Glucose-Depletion Suppresses Synaptic Transmissions in Rat Dorsolateral Septal Nucleus

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Summary Removal of extracellular glucose hyperpolarized the postsynaptic membrane of dorsolateral septal nucleus (DLSN) neurons. Glucose-depletion suppressed the excitatory postsynaptic potential (EPSP) and the late hyperpolarizing potential (LHP) without affecting the responses induced by glutamate or baclofen. Glibenclamide did not antagonize the effects produced by glucose-depletion. These results suggest that glucose is necessary to maintain the membrane excitability and transmitter-release in rat DLSN.

Key words: rat septal neurons, hypoglycemia, synaptic transmission.

Neurons in the central nervous system (CNS) are known to be sensitive to the change in extracellular glucose concentration; an increase in glucose depolarizes the neurons and augments the discharge frequency, while glucose-depletion hyperpolarizes and suppresses the firing activity (ONO et al., 1982; SPULER et al., 1985). Ashford and his colleagues (ASHFORD et al., 1990a, b) have demonstrated that the increase in neuronal excitability by glucose in rat hypothalamic neurons is mediated by the blockade of adenosine triphosphate (ATP)-sensitive potassium (K\textsubscript{ATP}) channels, which have been found in various tissues (NOMA, 1983; ASHCROFT et al., 1984, 1988; COOK and HALES, 1984; DUNNE et al., 1986; SPRUCE et al., 1987; DE WEILLE et al., 1988, 1989; BEN-ARI, 1989; ASHFORD et al., 1990a, b). However, little is known about the functional role of extracellular glucose on synaptic transmission in the CNS. The present study showed that depletion of glucose caused not only a hyperpolarization of postsynaptic membrane but also a presynaptic inhibition of excitatory and inhibitory postsynaptic potentials in the rat dorsolateral septal nucleus (DLSN).

Septal slices were obtained from male Wistar-Kyoto rats (120–300 g) sacri-
ficed by decapitation. The isolated brain tissue was immediately immersed in a cold (4–6°C) artificial cerebrospinal fluid (ACSF), which was continuously bubbled with 95% O₂ and 5% CO₂ gas mixture. Transverse slices (500 µm in thickness) were prepared as previously described (Hasuo et al., 1988) using a vibroslice (Campden Instruments) instrument. The ACSF was pH 7.4 and of the following composition (in mM): NaCl 117, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 25, NaH₂PO₄ 1.2, and d-glucose 11. A glucose-free solution was made by replacing glucose with an equimolar sucrose. A calcium-free solution was made by removal of extracellular calcium and 10 mM magnesium was added. Slices containing the DLSN were continuously superfused with prebubbled ACSF at 32°C in a recording chamber. Intracellular recordings were made by using glass microelectrode filled with 4 M potassium acetate (a tip resistance of 90–140 MΩ). Neuronal signals were recorded with Axoclamp 2A (Axon Instruments). A concentric bipolar electrode was used for focal stimulation (monophasic pulses with 0.2 ms duration) of fimbria/fornix pathway. Applied drugs were picrotoxin (Sigma), tetrodotoxin (TTX, Sankyo), and glibenclamide (gift from Hoechst AG, Frankfurt, Germany).

Stable intracellular recordings were performed from 39 septal neurons for 1–4 h. These cells showed the resting membrane potentials of −58 ± 8 mV (mean ± S.D.) (n = 28) and the input resistances of 117 ± 16 MΩ (n = 28) (Hasuo et al., 1988). When glucose (11 mM) was removed from superfusing solution (1–10 min), the membrane potential was hyperpolarized by 10 ± 2 mV (n = 21) in 78% of the neurons, the membrane input resistance was decreased by 52 ± 11% (n = 12), and the spontaneous firing ceased (Fig. 1A). These effects were reversible. Recovery from the hypoglycemia-induced hyperpolarization was not observed when 2-deoxyglucose (11 mM), an antimetabolic glucose, was introduced to the superfusate instead of glucose. The hyperpolarization was not blocked but rather enhanced in a calcium-free solution (zero calcium and 10 mM magnesium) containing TTX (1 μM). Voltage-current relationships (V–I curves) were examined in a septal neuron first in the normal ACSF solution (glucose, 11 mM) and then in the glucose-free solution. The V–I curve obtained in the glucose-free solution was less steep than those in the normal solution and both curves crossed at −89 mV. Thus the reversal potential of the hypoglycemia-induced hyperpolarization was estimated to be −90 ± 10 mV (n = 4). This value seemed to be close to potassium equilibrium potential. These results suggest that glucose-depletion increases potassium conductance and hyperpolarizes DLSN neurons.

