Glucose-Induced Intracellular Ion Changes in Sugar-Sensitive Hypothalamic Neurons

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Silver, Ian A. and Maria Erecinska. Glucose-induced intracellular ion changes in sugar-sensitive hypothalamic neurons. J. Neurophysiol. 79: 1733–1745, 1998. In the lateral hypothalamic area (LHA) of rat brain, ~30% of cells showed sensitivity to small changes in local concentrations of glucose. These “glucose-sensitive” neurons demonstrated four types of behavior, three of which probably represent segments of a continuous spectrum of recruitment in response to ever more severe changes in blood sugar. Type I cells showed maximum activity ≤5.6 mM blood glucose but became completely silent at hyperglycemia of 10–12 mM (normoglycemia 7.6 ± 0.3 mM; mean ± SD). Type II and III neurons exhibited a wider range of response. Type IV neurons (5–7% of glucose-sensitive neurons) paralleled the behavior of sugar-sensitive cells in ventromedial hypothalamic nucleus (VMH). In VMH, ~40% of cells responded to changes in blood glucose over a range of concentrations from 3.6 to 17 mM, by increasing their firing rate as sugar level rose and vice versa. Ionic shifts during increases in blood (brain) glucose levels were similar in LHA types I–III but fastest in I and slowest in III. [Na+] fell by 5–9 mM, [K+] rose by 6–8 mM, and plasma membrane hyperpolarized by 5 mV. [Ca2+] declined by 15–20 nM in line with membrane hyperpolarization. In VMH and type IV LHA cells, [K+] fell 3–8 mM and plasma membrane depolarized –3 to –5 mV as blood/brain glucose concentration increased from 7.6/2.4 to 17.6/4.2 mM, whereas [Ca2+] increased from 125 to 180 nM as a consequence of falling membrane potential. During falls in blood/brain sugar concentration the effects in both VMH and LHA cells were reversed. The findings are consistent with the ionic shifts in types I–III LHA cells being dependent on alterations in Na/K-ATPase activity, whereas those in VMH and type IV LHA cells could be caused by modulation of ATP-dependent K⁺ channels. A possible mechanism for linking the effects of small changes in glucose to ATP generation, which could bring about the above phenomena, is the interposition of a “glucokinase-type” enzyme in a role similar to that which it has in glucose-sensing pancreatic β-cells.

INTRODUCTION

It generally is accepted that the two main centers that control feeding behavior in animals are located in the basal hypothalamicus, with the lateral hypothalamic area (LHA) and the ventromedial hypothalamic nucleus (VMH) exhibiting a reciprocal relationship. Although the LHA, among its other vegetative functions, plays a key role in stimulation of appetite and food intake, the VMH exerts the opposite effect (Bray and York 1979; Nagai and Nakagawa 1992). One of the major endogenous regulatory factors that modulate the activity of these centers is the concentration of blood glucose. More than 25 years ago, Oomura and coworkers (1969) found that ~30% of LHA neurons responded to a rise in extracellular glucose level with a decrease in firing rate (i.e., inhibition of activity), whereas ~40% of VMH neurons showed an increase in discharge frequency (i.e., stimulation of activity), under the same conditions. It also was demonstrated that local application of glucose hyperpolarized the sensitive LHA cells, an effect that was prevented by ouabain and azide (Oomura et al. 1974). In view of this sensitivity to a blocker of the Na/K ATPase (ouabain) and an inhibitor of energy production (azide), combined with an apparent lack of change in membrane permeability, it was postulated that glucose exerted its inhibitory influence via stimulation of the Na/K pump activity.

In slices of nucleus tractus solitarius, which also contains neurons sensitive to changes in extracellular glucose concentration, those that were stimulated by a rise in sugar level (VMH-type) became depolarized by 3–10 mV and their membrane conductance increased by 50–82% (Mizuno and Oomura 1984). Conversely, a decrease in glucose to 3 mM hyperpolarized these same cells and reduced their membrane conductance. The authors postulated that such behavior most likely was due to alterations in the membrane permeability to potassium. Similar changes in membrane voltage consequent on either a rise or a fall in glucose level were demonstrated more recently in VMH cells in a hypothalamic slice preparation (Ashford et al. 1990). Moreover, cell-attached recordings from these neurons revealed the presence of potassium channels which were closed by a rise in either [ATP] or [glucose]. It was suggested that closure of the ATP-sensitive K⁺ channels is responsible for the increased firing seen in hyperglycemia.

Our earlier investigations on neuronal responsiveness to changes in their external environment (Silver and Erecinska 1990, 1992, 1994) showed that most central nervous system (CNS) neurons are remarkably insensitive to alterations in the extracellular concentration of glucose and that the latter has to fall <0.2 mM before ion gradients begin to collapse (Silver and Erecinska 1994). The sensitivity of the hypothalamic cells to small perturbations is thus in sharp contrast to the general pattern of neuronal behavior. Yet except for the very few reports cited above, there is no information on the mechanisms responsible for this unique “reactivity.” The current study was undertaken to characterize the ionic changes occurring in LHA and VMH neurons in intact rat brain, during physiological alterations of blood glucose level, to gain some insight into the molecular basis of glucose sensing by the CNS.
METHODS

Preparation of the rats

Measurements were made on 296 white Sprague-Dawley-derived rats of both sexes; body weight 250–300 g anesthetized by intraperitoneal injection of pentobarbitonal sodium (60 mg/kg as a 6% aqueous solution; Nembutal, Abbott Laboratories) together with atropine sulfate (0.3 mg/kg; Roche). Supplementary pentobarbitone was administered as required. Local analgesic (lignocaine 2%; Xylocaine, Astra Pharmaceuticals) was infiltrated subcutaneously over the scalp and at the prospective pressure points of the head holder. The level of anesthesia was assessed constantly by reference to the electrocorticogram (ECOG), electrocardiogram (EKG), and blood pressure traces, and supplementary doses of anesthetic given when required.

A tracheostomy was performed. The caudal femoral vein was cannulated and connected to a Gould-Statham P50 miniature blood pressure transducer. Catheters were inserted into the left femoral vein and artery. The venous line was used for administration of fluids, collection of venous samples and return of blood from the glucose measuring device (see further text).

