Acta Biologica Hungarica 55 (1–4), pp. 195–200 (2004)

HYPERPOLARIZATION BY GLUCOSE OF FEEDING-RELATED NEURONS IN SNAIL*

MAGDA ALANIA,[1,2] VARYA DYAKONOVA[1] and D. A. SAKHAROV[1]**

[1] Laboratory of Comparative Physiology, Institute for Developmental Biology, RAS, 26 Vavilov str., Moscow, 119909 Russia
[2] Laboratory of Physiology and Pathology of Brain, Tbilisi State University, 2 University str., Tbilisi, 380043 Georgia

(Received: August 31, 2003; accepted: December 1, 2003)

In the pond snail *Lymnaea stagnalis,* D-glucose action was investigated on electrical activity of identified central neurons.

In the CNS preparations isolated from specimens that starved for 24–96 h, D-glucose added to a standard or HiDi saline at 500–700 μg/ml effectively hyperpolarized ca. 90% of feeding related neurons B1, SO and CGC. However, not all feeding-related neurons examined were responsive to glucose.

Experiments on cells of the serotonergic Pedal A cluster have shown that hyperpolarizing action of D-glucose is retained following complete isolation of «hunger» neurons. Threshold concentration producing 1–3 mV hyperpolarization was ca. 50 μg/ml.

The results suggest a direct glucose involvement in the mechanisms that control feeding behavior in *Lymnaea.*

Keywords: Glucoreceptors – behavior – neuroethology – *Lymnaea stagnalis*

INTRODUCTION

Among neuroethological model systems for generation of motor behavior, the neural network which controls feeding movements in the pond snail *Lymnaea stagnalis* is one of the best-investigated (see [3], for a review). However, its dynamic modulation associated with hunger and satiety has not yet been fully investigated. Early findings imply a role for glucose in modifying expression of the feeding behavior in this mollusc. It has been demonstrated that the hemolymph glucose concentration rapidly reflects the quality and quantity of the food and may change dramatically, from 15–20 μg/ml in starved specimens to 760 μg/ml in well-fed ones [10]. Evidence for the presence of glucoreceptors in the *Lymnaea* CNS is provided by the reports of depolarizing glucose action on neurosecretory cells producing insulin-like hormone [6]. Here, we examined effects of D-glucose on feeding behavior related neurons, both *in situ* and after complete isolation.

* Presented at the 10th ISIN Symposium on Invertebrate Neurobiology, July 1–5, 2003, Tihany, Hungary.

** Corresponding author; e-mail: compar@pochtamt.ru

0236-5383/$ 20.00

MATERIALS AND METHODS

Mature specimens of *Lymnaea stagnalis* (Pulmonata Basommatophora) were taken from a breeding colony. The CNS was dissected from an animal anesthetized with injection of 0.1 mM $MgCl_2$. It was then placed into 2.5 mg/ml solution of pronase E (Sigma) for 15 min, washed in a standard snail Ringer (50 mM NaCl, 1.7 mM KCl, 4 mM $CaCl_2$, 1.5 mM $MgCl_2$, 10 mM Tris, pH 7.6) and pinned down in a Sylgard lined 4 ml chamber**.** Afterwards, the connective tissue sheath was removed, and the preparation was allowed to recover for 1 h before use. A standard setup for microelectrode recording was used, glass microelectrodes, 10–20 MΩ, were filled with 3 M KCl. Intracellular recording from neurons *in situ* was using the CNS preparation taken from food deprived (n = 34) or control, well-fed snails (n = 7). The preparation was continuously superfused at 1 ml/min with the Ringer solution or, alternatively, with high Mg^{2+}/high Ca^{2+} (HiDi) saline (50 mM NaCl, 1.6 mM KCl, 14 mM $CaCl_2$, 8 mM $MgCl_2$, 10 mM Tris, pH 7.6) (n = 4) to raise the threshold for polysynaptic connections [4]. The effect of D-glucose (Reakhim) was tested on the cells by dissolving it in normal or Hi-Di saline and pumping this into the bath. Isolated neurons (n = 32) were taken from the pedal serotonergic cluster PeA. Using microelectrode as a pull, the neuron selected for isolation was gently pulled out of the tissue until separation of the proximal neurite from the neuropile [2]. The neuron isolated this way was placed into continuous stream of a Ringer solution (50 mM NaCl, 1.6 mM KCl, 4 mM $CaCl_2$, 8 mM $MgCl_2$, 10 mM Tris, pH 7.4) at 0.75 ml/min. Prior to glucose application, the cell was kept chemically unstimulated for at least 20 min until its background electrical activity became stable. All data were stored in PC files. Significance of differences in spiking frequency prior to and after glucose administration was tested by nonparametric Krusskal-Wallis ANOVA test with a use of "STATISTICA" program (StatSoft, Inc. 1993).

