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Research report

Litter size reduction alters insulin signaling in the ventral tegmental area and influences dopamine-related behaviors in adult rats

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HIGHLIGHTS

- Induces life-long obesity.
- Alters insulin signaling in the ventral tegmental area.
- Alters the functioning of mesolimbic dopamine pathway in response to palatable food.

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ABSTRACT

Postnatal overfeeding is a well-known model of early-life induced obesity and glucose intolerance in rats. However, little is known about its impact on insulin signaling in specific brain regions such as the mesocorticolimbic system, and its putative effects on dopamine-related hedonic food intake in adulthood. For this study, rat litters were standardized to 4 (small litter – SL) or 8 pups (control – NL) at postnatal day 1. Weaning was at day 21, and all tests were conducted after day 60 of life in male rats. In Experiment 1, we demonstrated that the SL animals were heavier than the NL at all time points and had decreased AKT/pAKT ratio in the Ventral Tegmental Area (VTA), without differences in the skeletal muscle insulin signaling in response to insulin injection. In Experiment 2, the standard rat chow intake was addressed using an automated system (BioDAQ, Research Diets®), and showed no differences between the groups. On the other hand, the SL animals ingested more sweet food in response to the 1 min tail-pinch challenge and did not develop conditioned place preference to sweet food. In Experiment 3 we showed that the SL rats had increased VTA TH content but had no difference in this protein in response to a sweet food challenge, as the NL had. The SL rats also showed decreased levels of dopamine D2 receptors in the nucleus accumbens. Here we showed that early postnatal overfeeding was linked to an altered functioning of the mesolimbic dopamine pathway, which was associated with altered insulin signaling in the VTA, suggesting increased sensitivity, and expression of important proteins of the dopaminergic system.

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1. Introduction

Overweight, even at very young ages, is becoming increasingly prevalent in developed and developing countries [1,2]. Rapid weight gain from ages 0 to 4 months has been shown to be associated with overweight at age 7 and in young adulthood [3], demonstrating that the first few months of life constitute an important period for the development of overweight in childhood and its long-term health consequences.

Environmental modifications during 'critical periods' of development, especially early overfeeding and rapid weight gain early in life, may permanently increase the risk of overweight and associated diseases [4–7]. An established animal model for the study of these effects is to raise rats in 'small litters', as opposed to

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normal litters [8–11]. The artificial reduction of the natural litter size to half the number of pups per nest promotes early overfeeding and consequent early hyperglycaemia, rapid fat accumulation, and obesity [8–10]. Into adulthood, these rats demonstrate persistent alterations such as overweight, hyperphagia, glucose intolerance, hyperinsulinemia, dyslipidemia [10], increased blood pressure and glomerulosclerosis [12]. Epigenetic modifications seem to mediate these findings with hypermethylation of the insulin receptor promoter in the hypothalamus [13] and of the two Sp1-related binding sequences essential for the mediation of leptin and insulin effects on hypothalamic POMC expression [14].

Although the impact of early overfeeding on the homeostatic regulation of food intake is very well known, no studies have investigated the potential effect of postnatal overfeeding on the hedonic component of food intake. The ventral tegmental area (VTA)–nucleus accumbens (NAC) circuitry is crucial in promoting the intake of palatable foods [15–17]. Sweet and fatty foods potentiate the release of dopamine (DA), induce more pleasurable subjective feelings, and therefore are more rewarding [15,18]. Interestingly, neuropeptides that regulate energy intake and expenditure through the hypothalamus, such as insulin, also modulate the activity of dopaminergic neurons and their projections into regions involved in the rewarding processes underlying food intake [19–22]; insulin action on its receptor in the VTA is associated with a decrease in the response of the nucleus accumbens to food cues [21].

Therefore, in the current investigation we hypothesized that alterations in the neonatal nutritional environment, induced by raising the rats in litters of different sizes, would alter insulin signaling in specific brain regions such as the VTA, in which this hormone modulates the activity of dopaminergic neurons. Consequently, we expected an altered behavioral response to sweet food in adult rats (increased consumption in response to acute stress, but decreased ability to develop conditioned place preference to sweet food). In addition, we hypothesized that these alterations would be reflected in changes in the central regulation of the dopaminergic mesocorticolimbic pathway (TH in the VTA and D2 receptors, TH and pTH in the nucleus accumbens) at baseline and in response to a sweet food challenge.

