Synaptic Adaptation to Repeated Hypoglycemia Depends on the Utilization of Monocarboxylates in Guinea Pig Hippocampal Slices

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This report provides in vitro evidence that synaptic activity becomes resistant to repeated hypoglycemia, i.e., hypoglycemic synaptic adaptation occurs. Synaptic function was estimated by the amplitude of the postsynaptic population spike (PS) recorded in the granule cell layer of guinea pig hippocampal slices. ATP, phosphocreatine (PCr), glycogen, and glucose concentrations were measured to investigate energy metabolism homeostasis. Glucose deprivation produced a complete elimination of the PS amplitude, with a 50% inhibition by 10.6 min, and a ~15% reduction in ATP and PCr concentrations. Low-glucose (0.5–1 mmol/l) medium gradually depressed the PS. After recovery from glucose depletion, repeated glucose deprivation produced a slowly developing depression of PS, with a 50% inhibition by 36.5 min. However, ATP and PCr concentrations were maintained. Incubation in secondary low-glucose medium maintained PS amplitude. Hippocampal glycogen and glucose concentrations promptly decreased during repeated glucose deprivation, indicating that glycogenolysis does not fuel synaptic adaptation to repeated hypoglycemia. Synaptic function during repeated glucose depletion was reversibly depressed by addition of α-cyano-4-hydroxycinnamic acid or 3-isobutyl-1-methylxanthine, inhibitors of the monocarboxylate transporter. Replacement of extracellular glucose with Na-lactate or Na-pyruvate sustained synaptic transmission after transient glucose depletion. These results indicate that synaptic utilization of monocarboxylates sustains hypoglycemic synaptic adaptation.
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Prospective studies of diabetes and its complications clearly demonstrate that glycemic control prevents or delays several long-term complications of the disease. However, attempts to achieve near-normal blood glucose levels increase the risk of hypoglycemia, the most common and serious iatrogenic morbidity in patients with diabetes. Because glucose is the principal metabolic fuel of the brain, acute hypoglycemia can cause profound but reversible autonomic and neuroglycopenic symptoms (1). Hypoglycemia induces counterregulatory hormonal responses, which produce sympathetic symptoms. Neurobehavioral deficits associated with insulin-induced hypoglycemia frequently arise on tasks that require sustained attention, rapid decision making, analysis of complex visual stimuli, mental flexibility, and memory of recently learned information (1,2).

The main defense against severe hypoglycemia is the subjective recognition of the onset of falling glucose levels. Some diabetic patients lose this symptomatic awareness and show reduced neuroendocrine responses to hypoglycemia, which is defined as hypoglycemia unawareness. In response to repeated hypoglycemia, the glucose threshold at which the brain detects hypoglycemia and triggers neurohumoral responses is shifted downward in nondiabetic individuals and in patients with diabetes (3–5). Glucose-sensing areas of the brain, presumably including the ventromedial hypothalamus, are responsible for the detection of hypoglycemia and for triggering hormonal responses (6). Thus, glucose-sensing neurons are believed to adapt to repeated hypoglycemia. It is less clear whether recurrent severe hypoglycemia alters the plasma glucose level at which cognitive function becomes impaired. Some studies have shown profound cognitive impairment in response to repeated hypoglycemia (7), but others have reported that recurrent hypoglycemia protects the brain in patients with diabetes (4,5,8). Studies analyzing alterations in the P300 event-related potential, an objective and more sensitive measure of cognitive function than neuropsychiatry tests, did not detect differences in cognitive function between patients who were subjected to recurrent hypoglycemia and control subjects (9). Neural activity, as measured by analysis of inferior colliculi auditory-evoked potentials (ICAPs), was studied recently in awake diabetic BB rats (10). Whereas lowered plasma glucose delayed ICAP latencies in chronic hyperglycemic rats, ICAP latencies in recurrent hypoglycemic rats were unchanged, suggesting that antecedent hypoglycemia attenuates brainstem dysfunction associated with mild insulin-induced hypoglycemia. Thus, whether neural activity involved in cognitive function can adapt to repeated hypoglycemia remains unclear. This controversy may result from technical limitations to the measurement of subtle changes in human subjects and in in vivo animal experiments.

To overcome limitations of previous studies and to

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4-CIN, α-cyano-4-hydroxycinnamic acid; IBMX, 3-isobutyl-1-methylxanthine; ICAP, inferior colliculi auditory-evoked potential; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; PCr, phosphocreatine; PS, population spike.

