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Chronic stress reduces body fat content in both obesity-prone and obesity-resistant strains of mice

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Abstract

Unpredictable stressors have been used to assess the effect of stress on energy metabolism in obesity-prone (C57BL6J) and obesity-resistant (AJ) mice. Mice were exposed for 25 days to a stress protocol. Both strains of mice were divided into groups of control and stressed mice, which had access to either a high-fat or a high-carbohydrate diet. Twenty-four hours after the last session of stress, mice were sacrificed for blood and brain collections. Insulin, corticosterone, and glucose concentrations in plasma were measured, and expressions of corticotropin-releasing factor (CRF) in the paraventricular hypothalamic nucleus (PVH) and the central amygdala (CeA) were determined by in situ hybridization. Stressed mice in all groups had lower body fat contents than control mice, and all mice fed with the high-fat diet had heavier retroperitoneal and inguinal fat pads than mice fed with carbohydrate. CRF mRNA level in the CeA was lower in B6 mice than in AJ mice. Stressed mice had a lower expression of CRF in the CeA than control mice. In conclusion, chronic stress reduces body fat content in obesity-prone as well as in obesity-resistant mice.

Keywords: Unpredictable stress; Energy balance; CRF; Fat content; Obesity-prone

Introduction

There is strong evidence from investigations carried out in laboratory rodents that stress induces catabolic effects (Armario et al., 1985; Harris et al., 1998; Krahn et al., 1990; Marti et al., 1994; Michel and Cabanac, 1999a,b; Rybkin et al., 1997; Smagin et al., 1999; Yahata et al., 1987). Acutely, stress activates the corticotropin-releasing factor (CRF) system, which orchestrates the hormonal and behavioral response to stress (Sutton et al., 1982; Weninger et al., 1999). CRF has been recognized for years as a major anorectic agent (Cole et al., 1990; Koob and Thatcher-Britton, 1985; Murakami et al., 1989; Vale et al., 1981). Infusion of CRF into the brain decreases food intake, body weight gain, as well as the body weight set point (Britton et al., 1982; Cabanac and Richard, 1995; Morley and Levine, 1982; Rivest and Richard, 1990). CRF mediates stress-induced anorectic effects (Koob and Heinrichs, 1999; Menzaghi et al., 1993) that generally persist for quite a few hours after CRF administration. Not too surprisingly, day-to-day repetition of stress generally leads to a negative energy balance (Favre and Vermorel, 1975; Harris et al., 2002a,b; Michel and Cabanac, 1999b; Rybkin et al., 1997). Data from Harris (Harris et al., 1998) have even demonstrated that a 3-day exposure to stress can lead to a long-term retardation in weight growth in rats.

In addition to the demonstration for an association between stress and negative energy balance, there is also evidence from human studies to suggest that stress is positively associated with the development of obesity. Recently, Michel et al. (2003) demonstrated that stress leads to either body weight gain or body weight loss depending on whether stress is applied with a high-energy diet, or with a chow diet to obesity-prone animals. We indeed reported that 20 min of restraint stress in obesity-prone rats fed an energy-dense diet, led to an excessive body weight gain over the

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subsequent 9 days. It remains to be demonstrated whether the chronic exposure to stress would also promote positive energy balance in obesity-prone animals.

The present study was aimed at investigating fat deposition and food intake in obesity-prone (C57BL6J) and obesity-resistant (AJ) mice, which were chronically exposed to stress. C57BL6J (B6) mice are known to become obese when placed on a high-fat diet (Kraegen et al., 1986; Storlien et al., 1986; Surwit et al., 1988). This dietary effect is sustained as long as the energy-dense diet is provided. If the animals are returned to a standard chow diet, their body weights drop to a normal non-obese state (Parekh et al., 1998). On the other hand, AJ mice remain lean even when offered a high-fat diet. For this study, we designed an original stress protocol made of five stressors known to initiate hormonal stress responses. The stressors were selected from the literature (Barr and Phillips, 1998; Briese and Cabanac, 1991; Michel and Cabanac, 1999b; Monnikes et al., 1993). As B6 are obesity-prone when subjected to a high-fat diet, we hypothesized that a mild chronic stress would increase their food intake and facilitate their body weight gain. Finally, CRF mRNA contents in the paraventricular hypothalamic nucleus (PVH) and central nucleus of amygdala (CeA) were measured under the various conditions of the study. PVH is the site of the hypophysiotropic CRF and the CeA is known to be responsible for the behavioral response to stress and the fear conditioning response (Makino et al., 1999; Roozendaal et al., 1991; Roozendaal et al., 1990), and thus were the main areas of interest.

