept has been confirmed by the literature (Saper et al., 2002; Lundy, 2008; Yarmolinsky et al., 2009).

We observed that rats given unlimited access to standard chow eagerly consume vanilla sugar (VS) pellets and show a dopaminergic response to VS consumption in the medial prefrontal cortex and shell portion of the nucleus accumbens (NAcS) (Danielli et al., 2010). This dopaminergic response is expressed as a transient increase in extraneuronal dopamine concentration (measured by microdialysis) associated with consistent modifications in the phosphorylation pattern of some cAMP-dependent protein kinase (PKA) substrates in both areas (Rauggi et al., 2005; Danielli et al., 2010). In particular, an increase in the levels of dopamine and cAMP-regulated phosphoprotein of Mr 32,000 (DARPP-32) phosphorylated at threonine (Thr) 34, accompanied by a decrease in the levels of DARPP-32 phosphorylated at Thr75, is observed 30 min after VS consumption and phosphorylation levels are back to basal values within 1 h. These phosphorylation changes are then followed in the NAcS by opposite modifications 2–3 h after the meal (Danielli et al., 2010). This biphasic sequence in DARPP-32 phosphorylation changes is only observed in the NAcS of non food-deprived rats, whereas in fasted animals only the early modifications are observed (Danielli et al., 2010). A second consecutive consumption of VS few hours later is followed by an increase in extraneuronal dopamine significantly reduced compared to the first response, selectively in the NAcS, and this phenomenon is defined rapid habituation (Bassareo and Di Chiara, 1999). Rapid habituation, expressed in terms of decreased dopamine release (Bassareo and Di Chiara, 1999) and reduced DARPP-32 phosphorylation changes (Danielli et al., 2010), has been observed in non food-deprived rats consuming two consecutive palatable-caloric snacks while it does not develop in fasted animals (Bassareo and Di Chiara, 1999; Danielli et al., 2010). The sequence of early and delayed phosphorylation changes is triggered by dopamine D_1 receptors stimulation, since it is prevented when SCH 23390, a selective dopamine D_1 receptor antagonist, is administered 5 min after palatable food consumption (Rauggi et al., 2005; Danielli et al., 2010). SCH 23390 administration also prevents the development of rapid habituation (Danielli et al., 2010), an effect reminiscent of the fact that SCH 39166 infused in the NAcS 5 min after palatable food (conditioned stimulus) consumption seems to disrupt the formation of a short-term gustatory memory trace of the conditioned stimulus, since it impairs conditioned taste aversion learning (Fenu et al., 2001). Whether rapid habituation develops following two consecutive consumptions of a non-caloric palatable food in non food-deprived and in fasted rats is an open question. This is a relevant issue since in non food-deprived animals palatability plays a relevant role in the response to food consumption, while in food-deprived animals, or animals with limited access to standard chow, food preference and food consumption are largely influenced by post-ingestive sensations, i.e., the caloric content of food (Beeler et al., 2012). Indeed, no clear differences have been observed in sucrose and saccharin self-administration in non food-deprived rats (Stephens and Brown, 1999). Thus, development of rapid habituation, that likely follows the integration of palatability and post-ingestive sensations, may codify a "non needed" stimulus and contribute to the complex regulation of energy homeostasis. If this were the case, the consumption of a palatable caloric, or a palatable non-caloric snack would induce distinct neurochemical and behavioral responses in fasted rats.

The aim of this study was to address this issue and for this purpose we studied the dopaminergic response to two consecutive caloric, caloric-palatable, or non-caloric-palatable snacks in the NAcS of non food-deprived or fasted rats. Moreover, we trained non food-deprived rats to self-administer sucrose or saccharin. When a steady response on fixed-ratio (FR) 5 schedule was attained, rats were tested on a progressive ratio (PR) schedule of reinforcement and the breaking point (BP) score was recorded. BP measures how much effort animals are willingly to exert in order to obtain the reinforcing stimulus (Salamone et al., 2012); that is, BP is also an index of actual animal motivation. BP scores for sucrose or saccharin were then determined after an 18-h fast.

EXPERIMENTAL PROCEDURES

Animals

Experiments were carried out on male Sprague–Dawley rats (Charles River, Calco, Italy), weighing 200–225 g when the experimental procedures began, allowing 10 days of habituation to the animal colony. Animals were housed 4–5 per cage in an environment maintained at a constant temperature and humidity with free access to food and water. A 12-h reverse light/dark cycle (7:00 a.m. lights off, 7:00 p.m. lights on) was used. Experiments were carried out from 9:00 a.m. to 5:00 p.m. under a red light and controlled noise conditions. The procedures used were in accordance with the European legislation on the use and care of laboratory animals (EU Directive 2010/63) and the guidelines issued by the National Institutes of Health, and they were approved by the University of Siena Ethics Committee. All efforts were made to minimize the number of animals used and their suffering.

Microdialysis procedure

Anesthetized rats (pentobarbital 50 mg/kg, scopolamine 0.4 mg/kg, i.p.) were placed in a stereotaxic instrument and a concentric vertical probe was lowered into the NAcS (AP