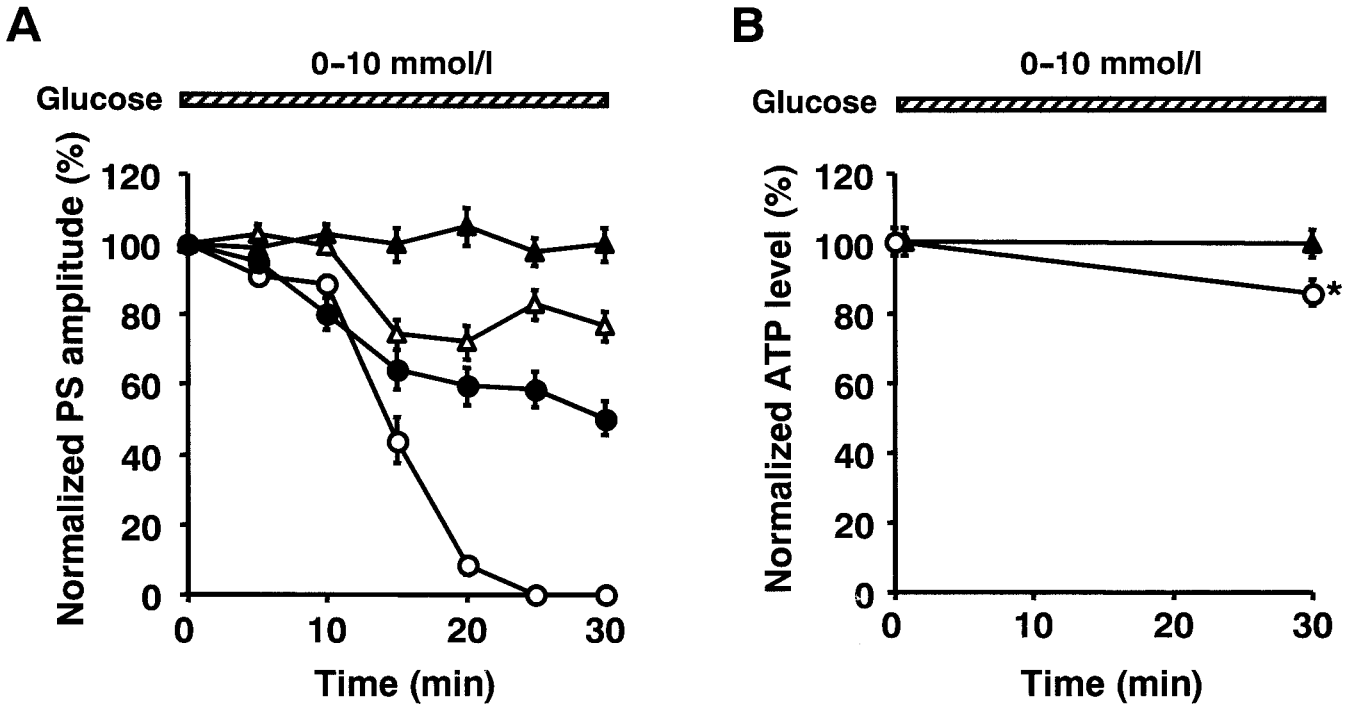

FIG. 1. Effect of glucose deprivation on synaptic activity and high-energy phosphate levels. *A*: Extracellular recordings of the PS were obtained in the granule cell layer of the hippocampal dentate gyrus. Hippocampal slices were prepared from guinea pig brains by a standard technique (11). Removal of exogenous glucose from standard medium produced a complete inhibition of the PS within 30 min, with a 50% inhibition at 10.6 min (○; $n = 15$). Incubation in 1 mmol/l (△; $n = 7$) or 0.5 mmol/l (●; $n = 10$) exogenous glucose solution depressed PS amplitudes to 76.6 ± 4.2 or 50.3 ± 5.0% of the control amplitude, respectively. In contrast, slices perfused with standard medium maintained normal evoked activity (▲; $n = 5$). The temperature of the medium was maintained at 35°C throughout the experiments. *B*: Preservation of ATP content in whole hippocampal slices was examined after glucose deprivation. Two hippocampal sections were then homogenized in ice-cold 0.5 mol/l perchloric acid plus 1 mmol/l EDTA and centrifuged, and the pellets were solubilized in 1 mol/l NaOH and subjected to protein assay by the Lowry method (12). The supernatants were neutralized, centrifuged, and used directly for the assay of ATP, using a sensitive enzymatic method analyzing NADPH production (11). The control ATP content was 13.3 ± 0.6 mmol/kg protein (▲; $n = 39$). The ATP content after a 30-min incubation in glucose-depleted medium (○; $n = 20$) was decreased to 85.9 ± 3.7% of the control ATP level. *$P < 0.05$ compared with the control ATP value.

investigate the neural basis of brain adaptation to repeated hypoglycemia, we conducted a series of in vitro experiments to analyze synaptic function during repeated hypoglycemia in guinea pig hippocampal slices. We estimated synaptic activity in hippocampal slices using electrophysiological techniques and investigated whether repeated hypoglycemia can maintain synaptic function. In addition, we examined the metabolic substrates that fuel synaptic function during repeated hypoglycemia. The advantages of our experimental preparation are *1*) the ability to condition neuronal cells in response to artificially regulated hypoglycemia, *2*) the exclusion of molecular transport across cerebral vessels, and *3*) the ability to monitor simultaneously the energy metabolism homeostasis that sustains synaptic activity during repeated hypoglycemia.

RESEARCH DESIGN AND METHODS

Preparation of hippocampal slices and electrophysiology. Hippocampal slices were prepared from guinea pig brains (Hartley, Shizuoka, Japan) by a standard technique (11). All animals were treated according to the guidelines for animal experimentation at Kobe University School of Medicine. Guinea pigs (14–28 days old) were anesthetized with sodium pentobarbital and decapitated. The hippocampi were removed from the skull and sliced rapidly into 300- to 400-μm-thick transverse sections. The slices were placed in an incubation chamber containing standard medium (in mmol/l: 125 NaCl, 4 KCl, 1.24 KH_2PO_4, 1.3 $MgSO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose) and bubbled with 95% O_2 and 5% CO_2 at 35°C. At the time of study, hippocampal slices were transferred individually to a submersion recording chamber, which was perfused with the standard medium at a flow rate of 4 ml/min.