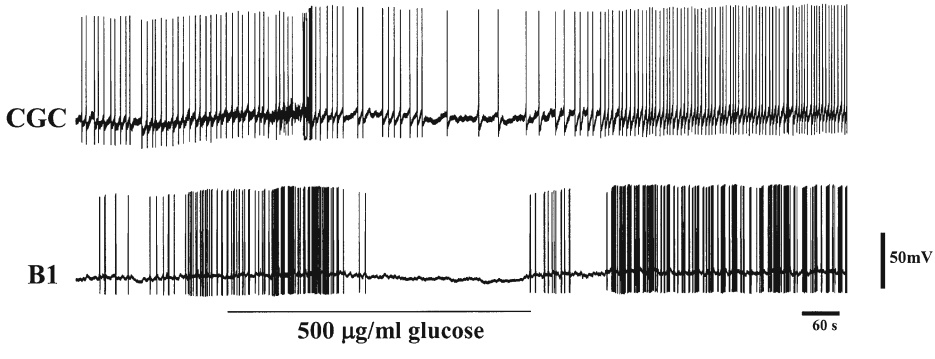

Fig. 1. Response of CGC and B1 neurons to addition of glucose (horizontal line) to a HiDi saline. CGC was depolarized prior to glucose application. The animal was deprived of food for 48 hours

RESULTS

Hyperpolarization by glucose of neurons in situ

In the isolated CNS, D-glucose (500–700 µg/ml) effectively inhibited certain, but not all, identifiable feeding related neurons examined. Non-responsive were buccal motoneurons B2 and B3. In contrast, the salivary gland motoneuron B1 was hyperpolarized up to 10 mV. Marked inhibition was also demonstrated by the cerebral serotonergic modulatory interneuron CGC (the cerebral giant cell), buccal modulatory interneuron SO (slow oscillator) and serotonergic motor neurons of the pedal A (PeA) cluster. The effects were retained in HiDi saline (Fig. 1) suggesting direct action of glucose on these neurons, not through the polysynaptic pathway. Inhibitory responses were maximal in preparations isolated from specimens that starved for 24–36 h; with longer starvation time, glucose action progressively decreased (Fig. 2).