EKG was recorded from left and right forelimbs via subcutaneous needle electrodes. The anesthetic was placed in a modified Balti-more stereotaxic head holder and positioned for use of stereotaxic coordinates as described by Swanson (1992). A median longitudinal skin incision was made over the skull, the periosteum reflected on each side of the central suture and two stainless steel miniature bone screws inserted into the frontal bones to serve as ECOG electrodes. A hole 2.8 mm wide and 4.0 mm long was drilled with the medial edge of the long axis parallel to and 0.5 mm lateral to the midline and the rostral edge 0.8 mm caudal to the bregma point. This opening was enclosed in an oval polycarbonate ring (4.0 × 25.5 mm ID; 2.5 mm depth, 1-mm-wall thickness), which was fixed to the skull with cyanoacrylate to form a well that was flushed with warm (38°C) artificial cerebrospinal fluid (ACSF) during recording. The stereotaxic coordinates used were 0.8–4.3 mm caudal and 1.0–2.5 mm lateral to the bregma point for LHA and VMH.

METHODS

Hypoglycemia was induced by subcutaneous injection of insulin (25 IU/kg) while reversal of hypoglycemia, or induction of hyperglycemia, were achieved by slow intravenous injection of 0.5 g glucose in 3 ml water.

Electrodes

Ion-specific probes were either double-barreled microelectrodes (tip diameter 0.08–0.15 µm, mean 0.10 µm) fabricated on a standard electrode puller from theta configuration borosilicate glass capillaries (1.5 mm OD, 1.0 mm ID) or multibarreled electrodes made from three to five glass capillaries (containing solid fibers) annealed together. The barrels to be filled with ion sensors were rendered hydrophobic by siliconization with the vapor of dimethyl-dichloro- or tri-n-butylchlororosilane and subsequently baked at 150°C for 1 h. The tips of electrodes used for intracellular recording were ground to a short chisel point on a dry, rapidly rotating, optical flat glass disk incorporating very fine diamond dust in such a manner that the “glucose” barrel (see further) was open to the opening of the “glucose” barrel (see further) on the most proximal part of the bevel. The reference barrels for recording membrane potentials/cell discharges were back-filled with filtered 3 M KCl. In multibarreled electrodes, a glucose barrel was filled with 1% (55 mM) glucose in ACSF. This barrel was used as a searching tool for the preliminary identification of glucose-responsive cells in the cerebral cortex. Where in plastic, 5% (55 mM) glucose in ACSF was used to identify the electrodes unless they were blocked by debris or silicone deposit. The sensors used were: for potassium, valinomycin as Fluka “cocktail A” (Wuhrmann et al. 1979) (Fluka No. 60031); for calcium, the ligand ETH 129 (N,N,N’,N’-tetracyclohexyl-3-oxapentanediamide) in the form of the “cocktail” described by Schefer et al. (1986) (Fluka 21193); and for sodium, Fluka 71716 Sodium ionophore I-Cocktail A containing the neutral lipophilic ionophore ETH 227 [N,N’,N’ -triethyl-N’,N’,N’-trimethyl-1,4,4’-propyldiynetris(3-oxobutyr- amide)] (Steiner et al. 1979). The ion-sensitive barrels were back-filled with the appropriate electrolyte for potassium, sodium, or calcium, i.e., 0.5 M KCl, 0.5 M NaCl, or a mixture of 0.1 M Mg acetate, 100 mM CaCl2, and 80 mM KCl, respectively. The last was found to be more reliable for use with the ETC 129 ligand for intracellular, calcium-sensitive probes than a simple solution of CaCl2.

Ion-sensitive electrodes were calibrated at 37.5°C, in terms of ionic concentration, in electrolyte mixtures that closely simulated their expected intracellular counterparts. Details of calibration procedures have been given by Silver and Erecinska (1990). All electrodes were calibrated individually, and only those showing a near-Nernstian response to changes in appropriate ion concentration and a lack of sensitivity to other ions were used for experimental measurements. For calcium-sensitive electrodes, a log/log linear response of between 25 and 29 mV/ decade and for Na+ and K+ electrodes 51–59 mV/decade were considered acceptable. Probes giving voltage changes outside these ranges, or that were nonlinear, were discarded. The impedance of all electrodes was measured before use; reference barrels were routinely 20–40·109 Ω and ion-sensitive barrels always ≥1010 Ω. The typical 87% response time of ion-selective electrodes in the recording system ranged from 500 ms (Ca2+) to 3 s ( Na+) and 5 s (K+) for an increase and 700 ms (Ca2+) to 5 s (Na+) and 8 s (K+) for a decrease, to an order of magnitude step change of concentration. All results were calculated from standard calibration curves constructed for the various cations as described earlier and are given as (free) ion concent-
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Glucose measurements

Extracellular hypothalamic glucose concentration was measured continuously by means of glucose-sensitive, ferrocene-coated, amperometric needle microelectrodes inserted into the diencephalon and fixed to the skull with dental acrylic (see preceding sections). These glucose sensors were as described by Silver and Erecinska (1994) and are based on oxidation of glucose to gluconic acid and hydrogen peroxide by glucose oxidase bound to a platinum black surface with immediate electrolysis of the H$_2$O$_2$ to O$_2$ and H$_2$O at the positively charged metal surface. The 1-$\mu$m tips of the electrodes were coated in four thin layers of cellulose diacetate, dried, and dipped into a 1% aqueous solution of ascorbate oxidase lateral hypothalamic area (LHA), sensitive to local, electroosmotic application of glucose (A, ↑) but not to a peripheral sensory input (ice on the tail; B, ↓) the other insensitive to local glucose application (C) but responsive to cold stimulus on the tail (D). Measurements of activity were made as described in METHODS. Where indicted by ↓, 1% glucose solution was expelled from the glucose-filled barrel by electroosmosis (10 s at $10^{-8}$ A) according to the method of Oomura et al. (1969, 1974).

Microfuge capillary tubes. An aliquot of 20 $\mu$L was centrifuged immediately, and the plasma kept on ice at 0°C until analyzed for glucose content in a standard clinical hexokinase-based colorimetric system (Technicon Instruments, Tarrytown, NY) to check that the in-line electrode was functioning reliably. Total blood sugar was determined periodically from the other 20 $\mu$L by the method of Trinder (1969) using an Instrumentation Laboratories (Warrington, Cheshire, UK) pediatric blood-glucose analyzer.

