EFFERENT POLYSYNAPTIC PATHWAYS FROM THE CNS TO THERMOREGULATORY END POINTS

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Brown adipose tissue (BAT) is a primary site contributing to thermal homeostasis through non shivering thermogenesis. It also plays a related and equally important role in diet-induced thermogenesis, dispersing excess energy and, in concert with food intake, regulating body weight. The central neural circuitry involved in the modulation of the activity of BAT via the sympathetic nervous system is relatively poorly defined. Detailed descriptions of these pathways has been limited by an inability of researchers to assign a "BAT- directed" tag to neurons deep within the CNS which are removed from their physiological endpoint by several synapses. The utilisation of neurotropic viruses which are transported retrogradely through chains of synaptically-connected neurons offers a solution to this problem. To this end, the Bartha strain of pseudorabies virus (PRV) has been used in the present experiments to map polysynaptic projections to the BAT in rats and, in conjunction with the immunostaining of candidate neuropeptides, these pathways (particularly in the hypothalamus) have been characterised in regard to their chemical phenotype. Sprague Dawley rats were anaesthetised with sodium pentobarbitone (60mg/kg ip) and PRV was injected into multiple sites in the interscapular brown fat. Rats were allowed to survive for varying periods to allow the virus to travel to different extents throughout the CNS. Rats were then re anaesthetised and perfused transcardially with 4% paraformaldehyde. Forty µm sections were cut throughout the brain, spinal cord and stellate ganglia. Both virus and a range of peptides were localised using appropriate antibodies and standard immunohistochemical techniques. The extent of double labelling of immunopositive profiles was assessed using fluorescence microscopy. Forty eight hours after inoculation of the BAT, virally-infected neurons were detected in the stellate ganglion and by 72 hours neurons infected with the virus were present in the ipsilateral thoracic spinal cord, but also in a range of medullary, pontine and hypothalamic sites considered "premotor" to sympathetic preganglionic neurons. These included the rostroventrolateral medulla, parapyramidal area, raphe pallidus and obscurus, A5 region, locus coeruleus, subcoeruleus, and peri aqueductal gray. The chemical signature of infected neurons in the stellate ganglia was consistent with recruitment of virus into fibres innervating adipocytes rather than blood vessels. In the hypothalamus, about 15% of neurons in the paraventricular nucleus contained oxytocin but did not express corticotropin releasing factor (CRF), galanin, vasopressin, cocaine amphetamine regulated transcript (CART) or melanin concentrating hormone (MCH). In the lateral hypothalamus however, MCH, CART and two members of the orexin family, orexin A and orexin B were abundantly co-expressed. Small numbers of infected neurons were found within the arcuate nucleus at the longest time interval studied and these contained leptin receptors and POMC. In the retrochiasmatic nucleus, by far the most prominent co-localisation occurred with CART accounting for approximately 85% of the neurons directed polysynaptically to the BAT. Orexin B and MCH are best recognised for their impact on feeding behavior and have been proposed as major mediators of this behavior via the lateral hypothalamus. Therefore the present data highlights an anatomical framework which may subserve thermogenesis but also indicates that regions, particularly in the lateral hypothalamus, known for their involvement in feeding may also contribute to thermogenesis. An intriguing possibility is that single neurons may coordinate both functions.

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