Hormonal, hypothalamic and striatal responses to reduced body weight gain are attenuated in anorectic rats bearing small tumors

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A B S T R A C T

Lack of compensatory or even reduced food intake is frequently observed in weight-losing cancer patients and contributes to increased morbidity and mortality. Our previous work has shown increased transcription factor expression in the hypothalamus and ventral striatum of anorectic rats bearing small tumors. mRNA expression of molecules known to be involved in pathways regulating appetite in these structures was therefore assessed in this study. Given that pain, pro-inflammatory cytokines and metabolic hormones can modify food intake, spinal cord cellular activation patterns and plasma concentrations of cytokines and hormones were also studied. Morris hepatoma 7777 cells injected subcutaneously in Buffalo rats provoked a 10% lower body weight and 15% reduction in food intake compared to free-feeding tumor-free animals 4 weeks later when the tumor represented 1–2% of body mass. No differences in spinal cord activation patterns or plasma concentration of pro-inflammatory cytokines were observed between groups. However, the changes in plasma ghrelin and leptin concentrations found in food-restricted weight-matched rats in comparison to ad libitum-fed animals did not occur in anorectic tumor-bearing animals. Real-time PCR showed that tumor-bearing rats did not display the increase in hypothalamic agouti-related peptide mRNA observed in food-restricted weight-matched animals. In addition, microarray analysis and real-time PCR revealed increased ventral striatal prostaglandin D synthase expression in food-restricted animals compared to anorectic tumor-bearing rats. These findings indicate that blunted hypothalamic AgRP mRNA expression, probably as a consequence of relatively high leptin and low ghrelin concentrations, and reduced ventral striatal prostaglandin D synthesis play a role in maintaining cancer-associated anorexia.

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1. Introduction

Healthy individuals increase food consumption after weight loss due to food deprivation or restriction until they have regained their initial weight (Dulloo et al., 2004). This weight loss-induced compensatory food intake depends on the hypothalamic action of hormones including ghrelin and leptin and of neurotransmitters, such as serotonin, which regulate the expression of the alpha melanocyte-stimulating hormone (α-MSH)-containing precursor proopiromelanocortin (POMC), neuropeptide Y and agouti-related peptide (AgRP) (Fekete et al., 2006; Seoane et al., 2003; Xu et al., 2008). Indeed, both a 24 h fast and a 1 month 50% food restriction increase hypothalamic expression of neuropeptide Y and AgRP and reduces that of POMC (Sucajtys-Szulc et al., 2010). Inhibition of melanocortin types 3 and 4 receptors (MC3&4R) by AgRP in the paraventricular hypothalamus increases food intake after deprivation-induced weight loss, an effect that is amplified by concurrent neuropeptide Y administration (Wirth and Giraudo, 2000). MC4Rs are also present in ventral striatal neurons containing gamma-aminobutyric acid (GABA) (Liu et al., 2003) known to express opioid peptides and receptors (Kalyuzhny and Wessendorf, 1998; Oertel and Mugnaini, 1984). Furthermore, ventral striatal administration of GABA or opioid receptor antagonists reduces deprivation-induced food intake (Bodnar et al., 1995; Kandov et al., 2006). Increased food intake after food restriction- or deprivation-induced weight loss in healthy animals thus seems to involve both hypothalamic and ventral striatal structures.

Cancer is often accompanied by retarded growth in children and adolescents and weight loss in adults (Kalyuzhny and Wessendorf, 1998; Mosby et al., 2009; Nething et al., 2007). In addition, many cancer patients also show reduced food intake. Although metabolic
manifestations in cancer patients can vary from prime anorexia to predominant muscle loss (Davis et al., 2004; Lasheen and Walsh, 2010), their concurrence leads to the anorexia–cachexia syndrome that provokes 10–20% of cancer deaths (Tisdale, 2002). Regardless of whether or not food intake is reduced, the compensatory increase of dietary intake in response to weight loss is lost in cancer patients (Boseus et al., 2001).

Brain MC4Rs are known to mediate anorexia–cachexia induced by large tumors (>5% of body weight) in rodents (Marks et al., 2001). However, tumor mass seldom exceeds 1% of host mass in cancer patients (Costa, 1977). Interestingly, we have previously shown that decreased weight gain and reduced food intake provoked by small tumors (1–2% of body weight) is accompanied by increased expression of Fos transcription factors in the paraventricular hypothalamus and ventral striatum (Konsman and Blomqvist, 2005). Given the role of hypothalamic melanocortin signaling and ventral striatal GABA-ergic and opioid transmission in regulating food intake after deprivation-induced weight loss in healthy animals (Kandov et al., 2006; Wirth and Giraudou, 2000), we hypothesized that AgRP, POMC, GABA or endogenous opioid expression differs between anorectic tumor-bearing animals and healthy food-restricted rats.