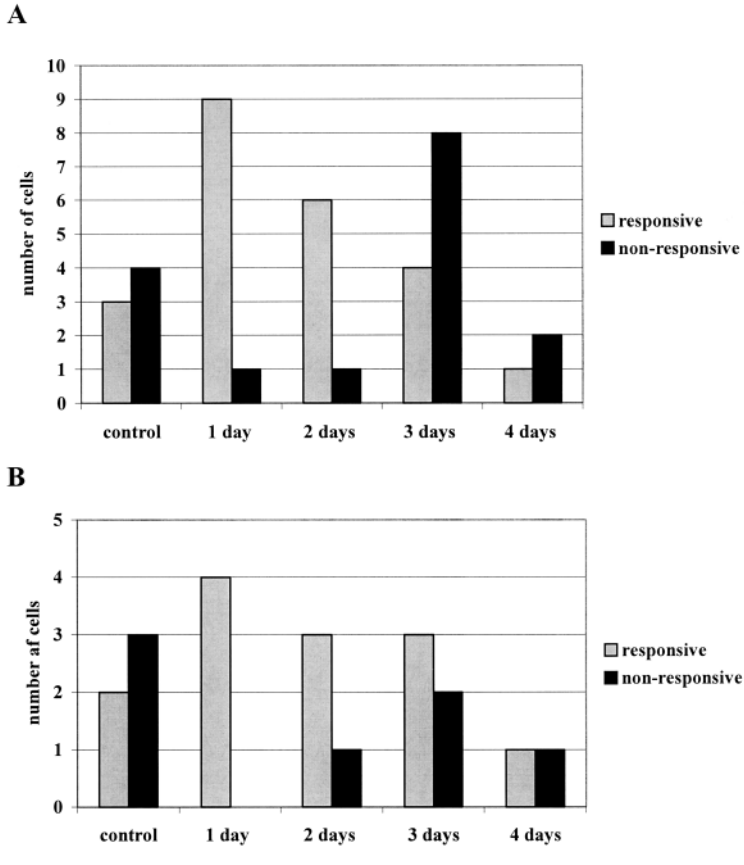

Fig. 2. A comparison of neurons responsive and non-responsive to glucose after 1, 2, 3 and 4 days of starvation. A: The cerebral giant cell (CGC). B: The salivary gland motoneuron (B1). Control, cells from satiated animals