Materials and methods

Animals and diets

Male B6 and AJ mice weighing 23.2–27.8 g, were pair-housed at 23°C in a humidity-controlled room. Lights were on at 0900 and off at 2100. Each strain was divided into 4 groups of eight individuals: high-carbohydrate control (CHO-Ctl), high-carbohydrate stressed (CHO-Str), high-fat control (FAT-Ctl), and high-fat stressed (FAT-Str). They were adapted to their ambient conditions for at least 7 days and to their specific diets for 3 days before the beginning of the stress protocol. Animals were offered purified diets. The high-carbohydrate diet consisted of starch (63%), corn oil (15%), casein (20%), methionine (0.3%), vitamins (1%), and minerals (0.6%). The high-fat diet was 65% lipid (half from corn oil and half from pure lard), 10% starch, 3.2% dextrose, 20% casein, 0.3% methionine, 1% vitamins, and 0.6% minerals.

Stress protocol

The animals were subjected to the following stressors: (i) conspecific exposure (care was taken to never pair the same mice) (Barr and Phillips, 1998); (ii) water avoidance (a mouse is placed on a wood cube surrounded by water) (Monnikes et al., 1993); (iii) cage inclination at 40° (Barr and Phillips, 1998); (iv) damp bedding (addition of water to the litter) (Barr and Phillips, 1998); (v) colonic temperature taking every 30 min (Michel and Cabanac, 1999b). The 5 stressors (one different stressor each day) were randomly assigned to each group so that each mouse had the five stressors over a period of 5 consecutive days in an unpredicted manner. In order to do so, random numbers were assigned to both stressors and animals, which were randomly picked up by a blind experimenter and then stressors were assigned to each group of animal in a random and unpredictable way. The exposure to each stressor lasted 2 h per day and took place at the beginning of the light cycle. The block of 5 stressors was repeated 5 times over 25 days.

Body weight, food intake, and tissue weight

Every morning, mice were weighed. The day-to-day food consumption was assessed by subtracting the food left uneaten from the amount of food provided to the animals. Twenty-four hours after the end of the treatment period, mice were anesthetized as quick as possible with a mixture of ketamine/xylazine for mice (0.1 ml/10 g). Then, they were decapitated and the inguinal (IWAT) and retroperitoneal (RWAT) white adipose tissue pads as well as the interscapular brown adipose tissue (IBAT) depot were removed and weighed. The soleus and vastus lateralis muscles were also removed and weighed.

Plasma determination

An intracardiac blood sample was taken in anesthetized mice, just before decapitation, for the determination of plasma corticosterone, insulin, and glucose. Blood was sampled without delay, usually 1 to 2 min after the injection of the anesthetics. Plasma glucose was determined (glucose oxydase method) using a glucose analyzer (Beckman, Palo Alto, Calif., USA). Serum corticosterone was determined by radioimmunoassay (Medicorp, Royalmount, Mtl., Canada) (sensitivity, 0.05 nmol/l; inter-assay coefficient of variation, 6.9%, intra-assay coefficient of variation, 7.3%). Insulin was determined by RIA with a reagent kit from Linco Research (St. Charles, MO, USA) (inter-assay coefficient of variation, 9%, intra-assay coefficient of variation, 2.9%) using rat insulin as a standard.

Brain preparation

Mice were anesthetized and without delay were perfused intracardially with 200 ml of ice-cold isotonic saline at first followed by 500 ml of paraformaldehyde (4%). Brain was removed at the end of perfusion and kept in paraformaldehyde (4%) pending its slicing. It was transferred to a