Recording

The ion-sensitive electrodes and their reference barrels were connected via chlorided silver wires to a capacitance-compensated, high-impedance (>10$^{14}$ Ω) DC amplifier, the output of which drove a storage oscilloscope, impulse rate meter, audioamplifier, chart recorder and, via an A-to-D converter, an Apple Quadra microcomputer running LabView 2.2 (National Instrumentation) virtual instrumentation. Intracellular recordings always included the membrane potential (the voltage from the reference barrel measured against ground potential), which was subtracted electronically from the output of the ion-sensitive probe. The output from the reference barrel also was used to record action potentials because the response of the ion-selective barrels was too slow for this purpose. Membrane resistance was measured routinely by current injection through the reference barrel.

A minimum recording period of 30 s was required before intracellular ion measurements were regarded as a reliable source of data, but recordings were made from most cells for ≥2–4 min. Recordings of extracellular action potentials from spontaneously active neurons were made via the reference barrels of the ion-sensitive electrodes before cells were penetrated (see Experimental design).

ECoG and EKG were recorded via appropriate standard amplifiers (Gould Electronics BV, Bilthoven, Netherlands).

Identification of anatomic location of recording sites

Reference barrels of electrodes were filled with electrolyte containing a 10% solution of the anionic fluorescent marker dye, Procion yellow, (M-4RS, I.C.I. Organics). At the end of a successful intracellular recording, dye was injected iontophoretically into the site with 100-ms current pulses of $2 \times 10^{-8}$ A at 5 s$^{-1}$ for 30–60 s, solely to assist identification of its anatomic position. The location

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Glucose-Sensitive Neurons</th>
<th>Glucose-Insensitive Neurons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type I</td>
<td>Type II + III</td>
</tr>
<tr>
<td>Pinch to tail</td>
<td>↑</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>0</td>
</tr>
<tr>
<td>Cold on tail</td>
<td>↑</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>0</td>
</tr>
<tr>
<td>Aromatic oil</td>
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<td>3</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>0</td>
</tr>
<tr>
<td>Breathing 10% CO$_2$</td>
<td>↑</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>145</td>
</tr>
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</table>

Responsiveness of cells was measured as a change in firing rate as described in Experimental procedures. LHA, lateral hypothalamic area; ↑, increase; ↓, decrease; 0, no change in firing rate. The definition of types I–III of neurons is given in the text. Of 837 cells evaluated, 591 were glucose insensitive, 169 were type I, and 117 were types II and III.
TABLE 2. Changes in firing rates, intracellular ion concentrations and membrane potentials in LHA neurons type II and IV during stepwise increases in glucose level

<table>
<thead>
<tr>
<th>Blood Glucose, mM</th>
<th>Firing Rate, spikes/s</th>
<th>[Na⁺], mM</th>
<th>[Ca²⁺], nM</th>
<th>[K⁺], mM</th>
<th>E, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>20 ± 5</td>
<td>23.8 ± 1.2</td>
<td>115 ± 5.7</td>
<td>70.4 ± 2.3</td>
<td>−68.6 ± 1.4</td>
</tr>
<tr>
<td>+2</td>
<td>+0.3</td>
<td>14 ± 4</td>
<td>22.0 ± 1.4*</td>
<td>110 ± 6.1*</td>
<td>73.4 ± 2.2</td>
</tr>
<tr>
<td>+5</td>
<td>+1.0</td>
<td>5 ± 2</td>
<td>20.2 ± 1.2</td>
<td>103 ± 5.1</td>
<td>76.4 ± 2.2</td>
</tr>
<tr>
<td>+10</td>
<td>+1.8</td>
<td>0 ± 0</td>
<td>17.8 ± 1.2</td>
<td>98 ± 6.2</td>
<td>80.2 ± 1.7</td>
</tr>
<tr>
<td>Type IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>14 ± 6</td>
<td>22.8 ± 1.2</td>
<td>140 ± 4.8</td>
<td>69.6 ± 1.5</td>
<td>−67.2 ± 1.4</td>
</tr>
<tr>
<td>+2</td>
<td>+0.3</td>
<td>19 ± 6</td>
<td>24.2 ± 1.2*</td>
<td>151 ± 3.7</td>
<td>68.0 ± 1.7*</td>
</tr>
<tr>
<td>+5</td>
<td>+1.0</td>
<td>25 ± 4</td>
<td>25.6 ± 1.4</td>
<td>161 ± 5.7</td>
<td>66.0 ± 1.3</td>
</tr>
<tr>
<td>+10</td>
<td>+1.8</td>
<td>31 ± 6</td>
<td>27.0 ± 1.1</td>
<td>174 ± 2.2</td>
<td>63.2 ± 0.7</td>
</tr>
</tbody>
</table>

Intracellular ion concentrations were measured with double- or multibarreled microelectrodes as described in Experimental procedures. Membrane potential (E) and firing rate were measured through the reference barrels of the ion-selective probes. Values are means ± SD for five cells in each category. All values are statistically significant compared with baseline, P < 0.05, except those marked *. Baseline glucose concentration in blood was 7.6 ± 0.3 (n = 175) and in brain 2.4 ± 0.13 (n = 133).

of the fluorescent spots also provided a means of estimating any brain shrinkage or distortion during subsequent processing and reconstruction. Although Procion yellow has limitations as a marker, other dyes that are more intensely fluorescent or localize more precisely have a greater tendency to block the tips of intracellular microelectrodes.

At the end of each experiment, an electrode was left at the final recording site, the animal killed with an anesthetic overdose, and the brain perfused through the carotid arteries with ice-cold 4% glutaraldehyde. The animal, in the headholder was placed in a cold room at 4°C. After 12 h, the electrode was removed, and the brain extracted from the skull and placed in cold 4% glutaraldehyde for a further 48 h. It then was cut serially in a cryostat to give 40-μm sections, which were stained with cresyl violet or toluidine blue. Recording points were identified from stereotaxic coordinates, electrode tracks, and microscopic examination of fluorescent markers under UV excitation. The anatomic positions of cells from which recordings were made were identified by reference to the appropriate sections in the rat brain maps of Swanson (1992).

