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Research Report

Expression of purinergic receptors in the hypothalamus of the rat is modified by reduced food availability

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Abbreviations:
AGRP, agouti gene related protein
ARC, nucleus arcuatus
DMH, dorsomedial hypothalamus
IR, immunoreactivity
LH, lateral hypothalamus
NAc, nucleus accumbens
NO, nitric oxide
NOS, nitric oxide synthase
NPY, neuropeptide Y
Ob-Rb_L, leptin receptor (long form)
PVN, paraventricular nucleus
VMH, ventromedial hypothalamus

ABSTRACT

ATP-sensitive P2 receptors are suggested to play an important role in the cerebral signal transduction. We examined the expression of the $P2Y_1$ receptor and the possibly downstream-related neuronal nitric oxide synthase (nNOS) in the hypothalamus of rats food-restricted for 3 or 10 days and rats refed after a restriction of 10 days. The restriction caused a reduction of the body weight and plasma triacylglyceride, an increase of non-esterified fatty acid levels correlating with a decrease of leptin levels and an enhancement of plasma corticosterone. All changes returned to basal levels after refeeding. The restriction induced an enhanced intake within 30 min after food presentation and a reduction in the latency. Interestingly, the latter was not abolished by refeeding. The daily food intake induced by refeeding was enhanced at the first day only. The expression of hypothalamic $P2Y_1$ receptor/nNOS mRNA and protein and of leptin receptor mRNA were enhanced after restricted feeding. These changes were abolished after 3 days of refeeding. Immunofluorescence studies indicated that $P2Y_1$ receptor and nNOS immunoreactivities are present in the dorsomedial, ventromedial and lateral hypothalamus and in the nucleus arcuatus. $P2Y_1$ receptor-positive cells were partially also nNOS-positive. The $P2Y_1$ receptor labeling was restricted to cell bodies of obviously non-glial cells, whereas nNOS labeling could be detected also at cellular processes of these cells. In the nucleus arcuatus, astrocytes were identified, expressing $P2Y_1$ receptors at cell bodies and cellular processes. The data suggest that restricted feeding may enhance the sensitivity of the hypothalamus to extracellular ADP/ATP by regulation of the expression of $P2Y_1$ receptors and possibly of their signal transduction pathway via nitric oxide production.

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1. Introduction

Food intake is modified by sensory information, hormonal signals related to energy balance and satiety signals (e.g., the anorectic peptide leptin) elicited by food ingestion. Today, purine nucleotides are not only accepted as sources of chemical energy but also as extracellular signaling molecules, probably involved in the regulation of processes such as the modulation of feeding (Krügel et al., 2004).

ATP released into the extracellular space may modulate the activity of central and peripheral neurons. It may act as a neurotransmitter by itself or in concert with classic transmitters as a cotransmitter (Burnstock, 2004). Adenine nucleotides exert their biological function by activation of cell surface P2 receptors. These receptors have a high affinity for ADP/ATP in contrast to the degradation product adenosine (Ralevic and Burnstock, 1998). They belong either to the P2X (ligand-gated cationic channels) or to the P2Y subclasses (G-protein-coupled receptors) (Ralevic and Burnstock, 1998; Burnstock, 2001), both widely distributed in peripheral tissues and the nervous system (Nörenberg and Illes, 2000; Moore et al., 2001). Recent studies indicate that P2 receptors in the nucleus accumbens (NAc) are involved in the modulation of neurotransmitter release (Krügel et al., 2003a), in the reinforcement of explorative behavior (Krügel et al., 2004) and modulation of feeding and feeding-evoked dopamine release (Kittner et al., 2000, 2004). Further, the expression of the mRNA of the ADP/ATP-sensitive $P2Y_1$ receptor in the NAc was altered in response to reduced food availability, possibly reflecting the habituation of behavior for food seeking and taking directed to achieve energy homeostasis and subsequent satisfaction (Krügel et al., 2003b). More recently, the hypothalamus has been regarded as a part of the extended limbic system responsible for emotion and motivation (Morgane et al., 2005).

