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Research Report

Acute brown adipose tissue temperature response to cold in monosodium glutamate-treated Siberian hamsters

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ABSTRACT

Neonatal monosodium glutamate (MSG) administration increases adiposity, decreases energy expenditure and is associated with arcuate nucleus (Arc) destruction. Disrupted brown adipose tissue (BAT) thermogenesis underlies some of these effects, although, interscapular BAT temperature (T_{IBAT}) has not been measured. Therefore, we tested the effects of neonatal MSG or vehicle administration in Siberian hamsters and, when they were adults, measured T_{IBAT} during acute cold exposure. The Arc and its projection to the hypothalamic paraventricular nucleus (PVH) are both components of the CNS outflow circuits to IBAT, with the latter implicated in BAT thermogenesis that could be compromised by MSG treatment. Using a viral transneuronal tract tracer, pseudorabies virus (PRV), we also tested whether the components of these circuits were intact. As adults, MSG-treated hamsters had significantly increased body mass and some white fat pad masses, markedly reduced Arc Nissl and neuropeptide staining, and PVH neuropeptide fiber staining. Cold-exposed (18 h at 5 °C) MSG- and vehicle-treated hamsters initially maintained T_{IBAT}, but the ability of the former waned after 2 h being significantly decreased by 18 h. PRV immunoreactive fibers/cells were not altered by neonatal MSG treatment despite substantial Arc and PVH destruction. MSG- and vehicle-treated hamsters given an exogenous norepinephrine challenge showed identical increases in the duration and peak of T_{IBAT}. Thus, the inability of MSG-treated animals to sustain T_{IBAT} in the cold is not due to any obvious MSG-induced deletions of central sympathetic outflow circuits to IBAT, but appears to be extrinsic to the tissue nevertheless.

1. Introduction

A greater appreciation of the CNS control of peripheral metabolism, including the link between brain and adipose tissues, has developed recently, especially the role of the sympathetic nervous system (SNS) in the regulation of lipid storage/mobilization and thermogenesis by white and brown adipose tissue (WAT and BAT), respectively (for reviews see: Bartness and Bamshad, 1998; Bartness and Song, 2007b; Himms-Hagen, 1991). The exact mechanisms underlying the ability of the SNS to control lipid mobilization/thermogenesis remain to be precisely defined, however.

The preponderance of research on the central control of energy intake and expenditure has focused on the hypothalamus

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(for review see: Cone et al., 2001), with an even narrower focus on the arcuate nucleus (Arc). The Arc initially received attention because lesions of this nucleus, produced by neonatal administration of monosodium glutamate (MSG), result in obesity (*e.g.*, Olney, 1969). The Arc has been the focus of further research because it has receptors for leptin (*e.g.*, Hakansson et al., 1996; Mercer et al., 1996), the adipokine (Zhang et al., 1994) that is thought to be important in energy balance (for review see: Arch, 2005). More specifically, the Arc contains two major cell types — neurons synthesizing orexigenic neuropeptides [neuropeptide Y (NPY) and agouti-related protein (AgRP)] and anorexigenic peptides [proopiomelanocortin (POMC) and cocaine-amphetamine related transcript (CART); for review see: (Cone et al., 2001)]. Both sets of neurons are targets for the actions of leptin on energy balance (for review see: Berthoud, 2002). Of the efferents from the Arc, there are prominent NPY/AgRP and CART/POMC projections innervating the hypothalamic paraventricular nucleus (PVH; Bai et al., 1985; Cowley et al., 1999). Although the roles of these Arc neural populations in altering energy intake are well-accepted, appreciation of their involvement in energy expenditure is increasing (*e.g.*, Bing et al., 1997; Kong et al., 2003; Morris et al., 1998; Schoelch et al., 2002).

A classic approach to understanding the role of any brain site for a given physiological response, such as energy balance, is to destroy it. Complete Arc electrolytic lesions, although difficult, have been accomplished (Choi et al., 1999), but carry with them a high probability of destroying the median eminence/pituitary gland which greatly complicates the interpretation of this type of Arc lesion. An alternative approach to Arc destruction is offered by neonatal administration of monosodium glutamate (MSG). MSG is able to penetrate the brain (*e.g.*, Olney, 1969) via the underdeveloped neonatal blood brain barrier (BBB) in several brain areas, including the area postrema (AP; *e.g.*, Leitner and Bartness, 2008a; Phelix and Hartle, 1990; Takasaki, 1978), but most notably in the Arc where up to 80–90% of neurons can be ablated (Olney, 1969). This neonatal MSG treatment produces a distinct adult phenotype of decreases in linear growth (Tamura et al., 2002), increases in body fat (Leitner and Bartness, 2008a) and sympathetic nervous system (SNS) dysfunction, most notably for the latter resulting in decreased BAT thermogenesis (*e.g.*, Moss et al., 1985b; Yoshida et al., 1984).