Extracellular recordings of the field postsynaptic population spike (PS) were obtained in the granule cell layer of the hippocampal dentate gyrus with 2 mol/l NaCl glass electrodes (resistance: 1–5 MΩ). Evoked synaptic responses were elicited with 0.1-msec constant-current pulses through a bipolar electrode placed in the perforant pathway. After establishing a stable baseline for at least 20 min as well as a control input-output curve, we monitored synaptic responses by applying single stimuli to the perforant pathway every 10 s at intensity sufficient to elicit a 60–70% maximal PS. To induce glucose deprivation, we removed glucose from the standard medium. To test the effect of lactate or pyruvate during glucose deprivation, we replaced glucose in the medium with 10 mmol/l Na lactate or 10 mmol/l Na pyruvate. Adding sodium lactate or sodium pyruvate did not influence the pH of the circulation medium. To exclude irreparable damage to the slices, we reintroduced standard medium containing 10 mmol/l glucose after test-medium treatment, and recovery of the PS amplitude was measured. All experimental media were bubbled with 95% O_2 and 5% CO_2, and the temperature of the media was maintained at 35°C throughout the experiments.

Quantification of hippocampal ATP, phosphocreatine, glycogen, and glucose concentrations. After preincubation in the standard O_2-saturated medium for at least 20 min, hippocampal slices were incubated in test medium containing 0–10 mmol/l glucose. Two hippocampal sections were homogenized immediately in ice-cold 0.5 mol/l perchloric acid with 1 mmol/l EDTA and centrifuged for 15 min at 2,000 rpm. Pellets were solubilized in 1 mol/l NaOH and used for protein assay according to the Lowry method (12). Supernatants were neutralized with 2 mol/l $KHCO_3$, centrifuged, and used directly for assay of ATP, phosphocreatine (PCr), and glucose. ATP, PCr, and glucose concentrations were determined by sensitive enzymatic methods that analyze NADPH production (11).

For glycogen assay, hippocampal slices incubated in test perfusion medium were homogenized in 150 μl of ice-cold 30 mmol/l HCl and heated at 100°C for 3 min, which promptly halts glycogen metabolism. Hippocampal homogenates were stored at −20°C until assays were performed. Glycogen assays were performed by the method of Passonneau and Lauderdale (13), in which 1 mol/l HCl completely hydrolyzes glycogen to glucose. Briefly, 50 μl of the suspension was removed and added to 50 μl of 0.5 mol/l NaOH for protein assay. The remainder was divided into 70- and 20-μl fractions (fractions A and B, respectively). Fraction A was added to 630 μl of 1 mol/l HCl and heated for

3 h at 100°C, and fraction B was not. Glucose in both fractions was quantified by the glucose-6-phosphate dehydrogenase/NADP fluorescence method (11). The glucose in fraction B, which reflects the endogenous glucose in hippocampal slices, was subtracted from the glucose in fraction A, which reflects the sum of endogenous glucose and glucose derived from glycogen hydrolysis. Standards were prepared from glucose or from bovine liver glycogen after desiccation at 120°C. It was found that desiccated glycogen hydrolyzed with HCl yields almost exactly the predicted amount of glucose (data not shown).

Materials. Glucose-6-phosphate dehydrogenase, hexokinase, creatine kinase, ADP, and NADP were purchased from Boehringer-Mannheim (Mannheim, Germany). Sodium lactate, sodium pyruvate, and α-cyano-4-hydroxycinnamic acid (4-CIN) were obtained from Sigma (St. Louis, MO), and all other chemicals were from Wako (Tokyo, Japan) or Nacalai (Kyoto, Japan). 4-CIN was dissolved in DMSO and applied to the perfusion medium at a final concentration of 200 μmol/l. 3-Isobutyl-1-methylxanthine (IBMX) was dissolved in 3.5 mol/l KOH and was applied to the extracellular solution at a concentration of 500 μmol/l.

Statistical analysis. Data are presented as means ± SE. Statistical analysis was performed by nonpaired t test. Treatment differences were considered significant at $P < 0.05$.

RESULTS

Effects of glucose deprivation on synaptic activity and high-energy phosphate levels. Similar to previously reported findings (11,14), a low glucose concentration in the extracellular solution produced a slowly developing decrease in synaptic response in the granule cell layer of guinea pig hippocampal slices (Fig. 1). Removal of glucose from the standard medium produced a complete elimination of the PS within 30 min, with a 50% reduction in amplitude by 10.6 min (Fig. 1*A*). Incubation in 1.0 or 0.5 mmol/l glucose-containing solutions for 30 min suppressed the PS amplitude to 76.6 ± 4.2 or 50.3 ± 5.0% of the control PS amplitude, respectively. In contrast, slices perfused with standard medium containing 10 mmol/l glucose maintained normal evoked activity.

