Effects of Blockers of Carbohydrate and Lipid Metabolism on Expression of mRNA of Some Hypothalamic Neuropeptides

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The effects of single injections of 2-deoxyglucose or 2-mercaptoacetate on the expression of mRNA of neuropeptide Y, pro-opiomelanocortin, and melanin-concentrating hormone in rat hypothalamus were studied by *in situ* hybridization in order to elucidate the role of these neuropeptides in the mechanisms of alimentary behavior caused by decreased levels of available fatty acids and glucose. The levels of neuropeptide Y mRNA in arcuate nuclei neurons are significantly increased under conditions of glucose deficiency, while the synthesis of melanin-concentrating hormone in the lateral hypothalamic neurons is increased in fatty acid deficiency. These data indicate that glyco- and lipodeprivation are different metabolic signals activating various neuropeptide systems responsible for alimentary behavior.

Key Words: *hypothalamus; neuropeptides; alimentary behavior; antimetabolites*

Injections of 2-deoxyglucose (2-DG) or 2-mercaptoacetate (MA), selective blockers of carbohydrate and lipid metabolism, respectively, notably increase food consumption [7]. These data allowed a hypothesis that peripheral signals on the glucose and fatty acid levels regulate activities of brain systems involved in the regulation of alimentary behavior [4].

The hypothalamus plays a key role in the integration of metabolic signals. Some recently isolated hypothalamic neuropeptides, such as neuropeptide Y (NPY), pro-opiomelanocortin (POMC) derivatives, and melanin-concentrating hormone (MCH) are involved in the regulation of alimentary behavior. NPY is a pancreatic polypeptide; chronic injection of NPY into brain ventricles leads to hyperphagia and accelerates body weight gain [11,13]. Expression of NPY mRNA and peptide concentration in the hypothalamic arcuate nuclei essentially increase during fasting; a similar picture was observed in animals with genetic obesity [5, 6]. Glucose utilization is an important signal for NPY production in the hypothalamus [1]. A POMC derivative α-melanocyte-stimulating hormone decreases food consumption by activating central melanocortin receptors [8]. In neurons of the lateral hypothalamic area MCH is involved in the regulation of energy metabolism and alimentary behavior. It was found that the level of MCH mRNA increased during fasting, and injection of this peptide into the brain ventricles stimulated food consumption [9,10]. These data on the regulatory role of neuropeptides in the mechanisms of alimentary behavior suggest that at least some of them can realize their function via monitoring of fatty acid and glucose utilization in the organism.

For elucidating the role of neuropeptides in the mechanisms of alimentary behavior caused by a decrease in the levels of available fatty acids and glucose we investigated the effect of single injection of 2-DG or MA on food consumption and expression of NPY, POMC, and MCH mRNA in rat hypothalamus.

MATERIALS AND METHODS

Sixteen male Sprague-Dawley rats weighing 300-350 g were used. The animals were kept under standard day/night regimen (light from 6.00 to 18.00) at 22°C with

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free access to water and food. Experiments were carried out in the daytime (12.00-15.00) when alimentary activity of rodents is low. Dry fodder was weighed before and after the experiment. Experimental rats (4 animals per group) were intraperitoneally injected with 2-DG (Sigma, 60 mg/kg in 1 ml normal saline) or MA (Sigma, 600 μmol/kg in 1 ml normal saline). Controls (n=8) were injected with 1 ml normal saline. The animals were decapitated 3 h postinjection and the brain was rapidly frozen for *in situ* hybridization. Cryostat sections of the hypothalamus (14 μ) dried on slides (Fisher Sci) were incubated for 16 h at 42°C with a radiolabeled probe complementary to nucleotides 1297-1344 for NPY mRNA, 1-33 for POMC mRNA, and 479-527 for MCH mRNA (Scandinavian Gene Synthesis). The probes were diluted in a hybridization mediumcontaining 50% formamide, 0.015 M citrate buffer, 0.02% bovine serum albumin, 0.02% ficoll, 0.02% polyvinyl pyrrolidone, 1% N-lauroylsarcosine, 10% dextrane, 500 mg/l denaturated salmon testis DNA, and 200 mM dithiotreitol. After hybridization the sections were washed for 1 h in citrate buffer at 55°C, dried on air, and coated with emulsion (Ulford). After exposure (from several days to 6 weeks for different peptides) the slides with sections were developed (Kodak), fixed in Kodak 3000 solution, stained with toluidine blue, and silver grains above stained cells were counted under a Nikon Microphot-FX microscope fitted with a dark-field condenser. The cell was considered labeled if the number of grains 5-fold surpassed the background. At least 200 cells per hypothalamus section from each animal were counted. The results were statistically processed using Student's t test.

