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Review

Delineating the regulation of energy homeostasis using hypothalamic cell models

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ABSTRACT

Attesting to its intimate peripheral connections, hypothalamic neurons integrate nutritional and hormonal cues to effectively manage energy homeostasis according to the overall status of the system. Extensive progress in the identification of essential transcriptional and post-translational mechanisms regulating the controlled expression and actions of hypothalamic neuropeptides has been identified through the use of animal and cell models. This review will introduce the basic techniques of hypothalamic investigation both *in vivo* and *in vitro* and will briefly highlight the key advantages and challenges of their use. Further emphasis will be place on the use of immortalized models of hypothalamic neurons for *in vitro* study of feeding regulation, with a particular focus on cell lines proving themselves most fruitful in deciphering fundamental basics of NPY/AgRP, Proglucagon, and POMC neuropeptide function.

1. Introduction

Positioned within the central nervous system (CNS), the hypothalamus has emerged as the master regulator of systemic energy homeostasis. Neurons comprising the hypothalamus exhibit extensive connections both within and between the hypothalamic nuclei, to other regions of the CNS, and to the periphery. This vast neuronal network together with the close proximity of the hypothalamus to blood brain barrier (BBB) ensures that processes involved in energy management are orchestrated in an organized, complemented, and timely fashion and co-ordinated according to nutritional, hormonal, and environmental cues. Decades worth of investigation have defined distinct hypothalamic nuclei and the neuronal populations pivotal to the regulation of a variety of physiological processes including reproduction, circadian rhythms, body temperature, and energy homeostasis. This review will describe the variety of *in vivo* and *in vitro* experimental approaches employed in the field of hypothalamic research, while highlighting the advantages and caveats to their usage. A focus will be placed on the evolution of hypothalamic neuronal cell models, particularly those expressing key feeding neuropeptides such as agouti-related neuropeptide (AgRP)/neuropeptide Y (NPY), proglucagon, and pro-opiomelanocortin (POMC), in order to highlight their extensive experimental potential and proven application to the study of energy regulation.

2. In vivo modes of hypothalamic investigation

2.1. Intracerebroventricular (ICV) injection and microinjection

Direct application of a desired chemical, biological, hormonal, or phototypic agent into the hypothalamus (micro-injection) or to the cerebral spinal fluid (CSF) of the cerebral lateral ventricles (intracerebroventricular; ICV) are techniques widely employed in hypothalamic investigation (Taib et al., 2013). Upon insertion of the cannula into the animal, agents can be administered bilaterally or unilaterally into specific hypothalamic nuclei, such as the arcuate nucleus (ARC), ventromedial hypothalamus (VMH), paraventricular nucleus (PVN), and dorsomedial hypothalamus (DMH) through the use of stereotaxic co-ordinates from the appropriate brain atlas during its installation. Surpassing the BBB, researchers have unimpeded access to hypothalamic tissue within the context of living animals and thus are poised to measure the direct physiological or pathophysiological consequence of the injected agent within the CNS. Significant advances in molecular genetics and

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the availability of complementary transgenic mice enable further targeting of specific neuronal populations within these hypothalamic nuclei and greatly expand the versatility of this technique. For example, Aponte and colleagues were able to examine the impact of ARC AgRP and POMC neurons on the feeding behavior of mice using a combination of genetic and ICV tools. Upon cannulation of mice expressing the light-activated cation channel channelrhodopsin-2 (ChR2) in AgRP or POMC neurons of the ARC hypothalamus only and their selective stimulation by light pulses stemming from an optic fiber revealed the different mechanisms employed by the anorexogenic and orexogenic neuronal populations (Aponte et al., 2011). From this work, they were able to determine that while POMC neurons exert their effect on feeding through the melanocortin pathway, AgRP neurons work independently from this system and directly modulate distinct feeding circuitry (Aponte et al., 2011). As commonly done in similar studies, the positional accuracy of the cannula and the correct administration of the stimuli to the hypothalamus was confirmed upon injection of a marker, in this case labeled beads, followed by histological analysis. Gross damages to the tissue upon cannula installation that may have influenced the study can also be ruled out histologically.

Although micro- and ICV injection gives researchers direct access to the hypothalamic tissue without interference from the BBB, it is an invasive and challenging technique. Firstly, relative to other routes of administration, cannulation requires extensive surgical skill and significant recovery times post-surgery for the animal prior to the commencement of experiments. Secondly, the technique is costly often requiring additional animal numbers to account for those that may succumb to the operation or hereafter, and higher housing budgets given that cannulated animals should be housed individually in wire-free cages to limit interference with their cannulas. Thirdly, while care is taken to ensure no gross abnormalities have been introduced into the brain tissue upon installation of the cannula, one cannot rule out minute tissue damage that may modulate brain physiology and compound conclusions of the study. Fourthly, unlike other routes of administration, such as intravenous injection, the injectable volume into the CNS of a rat or mouse is minute, given the CSF volume ranging between 40 and 90 μL, which obviously confounds the application of specific doses of reagents or use of those with limited solubility (Oshio et al., 2005; Davson, 1969). Finally, compounds administrated by ICV injection may not reflect true concentrations achieved by peripheral routes due to various properties of the BBB. For instance, a peripherally applied compound may be impenetrable to the BBB, non-uniformly transported throughout the CNS, or specifically accumulated within distinct regions under different metabolic states. On the other hand, injection into the CSF may impede the accompanying peripheral effects, particularly if the compound is occluded from entering the general circulation. Accordingly, comparison across studies on specific drugs or compounds and their resulting conclusions should be preceded with caution particular if they differ in their application routes unless care has been taken to account for the points listed above.