To determine whether the decay in synaptic transmission in response to glucose deprivation is accompanied by a reduction of high-energy phosphates, we investigated the preservation of ATP and PCr contents in hippocampal slices. Control ATP and PCr concentrations were 13.3 ± 0.6 and 29.2 ± 1.9 mmol/kg protein, respectively. Incubation in glucose-depleted solution for 30 min decreased the ATP content to 85.9 ± 3.7% of the control level ($P < 0.05$); (Fig. 1*B*). PCr was decreased to 84.5 ± 5.0% of the control level (data not shown; $P < 0.05$). Because a reduction in ATP and PCr has been estimated to occur throughout hippocampal slices, we determined the specific changes in ATP and PCr in the dentate gyrus (15). The dentate gyrus was dissected from hippocampal slices under a stereoscope and incubated in glucose-depleted solution for 30 min. ATP and PCr in the dissected dentate gyrus were decreased to 88.8 ± 5.9 and 84.7 ± 4.9% of the control levels, respectively (15), in good agreement with the ATP and PCr preservation levels determined in intact hippocampal slices. These results indicate that glucose deprivation produces complete inhibition of synaptic activity in the hippocampal dentate gyrus, accompanied by a significant reduction in ATP and PCr.

Repeated glucose deprivation produces hypoglycemic synaptic adaptation. We examined the effect of repeated glucose depletion on synaptic activity. After 30 min of glucose deprivation, hippocampal sections were incubated in standard medium containing 10 mmol/l glucose, resulting in recovery of the PS amplitude to 129.0 ± 5.9% of the control level (Fig. 2*A*). After stabilization of PS amplitude in standard medium, hippocampal slices were reintroduced to low-glucose medium. The repeated glucose depletion gradually inhibited PS amplitudes over 80 min, with a 50% inhibition by 36.5 min (Fig. 2*A*). However, in hippocampal slices subjected repeatedly to low glucose levels (1 or 0.5 mmol/l), PS amplitudes were maintained for >90 min (Fig. 2*A*).

Preservation of high-energy phosphates during repeated glucose deprivation is shown in Fig. 2*B*. After transient glucose deprivation, ATP concentration recovered to 96.7 ± 3.2% of the control level after a 30-min incubation in standard medium. Incubation in secondary glucose-free solution for

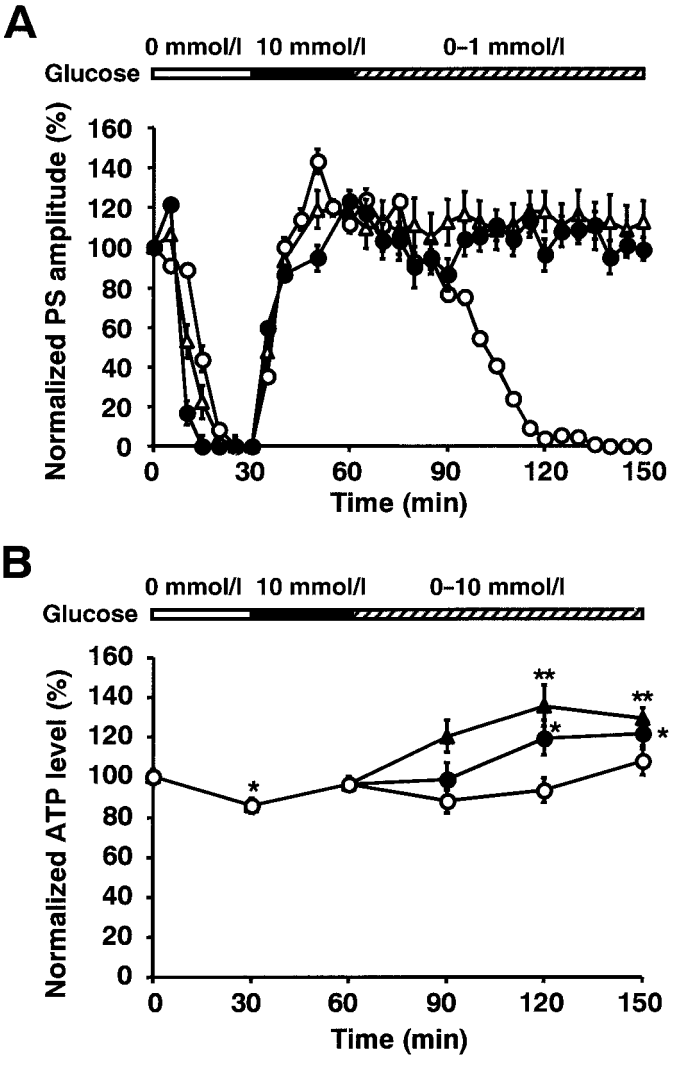

FIG. 2. Repeated glucose deprivation produces hypoglycemic synaptic adaptation. *A*: The effect of repeated glucose depletion on PS amplitudes was tested. PS recordings were obtained as described in Fig. 1*A*. Hippocampal slices were incubated in glucose-free medium, followed by incubation in standard medium (10 mmol/l glucose) for 30 min. Slices were then reintroduced to low-glucose solution. Subsequent glucose removal gradually depressed PS over 80 min, with a 50% inhibition at 36.5 min (○; $n = 8$). In contrast, incubation in 1 mmol/l (△; $n = 5$) or 0.5 mmol/l (●; $n = 7$) glucose medium maintained the PS amplitude for >90 min. *B*: Preservation of ATP content during repeated hypoglycemia (0 or 0.5 mmol/l) was determined. ATP quantification in hippocampal slices was performed as described in Fig. 1*B*. After a 90-min incubation in glucose-free medium, ATP concentrations remained unchanged: 108.1 ± 7.3% of control ATP level (○; $n = 16$). ATP levels in slices incubated in 0.5 mmol/l (●; $n = 14$) and 10 mmol/l glucose solution (▲; $n = 5$) were increased over control ATP levels. *$P < 0.05$, **$P < 0.01$ compared with control ATP values.