RESULTS

Three hours after injection of 2-DG or MA the weight of consumed fodder increased significantly (p<0.01) in comparison with the control (Fig. 1, *a*).

Autoradiographic label corresponding to NPY mRNA was detected in neurons of the median part of the arcuate nuclei (Fig. 2, *a*). Neurons expressing POMC mRNA were located in the ventrolateral part of the arcuate nuclei and in the periarcuate mediobasal hypothalamic area (Fig. 2, *b*). The hybridization signal corresponding to MCH mRNA was observed in neurons of the lateral hypothalamic area and *zona incerta* (Fig. 2, *c*). Quantitative analysis showed that injection of 2-DG increased the expression of NPY and MCH mRNA by 28.4 and 48.2%, respectively, while injection of MA caused a 30.1% increase in autoradiographic label only in the neurons synthesizing MCH (Fig. 1, *b*).

Activation of NPY mRNA synthesis in the arcuate nuclei neurons under conditions of blocked carbohydrate, but not lipid metabolism can reflect selective involvement of this peptide in the regulation of carbohydrate metabolism. The fact that NPY injection into the brain ventricles stimulates consumption of carbohydrate-rich fodder confirms this assumption [11]. Selective activation of NPY after blockade of carbohydrate metabolism also confirms the hypothesis that glucose and fatty acid deficiency stimulates food consumption by activating different neuronal pathways and neuropeptidergic systems [3]. Galaninergic neurons of the rostral hypothalamic paraventricular nuclei whose synthetic activity decreases after injection of MA can represent a neuropeptidergic system of the brain reacting to fatty acid deficiency [12].

The detected increase in the expression of MCH mRNA under conditions of glyco- and lipodeprivation indicates that MCH-ergic neurons of the lateral hypothalamic area can serve as a common component of different neuropeptidergic systems responsible for monitoring of fatty acids and glucose. It was previously hypothesized that neurons producing MCH take part in the integration of processes associated with emotional reactivity, excitation, and exploratory activity [2]. We can therefore suggest that the MCH-ergic sys-

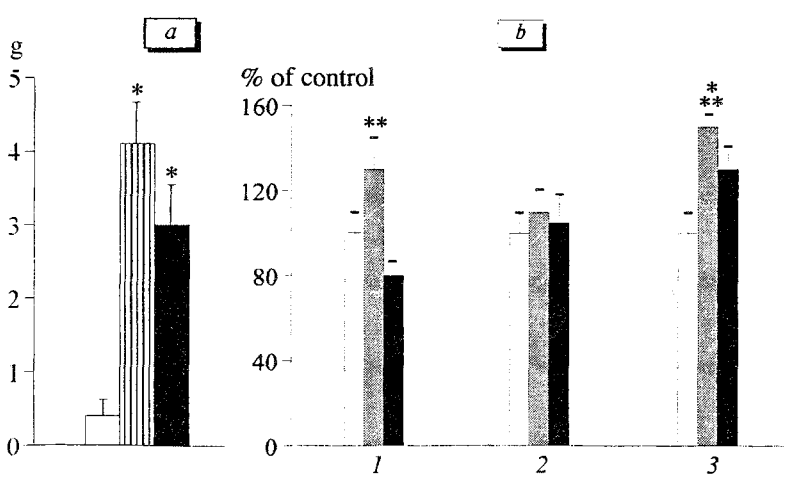

Fig. 1. Effects of 2-deoxyglucose (hatched bars) and 2-mercaptoacetate (dark bars) on food consumption 3 h after intraperitoneal injection (*a*) and on the level of neuropeptide mRNA expression (*b*) in rat hypothalamic neurons. Ordinates: weight of consumed fodder (*a*) and mean number of silver grains above the cells (*b*). *1*) neuropeptide Y; *2*) pro-opiomelanocortin; *3*) melanin-concentrating hormone. *p<0.01, **p<0.05 *vs.* control (light bars).