Orthodromic stimulation of fimbria/fornix pathway usually evoked an excitatory postsynaptic potential (EPSP) followed by an inhibitory postsynaptic potential (IPSP) and a late hyperpolarizing potential (LHP); the EPSP, IPSP, and LHP of DLSN neurons are mediated through activation of glutamate receptors and two separate γ-aminobutyric acid (GABA) receptor subtypes (GABA_A and GABA_B), respectively (Stevens et al., 1987; Hasuo et al., 1988). The full amplitude and time course of the EPSP are not apparent in normal solution, because of overlap with its following IPSP (Stevens et al., 1987). The effect of
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Fig. 1. A: Effects of glucose-depletion on the resting membrane potential and input resistance of a septal neuron. The resting membrane potential was $-62 \text{ mV}$ in the normal solution. The top line indicates the timing of the change of superfusing solutions. In the upper record, upward deflections represent spontaneously firing action potentials. Downward deflections with large and small amplitudes represent electrotonic potentials and synaptic potentials, respectively. Lower records show expanded trace of hyperpolarizing electrotonic potentials; records a–d were obtained at the times marked by corresponding letters in the upper trace. B: Effect of glucose-depletion on the EPSP and LHP obtained by stimulations of fimbria/fornix pathway. Arrowhead and closed triangle indicate the EPSP and LHP, respectively. The IPSP was blocked by the application of picrotoxin (100 $\mu$M). Records (i)–(iii) were obtained at the times marked by corresponding letters in the upper trace shown in A.

glucose-depletion on the EPSP was, therefore, examined in septal neuron treated with 100 $\mu$M picrotoxin, where the IPSP was suppressed. Figure 1B shows a potential sequence of the EPSP and the LHP. Hypoglycemic exposure for 10 min suppressed the EPSP and the LHP by $62 \pm 4$ ($n = 8$) and $70 \pm 8\%$ ($n = 8$), respectively. The suppression of these synaptic potentials recovered when glucose was re-applied to DLSN neurons. However, application of 2-deoxyglucose (11 mM) could not cause the recovery from the suppression.

We examined whether the glucose-depletion affected the postsynaptic potentials induced by glutamate and GABA$_\alpha$ receptor-agonists (Fig. 2B). Agonist-induced responses were recorded from DLSN neurons, to which glutamate and baclofen, a GABA$_\alpha$ agonist, were applied in the TTX (1 $\mu$M)-containing solution or in the calcium-free solution. Glucose-depletion for 5–10 min suppressed the glutamate-induced depolarization and the baclofen-induced hyperpolarization only by 15 ($n = 4$) and 23$\%$ ($n = 4$), respectively (Fig. 2B). These results suggest that glucose-depletion mainly affects presynaptically, resulting in the suppression of the EPSP.
Fig. 2. Effect of glucose-depletion on postsynaptic potentials (A) and responses to glutamate and GABA<sub>B</sub> receptor-agonists (B). Both data were obtained from a same DLSN neuron. A: EPSP-LHP sequences were obtained by the fimbria/fornix stimulations in the presence of picrotoxin (100 µM). Left and middle traces were taken before and 5 min after application of glucose-free solution, respectively. Right trace was obtained 10 min after the re-application of glucose. To record the middle trace, hypoglycemia-induced hyperpolarization was briefly nullified by intracellular injection of a depolarizing D.C. current. B: Glutamate (△) and baclofen (▲) were ejected from pipettes (filled with 1 mM glutamate and 100 µM baclofen, respectively) by pressure pulses (138 kPa for 40 ms). In middle traces, the resting potential was returned temporarily to the control resting potential level by applying DC current. Brief hyperpolarizing current pulses (200 ms) were applied to estimate the change of membrane resistance. Note that glucose-depletion depressed the EPSP and the LHP but did not affect significantly the glutamate-induced depolarization and the baclofen-induced hyperpolarization.

and LHP in rat DLSN.

