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Fos Expression in the Arcuate POMC-Neurons after Gastric Stimulation in the Rat

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The arcuate nucleus (AR) of the hypothalamus is a critical component of forebrain pathways that regulate a variety of neuroendocrine functions and plays a particularly important role in the regulation of feeding and metabolism. Expression of the immediate early gene product c-Fos is considered to be a marker for neuronal activation in different brain regions in response to afferent input. To determine neurochemical phenotype of hypothalamic arcuate neurons receiving input from the stomach, we carried out gastric stimulation in combination with c-Fos/peptide double-labelling immunocytochemistry. A fairly high number of α-MSH-immunoreactive (IR) neuronal perikarya and a high density of α-MSH-IR fibers are present in the hypothalamic AR. Quantitative analysis revealed that gastric distension upregulates the POMC derived α-MSH expression alone as well as c-Fos/α-MSH staining in AR neurons. These results demonstrate that arcuate α-MSHergic neurons are implicated in the dissemination of gastric distension signals in the brain.

Key words: arcuate nucleus, gastric stimulation, α-MSH, c-Fos-expression, rat.

Introduction

The arcuate nucleus (AR) of the hypothalamus is a critical component of forebrain pathways that regulate a variety of neuroendocrine functions [1]. The AR sends strong projections to other hypothalamic nuclei implicated in the regulation of feeding and metabolism. It consists of two neuronal populations: one of them stimulates food intake and expresses neuropeptide Y (NPY) and agouti-related peptide (AgRP) [2], while the other suppresses feeding and expresses proopiomelanocortin (POMC) and cocaine-amphetamine-related transcript (CART) [3]. Alpha-melanocytostimulating hormone (α-MSH) is one of the bioactive peptides derived from the prohormone POMC, which also participates in the control of food intake and body weight [1]. Therefore, it is important to understand the POMC gene regulation in the brain, as pharmacological manipulations of POMC expression/processing could be a potential strategy to combat obesity.

During the last decade, a new method for activity stains was developed [4], which allows expressing the immediate early gene product c-Fos and thus distinguishing functionally identified subpopulations of neurons.

The aim of the study was to assess quantitatively the distribution of c-Fos immunoreactive neurons in the hypothalamic arcuate nucleus after gastric distension, and to determine the proportion of α-MSHergic neurons activated by this stimulus.

Material and Methods

Twelve rats were anesthetized with ketamine (80 mg/kg i.p.). Cannulas were implanted in the stomach. After two a weeks latex balloon was inserted in the stomach and it was distended by filling the balloon with warm water. Rats were left for 60 min to allow c-Fos expression, and then they were perfused with fixative (4% phosphate-buffered saline paraformaldehide). Brains were removed, post fixated and 30 μm thick sections were cut in a cryostat at — 20°C. Sections were incubated for 24 h for double labelling, first with anti-c-Fos anti-serum, then with anti-α-MSH-antibody according to the protocol for avidin-biotin complex method. C-Fos staining reaction product was brown, while α-MSH was visualized with the SG chromogen as gray staining of the cytoplasm of the immunoreactive cells.

Results

A fairly high number of α-MSH-immunoreactive (IR) neuronal perikarya were present in the AR. In addition, a high density of α-MSH-IR fibers was particularly impressive. Numerous α-MSH-containing varicose neuronal fibers were found projecting to various parts of the hypothalamus — paraventricular nucleus, dorsomedial hypothalamic nucleus, perifornical area, lateral hypothalamus (LH), zona incerta etc. We found α-MSH-IR neurons mainly in the ventrolateral part of the AR. The overwhelming majority of distension-induced c-Fos-expression was in the lateral region of AR, where POMC neurons reside (Fig. 1). Many of the c-Fos-IR neurons were α-MSH/c-Fos double labelled. The absolute numbers of c-Fos-, α-MSH-positive neurons and double-labelled cells in each section were expressed as percentage from the total numbers of neuronal perikarya. The corpus distension produced significant increases in numbers of neurons expressing α-MSH (37.4%) above the control conditions (26-27.7%). The proportion of double-labelled cells in the AR of the stimulated group was higher (20.3% vs.17.3%) than in the control animals without gastric distension.

Discussion

The present study demonstrates that gastric distention, in the absence of other stimuli, leads to expression of c-Fos in the AR neurons. The vast majority of distension-induced c-Fos-expression was in the lateral part of AR, where neuronal perikarya expressing the POMC derived anorexigenic peptide α-MSH are situated. Gastric distention also increases the number of α-MSH neurons (37.4%) vs. 26% in animals with no distention. This effect leads to decreased appetite and food ingestion. Heisler et al. (5) have revealed that central serotonin (SER) systems are involved in the α-MSH activation via direct release of SER in the AR and binding to POMC neurons.

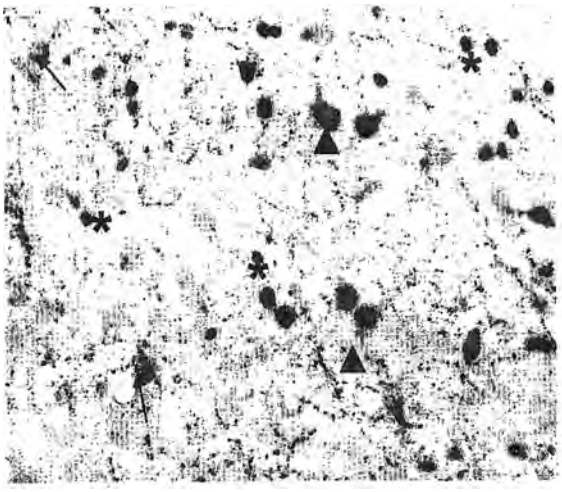

Fig. 1. Light microscope image of a frontal section of rat AR after gastric distension, processed for double immunocytochemistry for c-Fos and α-MSH. Neurons that were counted as α-MSH-positive but not activated by gastric distension (arrows), neurons that were α-MSH-negative but activated or c-Fos positive (asterisks), and neurons that were double-labelled (arrowheads) are indicated. × 160

The numerous α-MSH-containing fibers, we observed distributed throughout various hypothalamic nuclei, suggest that the neurotransmitter may influence the function of other brain structures, involved in the final physiological regulation of eating, and thus play a pivotal role in the control of energy balance and body weight. Our present findings suggest that populations of α-MSH neurons in the AR may provide a link between mediobasal hypothalamic satiety and LH phagic centers. These α-MSH-IR projections may underlie some of the extremely complex responses associated with hunger, food intake, and satiety.

In summary, we concluded that α-MSH arcuate neurons may be activated by stimuli that excite gastric mechanoreceptors (e.g., gastric balloon distension or ingestion of a large meal) and lead to cessation of feeding behavior.

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