Experimental design

Spontaneously active cells in the LHA and VMH were located by recording their (positive-going) extracellular action potentials through the reference barrel of the ion-sensitive probes. Responsiveness of these cells to various nonspecific peripheral stimuli was tested by application of cold (ice) and light pinch to the tail of the rat, followed by 10-s exposure of the nose at a distance of 1 cm to a neurosurgical swab (1 cm²) soaked in clove oil, and finally 10 s 10% CO₂ in the respired air. Approximately 65% of all cells
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TABLE 3. Changes in firing rates, intracellular ion concentrations and membrane potentials in LHA neurons type II and IV during stepwise decreases in glucose level

<table>
<thead>
<tr>
<th>Blood Glucose, mM</th>
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<th>[Na⁺], mM</th>
<th>[Ca²⁺], mM</th>
<th>[K⁺], mM</th>
<th>E, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>21 ± 6</td>
<td>22.4 ± 1.9</td>
<td>111 ± 5.9</td>
<td>70.0 ± 1.8</td>
<td>−67.8 ± 1.2</td>
</tr>
<tr>
<td>−2</td>
<td>23 ± 3</td>
<td>24.4 ± 1.9*</td>
<td>138 ± 17</td>
<td>67.6 ± 2.0*</td>
<td>−66.2 ± 0.7</td>
</tr>
<tr>
<td>−4</td>
<td>33 ± 10</td>
<td>28.0 ± 2.0</td>
<td>169 ± 12</td>
<td>63.2 ± 2.1</td>
<td>−64.0 ± 1.1</td>
</tr>
<tr>
<td>−6</td>
<td>0 ± 0</td>
<td>37.0 ± 2.3</td>
<td>321 ± 88</td>
<td>54.0 ± 2.0</td>
<td>−47.3 ± 1.5</td>
</tr>
<tr>
<td>Type IV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>19 ± 8</td>
<td>23.0 ± 1.4</td>
<td>140 ± 2.8</td>
<td>69.6 ± 1.7</td>
<td>−68.2 ± 1.7</td>
</tr>
<tr>
<td>−2</td>
<td>5 ± 4</td>
<td>21.6 ± 1.0*</td>
<td>129 ± 2.6</td>
<td>71.4 ± 1.9*</td>
<td>−69.6 ± 1.7*</td>
</tr>
<tr>
<td>−4</td>
<td>1 ± 1</td>
<td>20.4 ± 0.8</td>
<td>113 ± 3.0</td>
<td>73.0 ± 1.4</td>
<td>−71.0 ± 1.8</td>
</tr>
<tr>
<td>−6</td>
<td>0 ± 0</td>
<td>19.4 ± 0.8</td>
<td>106 ± 3.9</td>
<td>74.6 ± 1.2</td>
<td>−72.4 ± 1.4</td>
</tr>
</tbody>
</table>

Intracellular ion concentrations were measured with double- or multi-barreled microelectrodes as described in Experimental procedures. Membrane potential (E) and firing rate were measured through the reference barrels of the ion-selective probes. Values are means ± SD for five cells in each category. All values are statistically significant compared with baseline, P < 0.05, except those marked *. Baseline glucose concentration in blood was 7.6 ± 0.3 (n = 175) and that in brain 2.4 ± 0.13 (n = 133).

then were tested for glucose sensitivity by expelling glucose solution from the glucose-filled barrel by electroosmosis (10 s at 10 · 10⁻⁹ A) (Oomura et al. 1969, 1974). Cells that responded to local glucose application almost invariably proved subsequently to be sensitive to systemic changes in blood glucose; this indicated that they were responsive to sugar concentration and not to an artifact produced by the electrodes. If a cell responded to local glucose application by increased or decreased firing rate, the electrode was advanced in an attempt to penetrate it. This was successful with 30–40% of cells from which intracellular ion concentrations were recorded. Some cells that were not impaled continued to fire actively as the electrode tip moved past them. Those that remained

FIG. 3. Records from a triple-barreled microelectrode showing action potentials and cell membrane potential (top), together with intra- and extracellular concentrations (in mM) of potassium (middle) and calcium (bottom) in response to increased glucose concentration. These records are from a LHA type II cell. I, time of penetration of the cell. Left of |, extracellular recordings; right of |, intracellular recordings. Top: from reference barrel of the electrode and recorded on a time scale (seconds) that shows individual action potentials. Middle and bottom: displayed on a longer time scale (minutes) because of the relatively slow response times of the insensitive electrodes. One minute after the cell was penetrated, intravenous glucose infusion was started. Three segments of each trace were taken when blood glucose concentrations were those shown on the x axis, namely baseline (7.7 mM), +2 (9.6 mM), and +5 (12.5 mM). Insets: changes in [K⁺] and [Ca²⁺] at altered glucose concentrations shown in expanded scale on the x axis.
stable were used for extracellular recording of relatively long term firing rate changes, in response to systemic alterations in blood sugar (Figs. 2 and 7). Changes in blood and brain glucose concentration were produced by systemic injection of glucose solution or insulin as described above. Ideal intracellular recordings were those in which changes in ionic concentrations and membrane potentials could be followed in a series of individual cells during a control period and through administration of, and recovery from, an imposed alteration in blood sugar. While it is possible to produce relatively rapid increases in blood and brain glucose levels by intravenous injection of glucose solution, lowering of blood (and brain) sugar concentration after insulin administration inevitably takes many minutes during which there is frequently damage to cells impaled in the control period. For this reason, it was possible to gather complete sets of data during development of hypoglycemia from only relatively few cells, and it was necessary to supplement these observations with measurements on cells from which recordings had not been made in the control period but which provided data only during development of hypoglycemia and/or its reversal.

All procedures on animals were carried out as specified in licenses issued under the (British) Animals (Scientific Procedures) Act 1986.

**Results**

**Responses of LHA neurons to changes in blood glucose concentration**

The specificity of the response to an increase in blood glucose level, measured as a change in the rate of firing, was investigated in 877 LHA neurons (Table 1); 33% (286 cells) were sensitive to glucose, whereas 60% (591 cells) were not. Most of the glucose-insensitive cells responded to a number of other stimuli, such as cold applied to the tail (Fig. 1), scent of an aromatic oil, or 10% carbon dioxide in the inspired air, usually with an increased discharge frequency. The glucose-sensitive neurons, which apparently were scattered randomly throughout the LHA, exhibited a nonuniform behavior: cells termed type I (see further for the basis of classification) were highly selective for glucose because the majority did not respond to other stimuli (Fig. 1). Types II and III cells were less “specific” in that a number of them also responded to cold, pinch or increased CO2. Type IV cells were not sensitive to stimuli other than glucose.