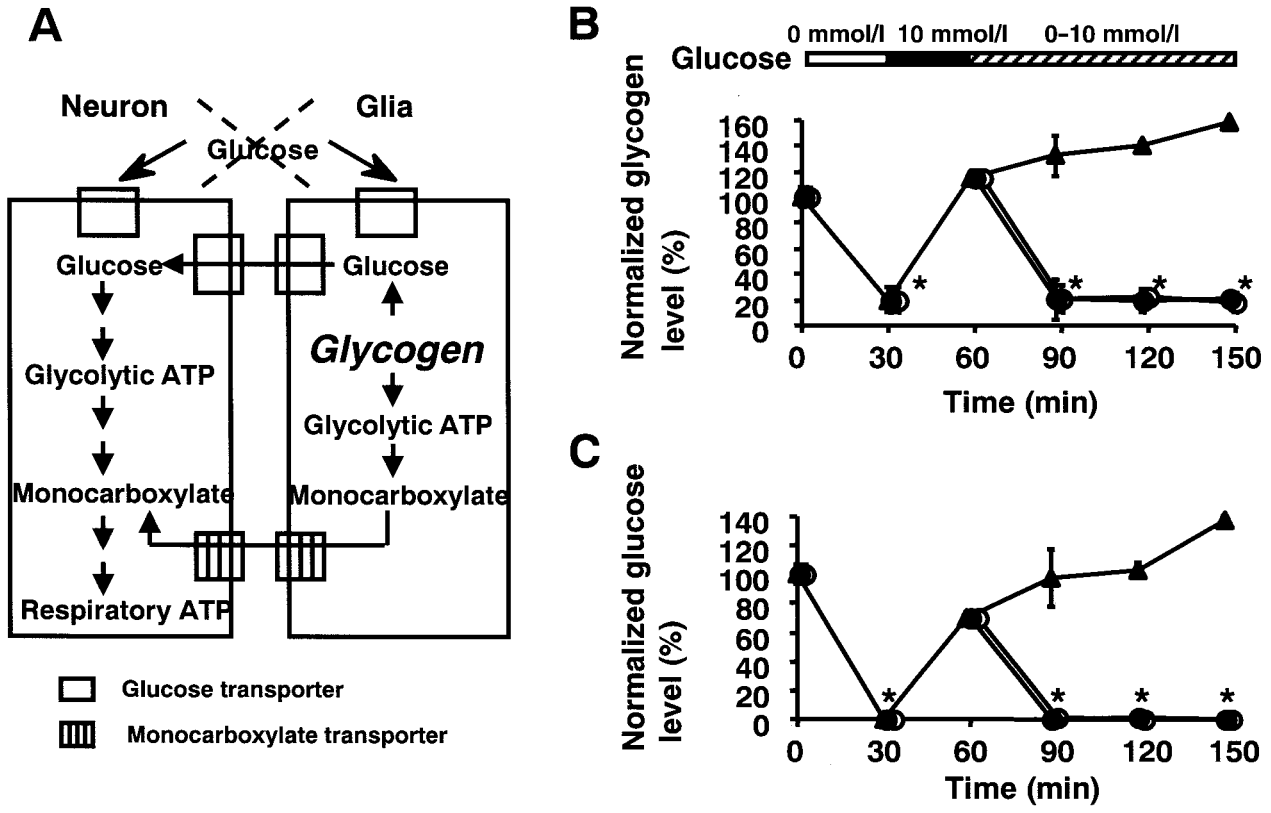

FIG. 3. Glycogenolysis does not support synaptic activity during repeated hypoglycemia. *A*: Schematic of glial-neuronal interactions during exogenous glucose deprivation. During glucose depletion, glial glycogen may support neurons by providing glucose or monocarboxylates. *B*: Glycogen concentration in whole hippocampal slices was examined during repeated glucose deprivation. Glycogen was determined by the method of Passonneau and Lauderdale (13). Control glycogen concentration was 224.9 ± 16.8 mmol glucosyl units/kg protein (n = 6). Hippocampal glycogen promptly fell to a low level upon the initial and the secondary glucose deprivation periods (○; n = 6). Glycogen concentrations also fell in the presence of 0.5 mmol/l glucose (●; n = 6) in secondary hypoglycemic medium, whereas they were elevated slightly in slices incubated in standard medium (10 mmol/l glucose; ▲; n = 6). *P < 0.01 compared with control level. *C*: Glucose concentration in hippocampal sections was determined during repeated glucose deprivation. Glucose was assayed using a sensitive enzymatic method (11), and the control concentration was 101.9 ± 6.6 mmol/kg protein (n = 6). Glucose was diminished to nearly 0 within 30 min of initial glucose removal, and glucose in slices incubated in secondary glucose-free medium (○; n = 6) or 0.5 mmol/l glucose medium (●; n = 6) was also decreased almost entirely within 30 min. Glucose was elevated slightly in slices incubated in standard medium (10 mmol/l glucose; ▲; n = 6). *P < 0.01 compared with the control concentration.