In the hypothalamus, both P2Y (Moore et al., 2000; Kittner et al., 2003) and P2X receptors (Xiang et al., 1998; Loesch and Burnstock, 2001; Wakamori and Sorimachi, 2004) are expressed. However, their physiological importance in the process of feeding is completely unknown. Based on in vitro investigations, it has been reported that ATP is released from hypothalamic neurons upon nerve stimulation (Potter and White, 1980; Sperlágh et al., 1998), and that this ATP may induce a direct excitation of hypocretin-1/orexin-A neurons in the lateral hypothalamus (Wollmann et al., 2005) as well as of acutely dissociated ventromedial hypothalamic neurons (Sorimachi et al., 2001). The stimulation of $P2Y_1$ receptors results in the activation of diverse signaling pathways, including the production of nitric oxide (NO) by nitric oxide synthase (NOS) (You et al., 1997; Malmsjo et al., 1999).

NO is thought to participate in the feeding drive (Morley et al., 1996), possibly by the facilitation of transmitter release in the hypothalamus (Prast and Philippu, 2001). It was reported that the inhibition of the NOS decreases food intake in obese and normal animals (Morley et al., 1996; Squadrito et al., 1994). Further, intracerebroventricular (i.c.v.) leptin injection has been documented to be capable of inhibiting diencephalic NOS activity and food intake in mice which could be abolished by L-arginine (Calapai et al., 1998). The neuronal (nNOS) type of NOS is widely distributed in the brain, e.g., hippocampus, cerebellum, striatum, amygdala and several hypothalamic nuclei (Endoh et al., 1994) and was shown to be coexpressed with the $P2X_2$ receptor subunit in the hypothalamus and brain stem of the rat (Yao et al., 2003). Various groups of NOS-positive neurons in the hypothalamus were found to colocalize NPY receptor Y1 staining in the ventromedial (VMH) and dorsomedial (DMH) hypothalamic nucleus, the nucleus arcuatus (ARC) and occasionally in the lateral hypothalamus (LH) (Fetissov et al., 2003).

We suggest that extracellular purinergic signaling may play a pivotal role in the hypothalamic regulation of feeding, at least partly via the production of NO. In this study, we focus on the expression of mRNA and protein for $P2Y_1$ receptors during restricted feeding and a subsequent refeeding interval. We also measured the respective expression of the nNOS. With immunohistochemical studies, we confirmed the colocalization of both proteins as an indication for a possible functional interaction. The process of chronic succession of hunger and satiety, which imitates natural conditions more appropriately than complete starvation, was evaluated by the recording of the body weight, the analysis of metabolic parameters, the measurements of plasma concentrations of corticosterone and leptin as well as by the determination of the hypothalamic expression of the signaling subtype of the leptin receptor (long form; Ob-R_L) during both feeding intervals. Chronic food restriction is a variable that may enhance the responsiveness to rewarding stimuli (e.g., food itself) depending on the extent of restriction (Carr, 2002); therefore, we have determined various behavioral data, such as the daily food intake, the food intake within 30 min after its delivery and the latency between food delivery and its first intake.

2. Results

Restricted feeding of rats caused a significant reduction of the body weight compared to that of the normally fed group ($P = 0.026$). Table 1 shows these values and their complete recovery after refeeding. The multiple comparison also revealed that there were no differences in the 24-h food intake between both groups before food restriction and after 3 and 10 days of ad libitum feeding (Table 1). However, at the first day of refeeding, the 24-h food intake of the restricted group was enhanced to 34.0 ± 2.39 g compared to the normally fed group which consumed 26.1 ± 1.8 g ($P = 0.008$) at the same day, as well as compared to the amount before restriction ($P < 0.001$) (not shown). The increased food intake at the first day of refeeding returned to normal values afterwards (Fig. 1A). No differences were found in the daily intake of water between both treatments (Table 1). Within the restricted group, an enhanced intake of water of 45.9 ± 3.8 ml was found only at the first day of refeeding compared to the intake of 31.0 ± 2.0 ml before restriction ($P < 0.001$) as well as versus ad libitum feeding at day 3 and 10 ($P = 0.02$ and $P = 0.012$). The restricted feeding caused an elevation of the plasma corticosterone which recovered by ad libitum feeding afterwards ($P = 0.006$) (Table 1). The plasma leptin was reduced by food restriction but also reached basal levels after refeeding ($P < 0.001$). The plasma glucose level did not change by the food restriction. The plasma triacylgyceride level was significantly reduced by the