In normoglycemia, LHA neurons fired spontaneously 6–33/s, and there was no obvious correlation between this (basal) rate of firing and the magnitude of response to a change in external glucose concentration. However, based on the type or sensitivity of this response, cells could be classified into four categories; types I–III were inhibited by systemic glucose changes imposed, one type of neuron was recorded from a single rat and hence the samples were independent of each other.

![FIG. 4. Records from a triple-barreled microelectrode showing action potentials and cell membrane potential (top), together with intra- and extracellular concentrations (in mM) of sodium (middle) and calcium (bottom) in response to hypoglycemia. These records are from a LHA type II cell. \( \downarrow \) time of penetration of the cell, intra- and extracellular recordings same as in Fig. 3. \( \uparrow \) designates the withdrawal of the electrode from the cell. Top: from the reference barrel of the electrode and recorded on a time scale (seconds) that shows individual action potentials. Middle and bottom: [K⁺] and [Na⁺], displayed on a longer time scale (minutes) because of the relatively slow response times of the ion sensitive electrodes. 30 s after the cell was penetrated intravenous insulin was administered (see methods). Four segments of each trace were taken when blood glucose concentrations were those shown on the x axis, i.e., baseline (7.6 mM), –1 (6.6 mM), –2 (5.6 mM), and –4 (3.6 mM).]
neurons, which were predominant (~60% of all glucose-sensitive cells), became completely inhibited (i.e., stopped firing) at a blood glucose of 10–12 mM, (brain glucose 3.2–3.4 mM; Fig. 5) i.e., only slightly above the physiological level in rats (7.6 ± 0.3 mM, n = 133, which corresponds to brain glucose concentrations of 2.4 ± 0.13 mM, n = 116). Type II neurons stopped firing when blood [glucose] reached ~17 mM (brain glucose of 4.2 ± 0.2 mM; Table 2), whereas type III still generated some discharges when blood glucose rose >17 mM (Fig. 6). Type IV cells (Table 2), which behaved “anomalously” and increased their firing rate in hyperglycemia, constituted only 5–7% of glucose-sensitive LHA neurons.

Early alterations in firing frequency also occurred when [glucose] was lowered (Fig. 2, 4–6; Table 3). Type I cells were again the most sensitive: they increased their firing rate almost to a maximum when blood glucose level fell from 7.6 to 5.6 mM (which corresponds to a decrease in brain glucose from 2.4 to 2.1 mM). Type II and III neurons showed more gradual changes, with type III exhibiting persistent activity at blood glucose levels lower than the baseline control value. Intracellular ion concentrations were measured with double or multibarreled ionselective electrodes as described in METHODS. E and cell action potential activity were measured through the reference barrels of the ionselective probes. Values given for each point represent means ± SD for 5 cells at each of the blood sugar levels below the symbols. Baseline blood sugar level (0) is 7.6 ± 0.3 (n = 175). Corresponding brain glucose concentrations can be found in Table 2. Values are means ± SD for 5 cells in each category. All values are statistically significant compared with baseline, P < 0.05, except those marked ns.
TABLE 4. Changes in firing rates, intracellular ion concentrations and membrane potentials in VMH neurons during stepwise increases and decreases in glucose level

<table>
<thead>
<tr>
<th>Δ Glucose, mM</th>
<th>Blood</th>
<th>Brain</th>
<th>Firing Rate, spikes/s</th>
<th>[Na⁺], mM</th>
<th>[Ca²⁺], mM</th>
<th>[K⁺], mM</th>
<th>E, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>11 ± 2</td>
<td>24.8 ± 1.6</td>
<td>125 ± 4.3</td>
<td>72.4 ± 2.7</td>
<td>−67.8 ± 1.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>+2</td>
<td>16 ± 3</td>
<td>24.3 ± 0.7*</td>
<td>137 ± 4.6*</td>
<td>71.0 ± 2.7*</td>
<td>−66.4 ± 1.5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+5</td>
<td>27 ± 1</td>
<td>23.5 ± 1.3</td>
<td>154 ± 6.5</td>
<td>70.6 ± 2.6*</td>
<td>−65.6 ± 1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>+10</td>
<td>33 ± 2</td>
<td>22.3 ± 1.2</td>
<td>180 ± 5.6</td>
<td>69.2 ± 2.7</td>
<td>−64.6 ± 0.8</td>
<td></td>
</tr>
<tr>
<td>Hypoglycemia</td>
<td>−2</td>
<td>6 ± 2</td>
<td>24.0 ± 1.3*</td>
<td>124 ± 4.8</td>
<td>73.5 ± 2.4*</td>
<td>−69.3 ± 1.9*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−4</td>
<td>1 ± 1</td>
<td>23.1 ± 1.4</td>
<td>118 ± 5.5</td>
<td>75.0 ± 2.3</td>
<td>−70.9 ± 2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>−6</td>
<td>0 ± 0</td>
<td>22.5 ± 1.5</td>
<td>108 ± 5.0</td>
<td>76.4 ± 2.3</td>
<td>−72.5 ± 2.3</td>
<td></td>
</tr>
</tbody>
</table>

Intracellular ion concentrations were measured with double- or multi-barreled microelectrodes as described in Experimental procedures. Membrane potential (E) and firing rate were measured through the reference barrels of the ion-selective probes. Values are means ± SD for either 16 (baseline) or 8 (all other) measurements. All values are statistically significant compared with baseline, P < 0.05, except those marked *. Baseline glucose concentration in blood was 7.6 ± 0.3 (n = 175) and that in brain 2.4 ± 0.13 (n = 133). VMH, ventromedial hypothalamic nucleus.