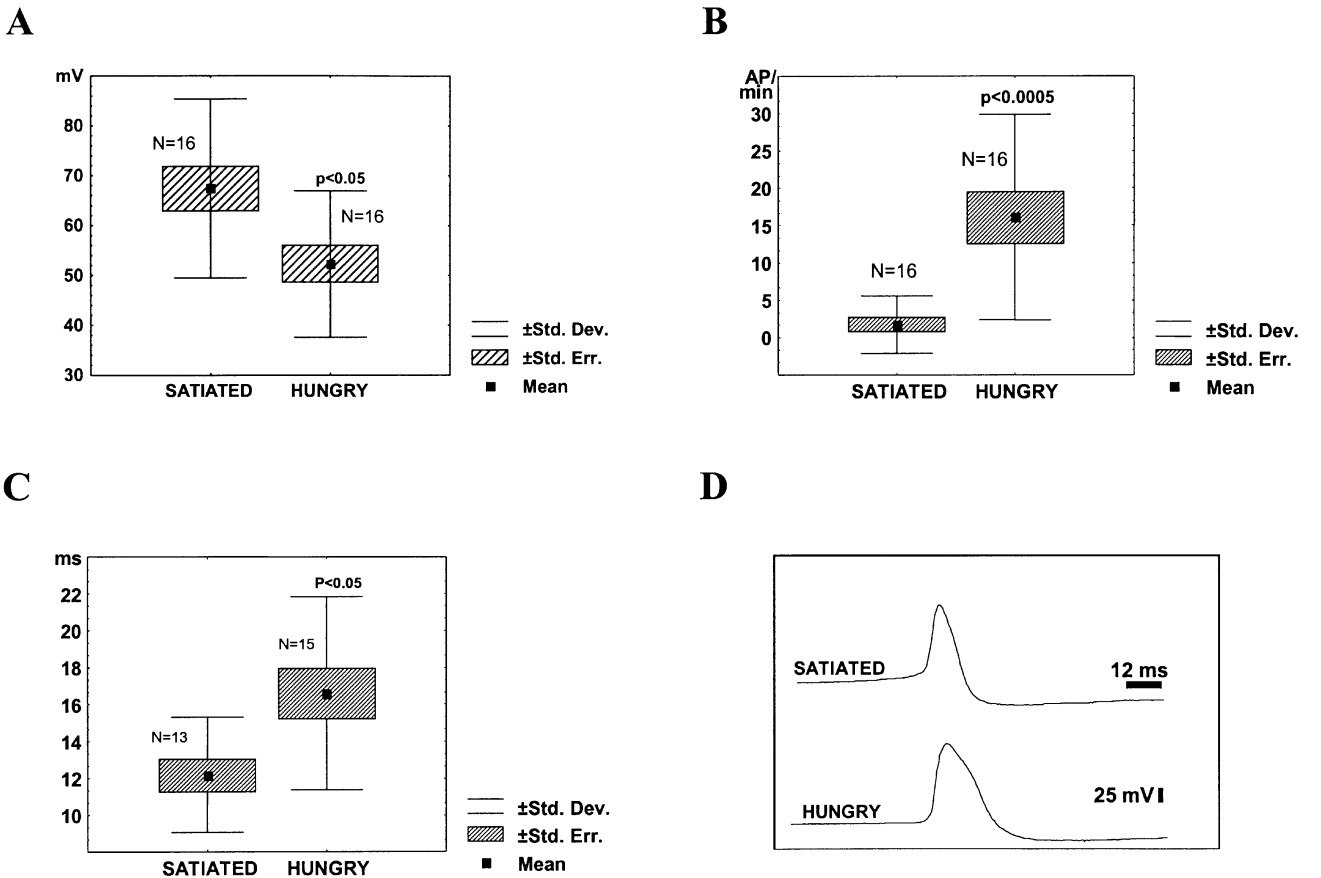

Fig. 3. A comparison of serotonergic PeA neurons isolated from satiated and hungry (36 hours) animals. A: The membrane potential. B: The rate of firing. C: The duration of action potential. D: An example of "satiated" and "hunger" action potentials

Hyperpolarization by glucose of isolated neurons

Effects of glucose were examined on selected neurons following isolation of the cell body with a proximal portion of the neurite. Neurons of the PeA cluster were used in these experiments. These cells innervate both the muscles and ciliated epithelium of the foot, and are known to participate in the control of locomotion [11]. Increased firing of the PeA neurons is an essential part of the mechanism of serotonergic arousal associated with search for food (for literature, see [1]).

Intracellular recordings from PeA cells *in situ* demonstrated that food deprivation for 36 h caused depolarization, increase in firing frequency and widening of the spike (see Hernadi et al., in this volume). Similar changes were found in single PeA neurons following isolation (Fig. 3). The neurons isolated from specimens that starved for 36 h had a lower membrane potential than those taken from satiated animals. The rate of firing was significantly higher, and action potentials wider, in «hunger» neurons. These characteristics were retained by isolated single neurons for the entire period of observation (20–30 min), thus indicating that starvation and satiety cause long-term changes at the membrane level.

Glucose had a hyperpolarizing action on isolated «hunger» PeA neurons (Fig. 4). Threshold concentration producing 1–3 mV hyperpolarization was ca. 50 μg/ml. The highest concentration tested, 200 μg/ml, caused 15 mV hyperpolarization and firing decrease. The effect was reversible as washing glucose away with glucose-free saline produced neuron depolarization and firing increase.

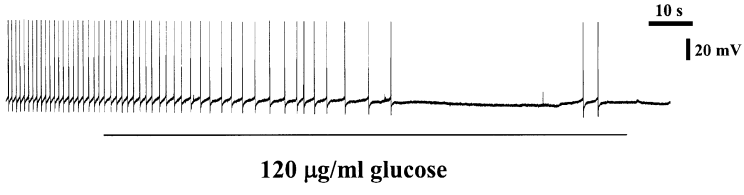

Fig. 4. Response of an isolated PeA neuron to addition of glucose (horizontal line). The snail starved for 24 hours

DISCUSSION

Glucose is the preferred carbon and energy source for most eukaryotic cells. It would be natural for a neural machinery controlling feeding behavior to be provided with glucoreceptors. Abundant evidence on this point was demonstrated by research on vertebrates including man (e.g. [7, 8, 9]). Our results extend these findings to behavior-related neurons of a model invertebrate, the pond snail. With respect to hunger and satiety, an opportunity to use this favorable preparation for performing a thorough neuronal analysis is thus presented.

Indeed, all neurons, which were found responding to glucose in our experiments, are known to be somehow involved in movements manifested during search for food,

namely, in the buccal rhythm (cells B1, CGC, SO [3]) and locomotion (cells PeA [11]). Hyperpolarizing glucose effect on these neurons would suggest that glucose directly mediates suppression by satiety of food searching movements. This suggestion appears to be in disagreement with observations on another model gastropod, *Aplysia californica,* where feeding behavior was found unaffected by glucose [5]. Future research may help to elucidate this problem. Our preliminary observations show that, in *Lymnaea*, the here described immediate suppression by glucose of firing in certain neurons is followed by prominent changes in network operation.

To summarize, the results of this study provide first evidence that gastropod molluscs possess central glucoreceptors mediating hyperpolarization by glucose of certain feeding-related neurons and suggest a complex glucose involvement in the mechanisms that control feeding behavior in *Lymnaea.*

ACKNOWLEDGEMENTS

Supported by RFBR grants 02-04-48091 (to DS), 03-04-63036K, IBRO Program «Against brain drain» (to MA and VD) and "Science support foundation" (to VD).

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