Antidiabetic sulfonylureas, such as tolbutamide and glibenclamide, have been shown to inhibit selectively the glucose-sensitive K<sub>ATP</sub> channels in insulin-secreting cells (Schmid-Antomarchi et al., 1987; De Weille et al., 1988; Sturgess et al., 1988). However, in DLSN neurons, glibenclamide (less than 20 µM) did not antagonize the hypoglycemia-induced membrane hyperpolarization and the suppression of postsynaptic potentials. At a higher concentration (more than 100 µM) glibenclamide and tolbutamide (100 µM–1 mM) depressed the hypoglycemia-induced hyperpolarization slightly. However, glibenclamide (100 µM) could not block the presynaptic inhibition of the EPSP and the LHP produced by hypogly-
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A

Glibenclamide (-)

11 mM Glucose

0 mM Glucose

11 mM Glucose


Glibenclamide (+)

20mV

60sec

B

Glibenclamide (+)

11 mM Glucose

0 mM Glucose

11 mM Glucose

10mV

200msec

Fig. 3. A: Effect of glibenclamide (100 μM) on the hyperpolarization induced by glucose-depletion. The resting membrane potential was −62 mV in the normal solution. The upper record shows the hypoglycemia-induced hyperpolarization taken in the absence of glibenclamide. In the lower trace, glibenclamide (100 μM) was applied to a glucose-containing ACSF for 10 min before application of the glucose-free solution. The top line indicates the timings of the change of superfusing solutions. Dotted lines indicate the resting membrane potential (−67 mV). Note that hypoglycemia-induced hyperpolarization was slightly depressed by glibenclamide (100 μM). B: Effects of hypoglycemia on the EPSP and the LHP examined in the presence of glibenclamide (100 μM). The resting membrane potential was −65 mV in the normal solution. EPSP-LHP sequences were evoked by electrical stimulations of fimbria/fornix pathway (at △) in the presence of picrotoxin (100 μM). The neuron was equilibrated with glucose-free solution for 10 min.

cemia (Fig. 3).

The present study showed that glucose-depletion produced a hyperpolarization due to an increase in potassium conductance, resulting in a suppression of spontaneous firing of septal neurons. Hypoglycemic exposure also caused a presynaptic inhibition of the EPSP and LHP, perhaps by decreasing the release of glutamate and GABA from presynaptic nerves terminals. There may be many possibilities to explain the mechanism underlying this presynaptic inhibition, such as a lack of energy to release transmitters from a nerve terminal, or a block of the conduction of action potential by a hyperpolarization of a nerve terminal. Direct blockade of Ca²⁺ influx is unlikely as the mechanism, because low glucose did not depress Ca spikes (Shoji, personal communication). Although further experiments are needed to clarify the mechanism for these inhibitions, the results in the present study suggest that extracellular glucose is required for maintaining the neuronal excita-
bility and synaptic transmission in DLSN.

Binding study has demonstrated that high affinity site for $^{3}$H-glibenclamide exists in septohippocampal area of the rat brain (De Weille et al., 1989; Mourre et al., 1989). Sulfonylureas have been found to block selectively the $K_{ATP}^{+}$ channel in various excitable tissues (Noma, 1983; Schmid-Antomarchi et al., 1987; De Weille et al., 1988; Sturgess et al., 1988). In hippocampal neurons, sulfonylureas blocked the $K_{ATP}^{+}$ channels activated by anoxia (Ben-Ari, 1989; Mourre et al., 1989) and by dopamine (D$_{2}$) receptor- and GABA$_{A}$ receptor- agonists (Ben-Ari, 1989; Roeper et al., 1990). However, the recent study by Ashford et al. (1990b) demonstrated that glibenclamide and tolbutamide did not block the glucose-regulated $K_{ATP}^{+}$ channels of inside-out membrane patches excised from freshly-dispersed rat ventromedial hypothalamic nucleus neurons. In the present study we also observed a partial blockade of the hypoglycemia-induced hyperpolarization, even when a high concentration of glibenclamide was applied. Perhaps, the affinity of glucose-regulated $K_{ATP}^{+}$ channels for sulfonylurea is different from that of $K_{ATP}^{+}$ channels activated by anoxia in central neurons.

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