Simultaneous measurements of firing rates and the concentration of one or more intracellular ions, together with membrane electrical potentials, were made in >100 glucose-sensitive LHA neurons. Because it was not possible to record [K⁺], [Na⁺], and [Ca²⁺], simultaneously in every cell, a few ion measurements from different cells of the same type have been combined. Examples of records from type II cells are shown in Figs. 3 and 4. In Fig. 3, the top trace is from the reference barrel and shows extracellular action potentials followed by cell penetration (⇓) and recording of membrane potential and intracellular action potentials. The middle and bottom traces are from the potassium and calcium-sensitive barrels, respectively, of the same electrode. Because of the relatively slow responses of the ion-sensitive probes, recordings from these are displayed on a longer time scale. One minute after the cell was penetrated, the rat was given glucose intravenously, and as the blood glucose level rose above the baseline value of 7.7 mM, changes in cell firing rate and ion concentrations occurred that are shown as excerpts that correspond to the increase in blood sugar indicated on the x axis. (The same traces but at higher amplification are shown in the insets.) Figure 4 shows continuous changes in [K⁺] and [Na⁺] together with excerpts of action potentials during decreases in blood glucose after injection of insulin. Similar protocols were followed for other cells and the results are summarized as means ± SD in Tables 2 and 3 and Figs. 5 and 6.
Intracellular ion concentrations measured in hypothalamic neurons of normoglycemic animals were very similar, if not identical, and were close to the values determined by us previously in cells in other regions of the brain (Erecinska and Silver 1992, 1994; Silver and Erecinska 1990, 1992). Neurons that were inhibited by a rise in [glucose] (97 cells of types I–III) showed very similar behavior patterns: the concentration of potassium rose whereas that of calcium and sodium fell (Figs. 5 and 6; Table 2). These alterations were relatively small: 6–10 mM for sodium and potassium and 15–20 mM for calcium. Membrane potentials became more negative by 4–7 mV, cells hyperpolarized and action potentials became fewer and larger.

In the very few neurons that were stimulated by a rise in glucose (type IV), changes in ion concentrations were in the opposite directions: sodium and calcium increased and potassium decreased while the cell depolarized by a few millivolts. An interesting difference between the type IV neurons and all other glucose-sensitive LHA cells was the resting concentration of calcium, which was ~30 nM higher, 140 versus 110 nM (Table 2).

Gradual changes in intracellular ion concentrations also were seen when blood glucose level was lowered progressively (Figs. 4–6; Table 3). The internal concentrations of sodium and calcium rose, whereas that of potassium fell and cells depolarized; all responses of type I cells were significant at a smaller decrement in [glucose] (decrease in blood glucose concentration by 2 mM; Fig. 5) than those of types II and III (Fig. 6; Table 3). The early increases and/or decreases in intracellular ions (Δ brain glucose −0.3 and −1.1 mM) were again relatively small. However, in severe hypoglycemia, when glucose concentration in brain fell to ~0.2 mM and lower (Δ brain glucose −2.2 mM), “catastrophic” changes began to occur: there were rapid rises in internal concentrations of sodium and calcium and a simultaneous loss of potassium with a fall in membrane potential as described previously (Silver and Erecinska 1992, 1994).

During decreases in blood (brain) glucose, type IV LHA neurons exhibited changes in ion concentrations that were opposite to those in types I–III; [Na⁺], and [Ca²⁺], declined, [K⁺], rose, and the plasma membrane hyperpolarized by 3–4 mV (Table 3). When the recordings carried out at the lowest brain [glucose], were continued for 3–5 min after the 0.2 mM level was reached, in three of five cells listed in Table 3, there were rapid falls in [K⁺], (to 52.3 ± 4.5 mM) and membrane potential (to −54.3 ± 4.7 mV) and increases in [Na⁺], (to 33.3 ± 4.0 mM) and [Ca²⁺], (to 289 ± 42 nM). In the remaining two cells, similar but slower changes were observed (results not shown).

Responses of intracellular ions to physiological changes in [glucose] were determined in 26 neurons classified as sugar-insensitive. The basal (normoglycemic) concentrations of [Na⁺], (24–27 mM), [K⁺], (67–71 mM), [Ca²⁺], (105–120 nM), and cell membrane electrical potentials (−65 to −69 mV) were not altered when blood glucose was either raised to 15–17 mM (4–5 mM in brain) or reduced to 1.5–2 mM (0.3–0.4 mM in brain). However, in very severe hypoglycemia (brain sugar level <0.3 mM), catastrophic ion movements were seen, similar to those that occurred under comparable conditions in glucose-sensitive cells.

Responses of VMH neurons to changes in blood glucose concentration

Of the 87 VMH neurons tested, 38 (43%) responded to a rise in external glucose concentration by an increase in firing rate. These cells were not sensitive to other stimuli employed. Only five of these glucose-sensitive neurons were of type I, i.e., those in which the change in the discharge rate reached a maximum after a relatively small rise in the sugar level (from 7.4 to 8–10 mM in the blood). Most neurons, 85%, were of type III and generated progressively higher discharge rates when blood glucose was gradually raised to ≥15 mM. No cells were encountered whose activity was inhibited by hyperglycemia.

Lowering external [glucose] had the opposite effect; even a very small decrease in blood sugar level, from 7.6 to 7.0 mM, reduced the rate of firing. At 5.6–6.0 mM blood glucose concentration, the discharge frequency fell by 40–60%, whereas at 3–4 mM, >90% of cells were silent (Table 4). An example of typical changes in neuronal activity, recorded extracellularly, during a continuous cycle from normo-, through hypo- to hyperglycemia, is shown in Fig. 7.

Concentrations of intracellular ions were measured with microelectrodes in 38 glucose-sensitive VMH neurons dur-
of 2 mM). This behavior indicates that these "sensors" can site and Ç
m glucose concentration. In both these regions, alterations in 2.5 ± 3
the neurons in hypothalamic areas LHA and VMH are en- therefrom) does not attain a maximum at the physiological,
VMH cells in intact rat brain in response to alterations in al. 1986) that the activity of this enzyme in several types of
transport of glucose from extracellular (middle), (top), and traces as in Fig. 8. Thirty seconds after the cell was penetrated,
and calcium (bottom) in response to hypoglycemia. These records are from a VMH cell. (Fig. 9 and Table 4). During hypoglycemia, intracellular potassium concentration increased, whereas that of sodium and calcium decreased. The plasma membrane hyperpolarized (Fig. 9 and Table 4).