BAT is the major organ responsible for non-shivering thermogenesis (*e.g.*, Foster and Frydman, 1978; for review see: Cannon and Nedergaard, 2004). The SNS innervation of BAT (Bamshad et al., 1999; Foster et al., 1982a,b) is critical for heat production during cold acclimation (Heldmaier et al., 1989; Himms-Hagen, 1986), overeating (Rothwell and Stock, 1982) and other conditions (for review see: Bartness and Song, 2005; Himms-Hagen, 1991). Of the BAT depots, interscapular brown adipose tissue (IBAT) receives the most attention because of its size, accessibility and clear innervation (for review see: Bartness and Song, 2005).

It appears that the impaired BAT thermogenesis in neonatally-treated MSG animals contributes to the genesis of their obesity in adulthood, most likely through reduction in the sympathetic drive to BAT (Morris et al., 1998), rather than an intrinsic defect in the underlying mechanisms controlling BAT thermogenesis [*e.g.*, the functioning of uncoupling protein-1 (UCP-1)]. That is, MSG-induced obesity is associated with decreases in BAT norepinephrine (NE) turnover (Nishioka et al., 1988; Rehorek et al., 1987; Yoshida et al., 1985), a neurochemical measure of sympathetic drive. These decreases in BAT NE turnover may be due to several factors including inappropriately low synthesis/release of NE from the SNS nerve terminals that innervate the tissue and/or MSG-induced disruption in the central SNS outflow circuits to BAT resulting in decreased NE release compared with animals that are neurally intact centrally.

Using the viral transneuronal retrograde tract tracer, pseudorabies virus (PRV), the CNS origins of SNS outflow circuits to IBAT have been determined in Siberian hamsters (Bamshad et al., 1999; Song et al., 2008), laboratory rats (Bamshad et al., 1999; Cano et al., 2003; Oldfield et al., 2002) and mice (Voss-Andreae et al., 2007). Among the many SNS outflow sites across the neuroaxis ultimately innervating IBAT, and of interest to the present study, are the Arc and one of its primary projection sites, the PVH (Bamshad et al., 1999; Bamshad et al., 1999; Song et al., 2008). Both the PVH (Freeman and Wellman, 1987; Yoshimatsu et al., 1993) and the Arc have been strongly implicated in the control of thermogenesis (Bing et al., 1997; Kong et al., 2003; Morris et al., 1998; Schoelch et al., 2002). Thus, the possibility exists that one of the extrinsic factors contributing to the ineffective IBAT thermogenic response to cold exposure of MSG-treated animals is the disruption of the brain–SNS–IBAT circuitry caused by the destruction of the Arc and/or its PVH projections.

Therefore, the present experiment tests IBAT function in adult hamsters that were treated with MSG- or its vehicle neonatally and that had temperature transponders implanted under IBAT for telemetric recording of IBAT temperature (T_{IBAT}) during cold exposure (18 h at 5 °C). To avoid the cold-induced increase in food intake that occurs in this species (Brito et al., 2008) that could dampen the cold-triggered stimulation of BAT thermogenesis, all animals also were simultaneously food deprived. Several days later we tested the intrinsic function of BAT thermogenesis by injecting NE systemically and measuring T_{IBAT}. It should be noted that, surprisingly, despite dozens of studies testing the response of BAT to several different stimuli in MSG-treated animals, direct measures of T_{IBAT} have not been tested and indeed, such measures are infrequently performed for any thermogenic challenge in any species. Instead, apparent surrogates of BAT thermogenesis are most frequently measured especially UCP-1 gene expression. MSG damage was quantified by standard histology for cells (Nissl staining) and more detailed analysis of the phenotype of the destroyed neurons using immunohistochemistry for a variety of neurochemicals. In a second experiment using PRV, we tested whether the SNS outflow circuitry from brain to IBAT in MSG-treated was compromised due to Arc destruction and/or its projections to the PVH, two sites that are part of the SNS outflow from brain to IBAT noted above (Bamshad et al., 1999; Song et al., 2008).

2. Results

2.1. *Verification of MSG-induced Arc, PVH and AP neuroanatomical damage*

MSG treatment significantly decreased cresyl echt violet Nissl staining, NPY- and AgRP-immunoreactive (ir) fibers throughout