30, 60, and 90 min resulted in unchanged ATP contents: 87.7 ± 5.4, 93.4 ± 6.1, and 108.1 ± 7.3% of the control ATP concentration, respectively (Fig. 2*B*). PCr concentrations were also maintained during secondary glucose deprivation, ranging from 85.3 ± 6.5 to 95.0 ± 1.6% of the control PCr level (data not shown). Incubation in 0.5 mmol/l glucose solution did not decrease ATP levels (Fig. 2*B*); rather, ATP levels were increased after 60 min (120.8 ± 5.9%; $P < 0.05$). Similarly, PCr concentrations in hippocampal slices incubated in 0.5 mmol/l glucose solution did not decrease; rather, they increased after 60 min (data not shown). Incubation in 10 mmol/l glucose solution increased both ATP ($P < 0.01$) (Fig. 2*B*) and PCr concentrations (data not shown) after transient glucose deprivation. These results indicate that synaptic activity becomes resistant to repeated hypoglycemia, (i.e., hypoglycemic synaptic adaptation occurs) and suggest that hippocampal neurons utilize an alternative endogenous fuel to support synaptic function during periods of hypoglycemia.

Glycogenolysis does not support synaptic function in response to repeated hypoglycemia. We next investigated putative metabolic substrates supporting synaptic potentials and levels of high-energy phosphates during repeated hypoglycemia. In the brain, glycogen is synthesized from glucose-6-phosphate, localized exclusively in glial cells, and utilized as the energy source for neuronal excitability and during glucose insufficiency (16–18). It is plausible that hippocampal slices recovering from transient glucose depletion absorb excess exogenous glucose and synthesize astrocytic glycogen, which is, in turn, utilized as the substrate to maintain synaptic activity during the subsequent hypoglycemia (Fig. 3*A*). To test this possibility, we measured glycogen and glucose in hippocampal slices during repeated hypoglycemia. Control glycogen and glucose concentrations were 224.9 ± 16.8 mmol glucosyl units/kg protein and 101.9 ± 6.6 mmol/kg protein, respectively. As shown in Fig. 3*B*, hippocampal glycogen promptly fell to a low level after initial glucose deprivation, and repeated glucose deprivation rapidly decreased glycogen to an almost identical level ($P < 0.01$) (Fig. 3*B*). In the presence of 0.5 mmol/l glucose in secondary hypoglycemic perfusion medium, glycogen fell to a similar low level ($P < 0.01$). It is not surprising that hippocampal glycogen concentration remained low after 30 min of glucose depletion, rather than dropping to 0, because glucose controls glycogen levels by binding to and inactivating the enzyme responsible for glycogen breakdown, phosphorylase A (17). Cultured astrocytes are not able to mobilize glycogen stores completely in the absence of