**DISCUSSION**

The present study is, to the best of our knowledge, the first work that examines, in a systematic manner and in considerable detail, the changes in intracellular concentrations of [Na⁺], [K⁺], and [Ca²⁺], and membrane potential as well as in neuronal firing rate that occur in LHA and VMH cells in intact rat brain in response to alterations in blood (and brain) glucose level. It shows that 30–45% of the neurons in hypothalamic areas LHA and VMH are endowed with exquisite sensitivity to increases/decreases in glucose concentration. In both these regions, alterations in cell firing rate and internal ion concentrations were observed at increments/decrements in the extracellular sugar level as small as 0.2–0.3 mM (or blood glucose decreases/increases of 2 mM). This behavior indicates that these "sensors" can respond to physiological shifts in sugar concentration and therefore have the capacity to be an integral part of the body homeostatic mechanisms. However, not all cells, in particular those in the LHA, exhibit the same degree of sensitivity. Although for the sake of simplicity in presentation of the data we categorized the LHA glucose-sensing neurons into four classes, our findings suggest that there is a continuous spectrum of responsiveness and that the stronger the stimulus (change in glucose concentration) the larger is the number of cells that are recruited to react. Some of the glucose-sensitive cells of types II and III also responded to peripheral sensory stimuli, and it is possible that such inputs may modulate their response to glucose.

Consistent with the data in the literature (Oomura et al. 1969), the majority of glucose-sensitive LHA cells hyperpolarized and decreased their firing rate when blood glucose level was raised and depolarized and increased discharge frequency when glucose concentration fell. Concomitantly, hyperglycemia was accompanied by a reduction in [Na⁺], and a rise in [K⁺], whereas the opposite events took place in hypoglycemia. This pattern of responses is consistent with stimulation and inhibition, respectively, of activity of the plasma membrane Na/K ATPase, as postulated by earlier authors (Oomura et al. 1969). The question that was not addressed previously by others but that can be answered based on our results, is how a change in external glucose level is "translated" into altered pump function.

The major determinants of the Na/K pump activity are the concentrations of intracellular sodium and extracellular potassium as well as [ATP] and the products of its hydrolysis, [ADP] and [Pi]. The first three are stimulatory, whereas the latter two are inhibitory (Def Weer 1970; Garay and Garrahan 1975; Glynn and Karlish 1975; Robinson and Flashner 1979). Transport of glucose from extracellular space into neurons is considered to occur via facilitated diffusion, which does not require sodium (Lund-Andersen 1979). Thus enhanced uptake of this sugar in hyperglycemia should not elevate [Na⁺], as indeed is indicated by our data. (Although an apparent lack of even transient increases in the intracellular level of sodium could argue against the existence in LHA sensor cells of a separate, Na-linked glucose carrier small changes occurring in <3 s might have been missed due to the relatively slow response of the Na electrode.) Similarly, an elevation in [K⁺], under the same conditions (Table 2; Figs. 3, 5, and 6) suggests that [K⁺], is unlikely to rise, whereas a possible decrease could reduce, but not augment, the pump activity. By contrast, a rise in ATP (and consequent decreases in [ADP] and [Pi]), if it occurs, should be stimulatory, whereas a decline should be inhibitory. The suggestion that such a mechanism for Na/K ATPase regulation exists is based on the findings (Ikehara et al. 1984; Soltoff and Mandel 1984; Tessitore et al. 1986) that the activity of this enzyme in several types of intact cells (in contrast to membrane preparations isolated therefrom) does not attain a maximum at the physiological, intra-cellular ATP level (2.5–6 mM for various cell types; 2.5–3 μmol/g wet weight, or 3.0–3.5 mM in brain) (Erecinski and Silver 1989, 1994; Siesjö 1978), even though both nucleoside triphosphate binding sites should be saturated with the substrate [Km for ATP of ~10 μM at the catalytic site and ~0.5 mM at the regulatory site (Glynn and Karlish...
1975; Robinson 1976)]. If such dependence on energy also occurs in LHA neurons and if changes in [glucose] continuously modify the intracellular concentration of ATP, then activity of the pump will increase above the basal level in hyperglycemia and decrease in hypoglycemia in agreement with our results. Although this scenario could account for the findings, it explains neither how a small change in glucose level brings about an alteration in intracellular energy content nor why there is a difference between glucose-sensitive LHA cells and the majority of CNS neurons that do not respond metabolically to small (physiological) alterations in sugar concentration.

It generally is accepted that cerebral metabolism of glucose, which is one of the determinants of tissue ATP level, is phosphorylation- and not transport-limited (Furler et al. 1991; Lund-Andersen 1979; Pardridge 1983; Robinson and Rapoport 1986). Two conclusions follow: that under physiological, normoglycemic conditions extra- and intracellular concentrations of glucose are similar and that activity of the phosphorylating enzymes, and not the neuronal plasma membrane transporters (Bell et al. 1993; Vannucci et al. 1997), is the rate-controlling step. The first is supported by the findings that total cerebral glucose content (which is mainly intracellular) (e.g., Duffy et al. 1972; Gjeede and Diemer 1983; Lewis et al. 1974; Mason et al. 1992) is very close to that in the extracellular space (Silver and Erecinska 1994). With respect to the second, glucose enters neurons predominantly on GLUT3, which has a relatively high affinity for its substrate ($K_m < 2 \text{ mM}$) (Colville et al. 1993): thus the transporter is likely to operate at a velocity not much below the $V_{max}$. Sugar phosphorylation is catalyzed by hexokinases and brain contains almost exclusively the type I enzyme, which, with a $K_m$ for glucose of 10–50 $\mu$M (Wilson 1985), should be saturated under physiological conditions.

It is clear that the above considerations, which are based on measurements in bulk tissue, apply to the majority of CNS neurons (which are not glucose sensitive) but not to LHA cells. In the latter, it is either the transport or phosphorylation of glucose that must be distinct. Moreover the velocity of either or both of these processes would have to vary over a broad range of sugar concentrations to modulate cellular metabolic rate and ATP content. Recent computer modeling studies (Sweet and Matschinsky 1995) of glucose metabolism in pancreatic $\beta$-cells, which also respond sensitively to alterations in the ambient glucose level (Meglasson and Matschinsky 1986), show that for transport not to be limiting its velocity has to exceed phosphorylation by a factor of 5. When the maximal rate of transport approaches that of phosphorylation, the former becomes a very sensitive effecter of fuel usage (Sweet and Matschinsky 1995). There is no information on what relations exist between the two processes in glucose-sensing hypothalamic neurons, but there is no indication in our results that transport of glucose involves a process other than facilitated diffusion, which varies linearly with changes in substrate concentration. Consequently, alteration of metabolism by $\Delta$glucose of 0.2–0.3 mM would be expected to be very small. Hence in our opinion, although glucose transport is a potential site of control, it seems unlikely to play a major role in affecting energy metabolism of glucose-sensitive hypothalamic cells.