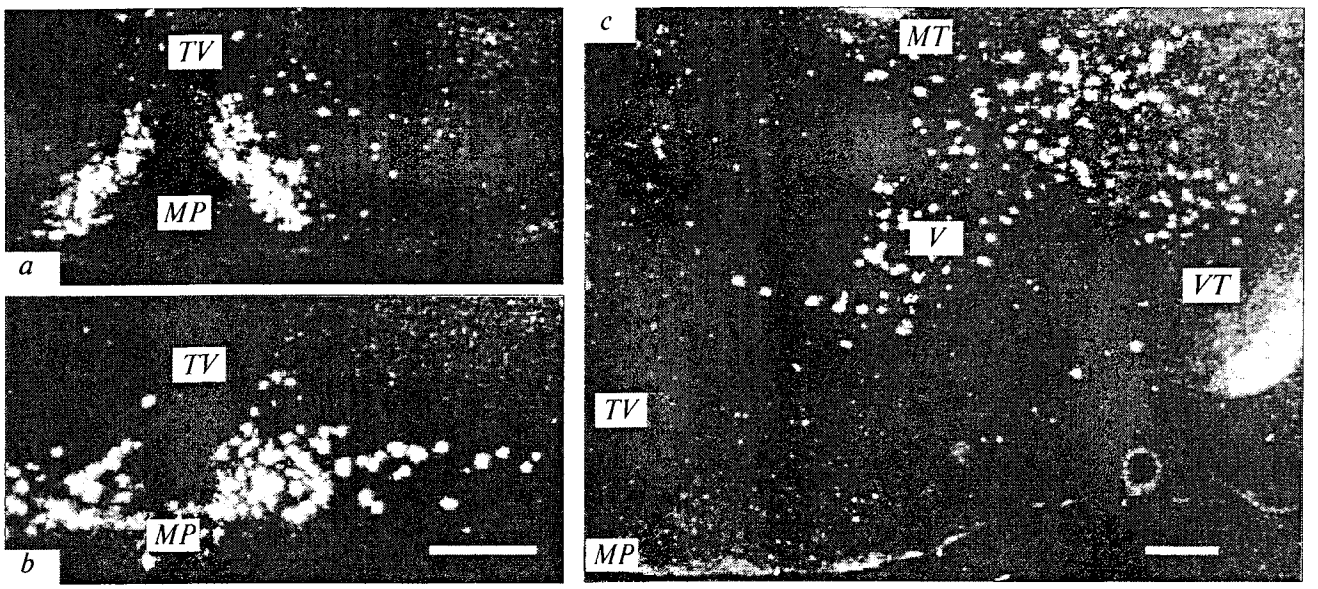

Fig. 2. Neurons expressing neuropeptide Y (*a*), pro-opiomelanocortin (*b*), and melanin-concentrating hormone (c) mRNA in rat hypothalamus. Photographs in dark field. *TV*) third ventricle; *MP*) medial protrusion; *V*) vault; *MT*) mammillothalamic tract; *VT*) visual tract. Mark corresponds to 200 μ.

tem of the lateral hypothalamic area as an emotional and exploratory component of alimentary behavior is involved in activation of the central systems regulating food consumption under conditions of deficit of the main energy substrates.

The data indicate the presence of neurons selectively reacting to changes in the carbohydrate metabolism in the hypothalamus (neurons of the arcuate nuclei reacting to NPY) and neurons reacting to deficiency of the main energy metabolites (MCH-ergic neurons of the lateral hypothalamic area). These neuronal systems can represent various components of the brain system regulating alimentary behavior by monitoring fatty acids and carbohydrates in the organism.

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