2.2. Lesions

Introduction of lesions by electrical or chemical means is an old technique used to measure the physiological relevance of particular hypothalamic nuclei and characterize phenotypic consequences upon its disruption *in vivo*. Application of excitotoxins or chemicals that lead to prolonged neuronal excitement and death is particularly widespread in the study of hypothalamic function and have involved the use of monosodium glutamate (MSG), N-methyl-D-aspartic acid (NMDA), ibotenic acid, and kainic acid (Chou et al., 2003; Clark et al., 1992; Natarajan and Wilkinson, 1997; Olazabal and Ferreira, 1997). Excitotoxicity from these compounds likely arises from ion misregulation, excessive and prolonged changes in membrane potential, activation of enzymes such as proteases and caspases, and loss of post-synapitc neuronal integrity (Beck et al., 2003; Olney et al., 1986; Dawson et al., 1991; Leist et al., 1998; Brecht et al., 2001).

Lesions can be targeted to specific hypothalamic nuclei, such as ARC, DMH, LH, PVN, and preoptic area (POA), using brain atlases and stereotaxic co-ordinates or to specific neuronal populations through the use of transgenetics (Chou et al., 2003; Clark et al., 1992; Natarajan and Wilkinson, 1997; Rockhold et al., 1990; Gerashchenko et al., 2001; Hasegawa et al., 2005). For instance, administration of diphtheria toxin (DT) into mice engineered to express DT receptors on AgRP neurons only, enable the specific ablation of AgRP-expressing neurons in the hypothalamus in order to characterize the impact of starvation at the cellular level (Wu et al., 2008; Gropp et al., 2005; Luquet et al., 2005; Buch et al., 2005). Additionally, the transgenetic introduction of the neurotoxin ataxin-3 into specific neuronal populations can also induce apoptosis (Yoshizawa et al., 2000). These techniques have been successfully applied for the study of AgRP, MCH, orexin, and POMC hypothalamic neurons and will likely be continually fruitful in hypothalamic investigation (Hara et al., 2001; Beuckmann et al., 2004; Alon and Friedman, 2006; Yamanaka et al., 2003; Bewick et al., 2005; Whiddon and Palmiter, 2013; Wu et al., 2012; Zhan et al., 2013).

Despite these advantages, the use of lesion-based studies does have its caveats. Firstly, unintentional damage likely accompanies the introduction of hypothalamic lesions, which can not only initiate variability across experimental animals, but also mediate a spectrum of undesired phenotypic consequences (Zaczek et al., 1980). Secondly, brain regions and neuronal populations are not equally susceptible to disruption and each must properly optimized for each experimental condition. For instance, ARC and SCN neurons have been shown to be exceptionally resistant to excitotoxic compounds (Kohler and Schwarcz, 1983; Bottum et al., 2010; Gojska and Belsham, 2014; Nazarians-Armavil et al., 2014). Further, the sensitivity of neuronal populations to different excitotoxins can also vary (Purser et al., 2013). Thirdly, the mode of lesion formation can dramatically influence experimental outcomes, as shown from various studies on ARC and LH neurons (Gojska and Belsham, 2014; Backholer et al., 2010; Williams et al., 2010). This phenomenon may result from the discrete versus diffuse damage boundaries from electrolytic or excitolytic lesions respectively, or the axon-preserving properties of some exotoxins, which generally target neuronal cell bodies only (Gojska and Belsham, 2014; Backholer et al., 2010; Williams et al., 2010).

3. In vitro modes of hypothalamic investigation

3.1. Organotypic slices

The organotypic technique has survived several decades of optimization and now is one of the leading methods to study hypothalamic function *in vitro* (see review Gahwiler et al., 1997). After generating a series of slices (<100–400 μm thick), they can be further dissected to obtain specific hypothalamic nuclei or regions and subjected to extensive manipulation for electrophysiological, chemical, and biological experimentation. This technique is particularly advantageous in the investigation of neuronal networks given the maintenance and accessibility of cyto-architecture and has been applicable to the study of various diseases of the CNS (Lossi et al., 2009). Combining this method with viral vectors to mediate expression of reporter genes in specific cell types or performing this technique on tissues isolated from transgenic mice