An alternative is that fuel-sensitive LHA neurons, like liver and pancreatic $\beta$-cells (Meglasson and Matschinsky 1984), possess glucokinase, a low-affinity (high $K_m$) hexokinase. This enzyme is not inhibited by glucose-6-phosphate and exhibits a Hill coefficient of 1.2–1.5 (Meglasson et al. 1983; Storer and Cornish-Bowden 1976), which amplifies metabolic changes in response to increases/decreases in substrate concentration. It has been suggested that the glucokinase is responsible for glucose-induced energetic alterations in $\beta$-cells (Meglasson and Matschinsky 1986) in which $[\text{ATP}]$ (or $[\text{ATP}] / [\text{ADP}]$) controls activity of the ATP-dependent $K^+$-channel (Ascroft 1988; Dunne and Petersen 1991). Our conjecture that glucose-sensitive LHA neurons contain an analogous sensor mechanism is supported by the recent finding that certain cells in the medial hypothalamus express a glucokinase gene, glucokinase mRNA, and glucokinase activity (Jetton et al. 1994). By modulating the concentration of cytosolic $[\text{ATP}] / [\text{ADP}]$ LHA glucokinase could alter the activity of the plasma membrane Na pump, which in many systems (Lynch and Balaban 1987; Takai and Tomita 1986; Weiss and Lamp 1989), including brain (Lipton and Robacker 1983; Raffin et al. 1988, 1992; Roberts 1993), is particularly sensitive to glycolytically generated ATP.

The glucose-sensitive VMH cells depolarized and increased their discharge frequency when glucose level was raised and hyperpolarized and decreased their firing rate when [sugar] was reduced. Concurrently, $[K^+]_i$ fell in hyperglycemia and rose in hypoglycemia. These changes are consistent with the closure and opening in the two conditions respectively, of the ATP-sensitive $K^+$-channels, as indeed has been demonstrated directly by electrophysiological studies (Ashford et al. 1990). The VMH ATP-sensitive potassium channels have a single channel conductance of $\sim 150 \text{ pS}$, and their $K_v$ for ATP ($\sim 3 \text{ mM}$) is considerably higher than that in skeletal (0.5 mM) and cardiac muscles (0.1–0.5 mM) and pancreatic $\beta$-cells (15–20 $\mu$M) (Ascroft 1988). This high $K_v$ for ATP, very close to the concentration of the latter in “resting” brain (Erecinska and Silver 1989, 1994; Siesjo 1978), means that small changes in energy level could alter potassium permeability and thus membrane potential in these neurons, very sensitively. Although no information currently exists on how glucose-responsive VMH cells control their energy metabolism, the existence of a glucokinase-like enzyme, similar to that outlined above for LHA cells, would allow for a continuous spectrum of changes in $[\text{ATP}] / [\text{ADP}]$ and account for our experimental findings. It is worth pointing out that this mechanism is very similar to that which has been postulated to control potassium channel activity, and hence physiological function, of fuel-sensing pancreatic $\beta$-cells (Ascroft 1988; Dunne and Petersen 1991).

Our results also show that alterations in $[\text{Na}^+]_i$ and $[K^+]_i$, which occur in the sensitive LHA and VMH neurons in hyper- and hypoglycemia, are accompanied by changes in intracellular calcium concentration. The pattern of behavior, a rise during depolarization and a decline on hyperpolarization, is consistent with an increase in the number of opened $Ca^{2+}$-channels when the membrane potential falls. These changes may be important because it has been reported recently that $[Ca^{2+}]_i$ controls the fraction of sodium channels
available for activation (Bulatko and Greeff 1995): a rise in the concentration of calcium increases the number of active Na\(^{+}\)-channels, whereas a reduction decreases it. Thus it is not only the state of polarization of the plasma membrane but also the [Ca\(^{2+}\)], which may be responsible for determining neuronal excitability (discharge frequency) at the various ambient glucose levels.

An important distinction between the majority of fuel-sensitive LHA neurons (types I–III) and those in the VMH is that in the former an increase in glucose metabolism, and a consequent rise in energy level, affect predominantly the Na/K pump activity, whereas in the latter the same metabolic events influence the membrane permeability to potassium. It follows that either ATP-sensitive K\(^{+}\)-channels are present only on the glucose-responsive VMH neurons or that in these cells the effects of ATP on the activity of the pump and the state of the channels are tilted in favor of the latter. However, 5–7\% of LHA cells (type IV) reacted to glucose in a manner opposite to that of the remaining 93–95\%. Thus in these “anomalous” cells, the predominant effect seems to be on potassium permeability, which is similar to that exhibited by the VMH neurons. Whether type IV LHA cells are a relic of evolution from more primitive organisms, where cells with different functions are not segregated to different areas, or form part of a smoothing feedback loop, is not known.

A final point to be considered is whether the rate and magnitude of the glucose changes imposed on the sensing cells in the present experiments can be considered normal. The alterations in blood sugar to which the rats were exposed covered the physiological range and extended beyond this to levels likely to be encountered in moderately stabilized diabetic patients. Rates of change in glucose concentration that were imposed were faster than those under physiological conditions in conscious subjects but similar to those which occur in poorly stabilized diabetics. It is, therefore, reasonable to expect that the responses recorded from sugar-sensing cells under the experimental conditions used in our study are normal and form part of the regulatory mechanism that controls blood-glucose concentration.

In conclusion, the current study has identified changes in intracellular concentrations of ions that occur in glucose-sensitive LHA and VMH neurons of rat brain on increases and decreases in blood (and brain) glucose level. Moreover, it revealed differences in sensitivities among individual cells. The importance of these findings lies in that they allow construction of an hypothesis for a cellular mechanism whereby an alteration in sugar concentration can be translated into either increased or decreased neuronal activity. Our work provides, therefore, a better understanding of physiological processes which maintain glucose homeostasis in mammals.

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