2. Methods

2.1. Animals

Wistar rat matrices (60 days, 180–200 g) were obtained from the Universidade Federal de Pelotas and acclimatized to our rat facility for 2 weeks prior to mating. All rats were housed in Plexiglas cages in groups of 2–4 rats, under a temperature of 25 ± 2 °C and humidity of 35%. The cage floor was covered with wood chips, and cleaning was done 2–3 times per week. Food and water were left ad libitum. After mating, the females were left in single cages until giving birth. At day 1 after birth, litters were culled to 4 animals (3 male and 1 female per litter) in the small litter group (SL), and to 8 pups (5–6 males and 3–2 females) in the normal litter group (NL). Weaning occurred at day 21, when rats were separated by gender. Only males were used in these studies. Body weight was measured weekly until day 84 of life, and at decapitation (day 110–120). In the different experiments we used 101 offspring from 38 litters, having no more than 2 pups from the same litter per group per experiment. All the experiments were performed after 60 days of life. When rats were subjected to more than one behavioral task, it was given at least one week of recovery between the tasks. The project was approved by the local Ethics Committee (Grupo de Pesquisa e Pós-Graduação – Hospital de Clínicas de Porto Alegre, project 09-409).

2.1.1. Experiment 1

2.1.1.1. Body weight, central (VTA) and peripheral (skeletal muscle) insulin signaling. Body weight was measured weekly from weaning until adulthood, using a digital scale with precision of 0.01 g. In adulthood, after 4 h of fasting, rats were injected IP with insulin (5 U/kg body weight) and the tissue was collected by fast decapitation 15 min afterwards. Muscle samples (medial gastrocnemius) or brains were flash frozen in isopentane under dry ice, and stored at −80 °C freezer until analysis. The brains were warmed to −20 °C and the brain regions (VTA) were macroscopically dissected through thick sections of 0.25 cm with the aid of an Atlas [23]. When identified, the region was carefully isolated and punches of 2 mm diameter were performed to collect the tissue. These punches were processed for Western blot analysis as described below.

2.1.2. Experiment 2

2.1.2.1. Standard rat chow intake, sweet food intake after tail pinch stress, conditioned place preference. Rat chow intake – After reaching 60 days of life, rats were transferred into cages equipped with a BioDAQ® food intake monitoring system (Research Diets). Rats were housed individually and provided access to standard rat chow (composition: 22% protein, 4.5% fat, 54% carbohydrate, 2.95 kcal/g; NUVILAB®) and water ad libitum, with a habituation period of 4 days. The subsequent 2 days were used for analysis. Total food intake and meal pattern were analyzed with the BioDAQ® system as previously described [24,25]. Briefly, the system uses a food hopper mounted on an electronic strain gauge-based load cell to measure food intake. The food hopper is weighed 50 times per second (accurate to 0.01 g), and the mean and standard deviation (SD) of intake over approximately 1 s are calculated by a peripheral computer. Feeding is signaled by a fluctuation in the food hopper weight (defined as SD > 2000 mg), caused by the animal eating, at which the date, time and hopper weight were recorded. The end of a feeding bout (but not necessarily a meal—see below) is signaled when the hopper is left undisturbed for 2 min (defined as SD < 2000 mg), at which time the duration of the feeding event and the amount eaten (initial hopper weight minus the final hopper weight) was calculated. Each feeding event record (cage/animal number, start date and time, feeding duration, final hopper weight, and amount eaten) is exported to a central computer, and a Microsoft Excel-based spreadsheet (Microsoft, Redmond, WA) is used for calculation of the desired parameters (see below) and summarization of data. Food spillage was monitored during the study, and no spillage was observed during the test periods. The amount of food eaten, meal size and meal frequency were calculated for the last 2-day period and stratified into nocturnal and diurnal periods. A meal was defined as a difference in hopper weight of >0.1 g, separated from other feeding bouts by >15 min [26,27], and meal size as the amount in grams that an animal ate divided by the number of meals in a period.

Sweet food consumption in response to tail pinch stress – The tail-pinch stress was chosen as a stressor because it increases dopamine release [28,29], and eating is the predominant response to tail-pinch [30–34]. Prior to testing for the sweet food consumption, the animals were habituated to the new food (Froot Loops®, Kellogs), as described before [35–37]. A subset of animals was submitted to tail pinch stress, which consisted of pinching the region between 2 and 3 cm from the base of the tail for the period of 1 min, with a wooden clothespin, and then returned to the homecage. A previously measured amount of Froot Loops, as well as standard chow, was left on the homecage, then sweet food intake and rat chow was measured 1 h after the tail pinch stress.

Conditioned place preference (CPP) to sweet food – The conditioned place preference task consisted of a 210 mm × 450 mm × 410 mm box divided equally into two compartments that were connected by a small opening

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