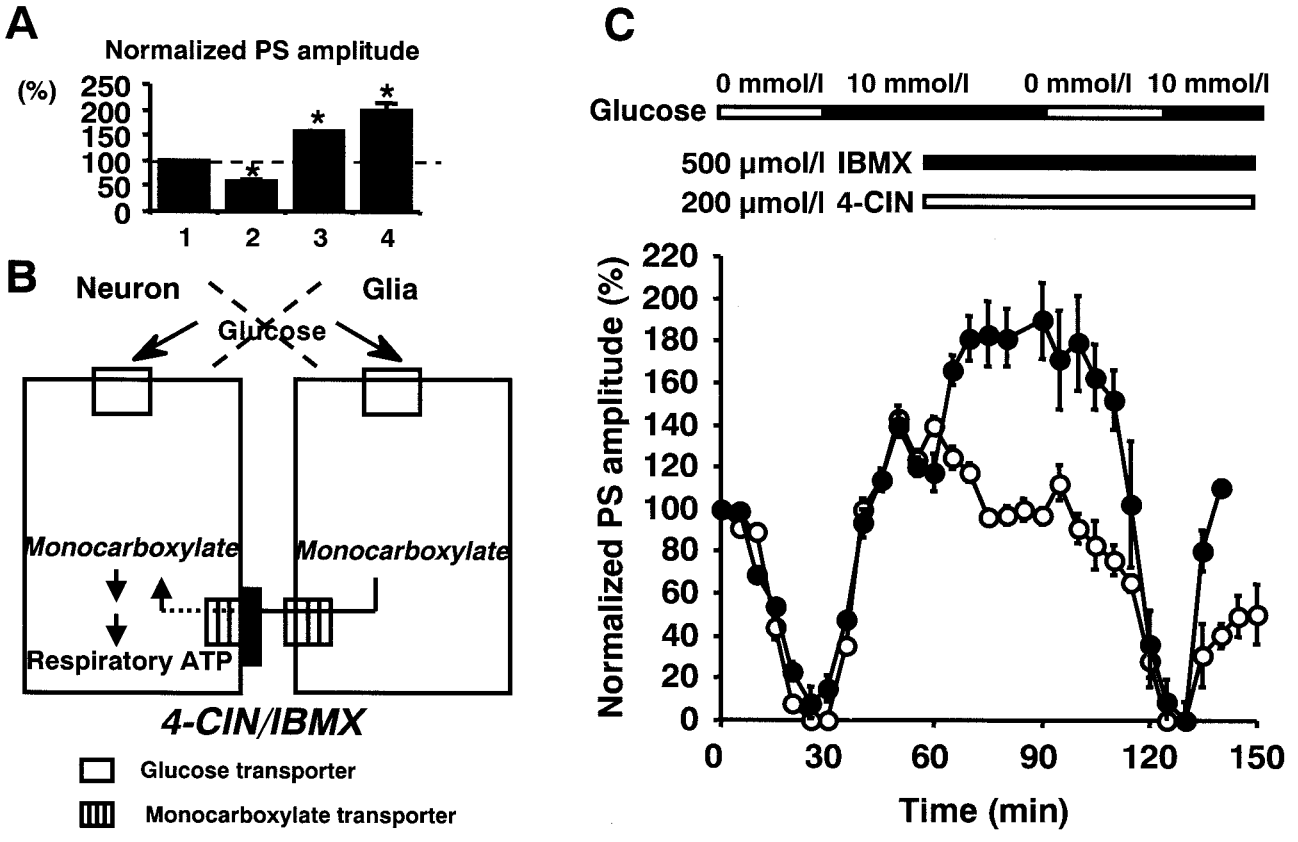

FIG. 4. Utilization of monocarboxylates contributes to synaptic adaptation during repeated glucose deprivation. *A*: The effects of monocarboxylate transporter inhibitors (4-CIN and IBMX) on 10 mmol/l glucose-supported PS were tested. 4-CIN was dissolved in DMSO and was applied to the perfusion medium. 0.1% DMSO did not affect PS amplitude (column 1) ($n = 5$), but addition of 200 μmol/l 4-CIN suppressed PS amplitude by 43.0 ± 6.8% (column 2) ($n = 5$). IBMX was dissolved in 3.5 mmol/l KOH and was applied to the perfusion medium. Addition of 3.5 mmol/l KOH increased PS amplitude by 158.8 ± 1.9% (column 3) ($n = 4$), and addition of 500 μmol/l IBMX enhanced PS by 198.2 ± 15.8% (column 4) ($n = 4$). *$P < 0.01$ compared with the control amplitude. *B*: Schematic of glial-neuronal metabolic interactions during repeated glucose deprivation. During glucose deprivation, glial monocarboxylates may be transported via monocarboxylate transporters to neurons and used as energetic substrates to support synaptic activity. *C*: The effects of 4-CIN and IBMX on PS amplitude after transient glucose deprivation were evaluated. PS recordings were obtained as described in Fig. 1*A*. Application of 200 μmol/l 4-CIN suppressed by 22.4 ± 3.9% the 10 mmol/l glucose–supported PS amplitude that had recovered after transient glucose depletion. Subsequent removal of extracellular glucose, in the presence of 200 μmol/l 4-CIN, induced a depression in PS amplitude. Reintroduction of 10 mmol/l glucose to the perfusion medium partially restored synaptic transmission (○; $n = 7$). Administration of 500 μmol/l IBMX enhanced PS amplitude by 183.2 ± 11.1%, and subsequent glucose deprivation induced similar decay of PS amplitude (●; $n = 6$).

glucose (17). Indeed, glucose levels were diminished to nearly 0 within 30 min during the initial and the secondary glucose deprivation periods ($P < 0.01$) (Fig. 3*C*). Glucose concentration in slices incubated in secondary hypoglycemic solution containing 0.5 mmol/l glucose also decreased almost entirely within 30 min ($P < 0.01$). These results indicate that glycogenolysis does not support hypoglycemic synaptic adaptation.

Utilization of monocarboxylates contributes to synaptic adaptation in response to repeated hypoglycemia. In the adult brain, monocarboxylates, including lactate, pyruvate, and β-hydroxybutyrate, can be used to produce high-energy phosphates (14,15,18–20) and protect against neuronal damage after hypoxic or ischemic insult (21,22). In the immature brain, monocarboxylates can be used to fuel synaptic transmission during periods of glucose deprivation (23). Therefore, we investigated whether monocarboxylates can be used to sustain synaptic activity in response to repeated hypoglycemia (Fig. 4*B*), using monocarboxylate transporter (MCT) inhibitors 4-CIN, IBMX, and phloretin (21). 4-CIN prevents monocarboxylate utilization by inhibiting the mitochondrial pyruvate carrier as well as by inhibiting the plasma membrane MCT. First, we tested the effect of 200 μmol/l 4-CIN on 10 mmol/l glucose-supported PS (Fig. 4*A*). Whereas 0.1% DMSO used for dissolving of 4-CIN did not affect PS amplitude (Fig. 4*A*, column 1), application of 200 μmol/l 4-CIN suppressed PS amplitude by 43.0 ± 6.8% (column 2) ($P < 0.01$). In contrast, 10 mmol/l glucose–supported PS recovered from transient glucose deprivation was suppressed by 22.4 ± 3.9% after the application of 200 μmol/l 4-CIN ($P < 0.01$) (Fig. 4*C*). After 10 mmol/l glucose–supported PS was stabilized in the presence of 4-CIN, extracellular glucose was removed (Fig. 4*C*). Secondary glucose depletion induced a depression in PS amplitude, which exhibited a similar time course to that observed in response to the initial glucose deprivation. Subsequent addition of 10 mmol/l glucose in the perfusion medium partially restored the PS amplitude.