Several tumors have been reported to produce the anorectic cytokine-like hormone leptin (Howard et al., 2010) or the cytokine endothelin-1 (Bagnato et al., 2008; Pfab et al., 2004), which, in turn, induces the production of pro-inflammatory cytokines (McMillen et al., 1995; Ruetten and Thiemermann, 1997) known to lower food intake (Konsman and Dantzer, 2001). Alternatively, endothelin-1 can act on sensory nerves to activate dorsal spinal cord regions processing nociceptive information (Hans et al., 2009). Moreover, the overall pattern of forebrain Fos expression during cancer-associated anorexia observed in our previous work (Konsman and Blomqvist, 2005) is reminiscent of that seen after sustained nociceptive stimulation of deep tissues (Monnikes et al., 2003; Ohtori et al., 2000). Since pain treatment reduces anorexia in cancer patients (Feuz and Rapin, 1994; Meuser et al., 2001) and nociception increases hypothalamic POMC expression (Seo et al., 2008), it may play part in cancer-associated anorexia. We therefore hypothesized that plasma concentrations of anorectic cytokines and hormones or dorsal spinal cord processing are increased in tumor-bearing animals.

To address these hypotheses, we evaluated hypothalamic and ventral striatal mRNA expression of molecules known to be involved in pathways regulating appetite in relation to plasma concentrations of metabolically active hormones and cytokines as well as spinal cord cellular activation patterns in our animal model of cancer-associated anorexia. In addition, we also studied hypothalamic and ventral striatal mRNA in a non-hypothesis driven manner as to identify new candidate molecules relevant to cancer-associated anorexia.

2. Material and methods

All surgical procedures and treatments were carried out according to the guidelines of the French National Committee for Animal Care and the European Convention of Vertebrate Animals Used for Experimentation as part of the European Council Directive 86/609/EEC of November 1986. Reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA), unless otherwise stated.

2.1. Animals

Twenty-two male Buffalo rats (160–230 g upon arrival; Harlan, Horst, the Netherlands) were housed in transparent cages with unrestricted access to food and water in a room maintained at 21.5–22.5 °C with lights on from 07:00 AM until 07:00 PM.

2.2. Tumor cell inoculation

Morris hepatoma 7777 cells characterized by glucocorticoid-inhibited alpha-fetoprotein production according to the supplier (LCG Standards, Molsheim, France) were stocked in liquid nitrogen and used within 6 months after receipt. After 4 weeks of culture in Dulbecco’s Modified Eagle’s Medium ( Gibco Invitrogen, Carlsbad, CA, USA) with 10% fetal bovine serum (Eurobio, Courtaboeuf, France), cells were harvested, washed with 0.2% EDTA and detached with 0.05% trypsin, centrifuged and transferred to sterile phosphate-buffered saline (PBS) without Mg<sup>2+</sup> and Ca<sup>2+</sup>. Seven rats were anesthetized with ketamine/xylazine (61 and 9 mg/kg ip, respectively; Rhône Mérieux, France and Bayer Pharma, Sens, France) and injected subcutaneously between the scapulae with ten million Hepatoma 7777 cells. Fifteen other animals received a subcutaneous injection of PBS under anesthesia.

2.3. Experimental groups and protocol

Food intake and body weight were measured every other day during the first 2 weeks after subcutaneous injection, daily during the third week and twice daily afterwards. When the difference in body weight gain relative to the start of the experiment between ad libitum fed tumor-bearing rats and PBS-injected animals reached 10%, eight rats of the latter group were subjected to a 33% food restriction (consisting of a daily portion of 12 g of food pellets, while free-feeding animals ate around 18 g per day) until they reached the same body weight as the former. Animals were then anesthetized by an intraperitoneal injection of sodium pento-barbital (Ceva Santé Animale, Libourne, France) and decapitated. Blood was collected in 10% EDTA-containing tubes (Sarstedt, Marnay France) for plasma hormone and cytokine assays and brains were rapidly removed and placed in ice-cold PBS. Epididymal, mediastinal and periareal fat pads were removed and weighed as their weight reflects body weight and correlates with leptin synthesis (Oliver et al., 2001).

2.4. Immunohistochemical detection of spinal cord cellular activation markers

Trunks containing spinal cord were fixed by intracardiac perfusion of 4% paraformaldehyde in 0.1 M PBS, after a short rinse with saline. Spinal cords were removed, post-fixed for 4 h, dehydrated in PBS containing 30% sucrose, frozen in liquid nitrogen vapors and cut into 30 μm sections, which were then stored and processed as previously described (Konsman et al., 1999). Increased expression of c-Fos and FosB in the dorsal spinal cord, are considered cellular activation markers of nociception (Coggeshall, 2005; Luis-Delgado et al., 2006). Immunohistochemistry was performed as previously described (Konsman and Blomqvist, 2005). Briefly, after several washes in 0.1 M Tris–buffered saline (TBS; pH 7.4), non-specific binding sites were blocked by a 45 min incubation in TBS containing 0.3% Triton X-100 and 1.0% bovine serum albumin (Euromedex, Souffelweyersheim, France). The first antibody (rabbit anti FosB (sc–48) or anti-c-Fos (sc–52), Santa Cruz Biotechnology, Santa Cruz, CA, USA) was then added diluted 1:2000 overnight. After rinses in TBS and a 30 min bath of 0.3% hydrogen peroxide, sections were incubated for 2 h with biotinylated donkey anti-rabbit immunoglobulin G (1:1000; Jackson West Grove, Pennsylvania, USA) and for 2 h with a complex of avidin and biotinylated peroxidase (1:1000; Vector laboratories, Burlingame, CA, USA) with intermittent washes in TBS. Finally, sections were stained using 3,3'-diaminobenzidine tetrahydrochloride as chromogen in the