The effect of IBMX on hypoglycemic synaptic adaptation was then tested. IBMX was dissolved in 3.5 mmol/l KOH, and 500 μmol/l IBMX was applied to the perfusion solution (Fig. 4*A*). Addition of 3.5 mmol/l KOH in standard solution increased the PS amplitude by 158.8 ± 1.9% ($P < 0.01$) (Fig. 4*A*, column 3), and additional administration of 500 μmol/l IBMX increased the amplitude to 198.2 ± 15.8%

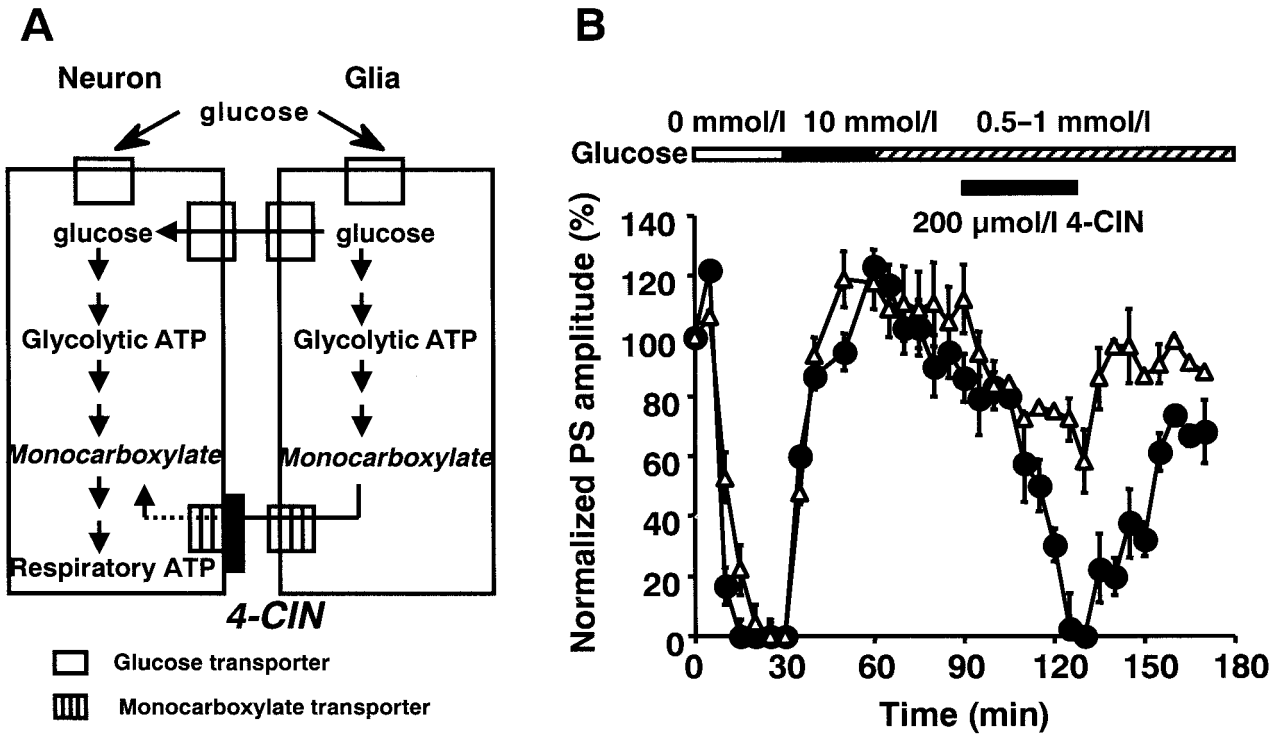

FIG. 5. Utilization of monocarboxylates contributes to synaptic adaptation during repeated hypoglycemia. *A*: Schematic of glial-neuronal interactions during repeated hypoglycemia. After transient glucose deprivation, glial monocarboxylates and extracellular glucose at a concentration of 0.5–1.0 mmol/l may support synaptic activity. *B*: The effect of 4-CIN on PS amplitude maintained in 0.5–1.0 mmol/l glucose was tested. PS recordings were obtained as described in Fig. 1*A*. After transient glucose deprivation, synaptic activity was sustained in secondary hypoglycemic solutions containing 0.5 mmol/l (●; $n = 6$) or 1 mmol/l glucose (△; $n = 6$). Application of 200 μmol/l 4-CIN suppressed PS amplitudes by 100 and 42.6 ± 10.6%, respectively.

of the control amplitude ($P < 0.01$) (Fig. 4*A*, column 4). In hippocampal slices recovered from transient glucose deprivation, 500 μmol/l IBMX in 3.5 mmol/l KOH increased the amplitude to 183.2 ± 11.1% of the control value ($P < 0.01$) (Fig. 4*C*). Secondary glucose depletion in the presence of 500 μmol/l IBMX produced a decay of PS amplitude in a time course similar to that observed in the presence of 200 μmol/l 4-CIN. We also tested the effect of 200 μmol/l phloretin and found it to decrease synaptic activity similar to 4-CIN during secondary glucose depletion (data not shown).

We further examined the effect of 4-CIN on PS maintained in secondary hypoglycemic medium containing 0.5 or 1.0 mmol/l glucose (Fig. 5). After transient glucose deprivation, administration of 200 μmol/l 4-CIN in 0.5 or 1.0 mmol/l glucose solution reversibly suppressed PS amplitudes entirely or by 42.0 ± 10.6%, respectively (Fig. 5*B*).

Finally, we investigated whether exogenous lactate or pyruvate substituted for glucose could sustain synaptic transmission in slices recovered from transient glucose deprivation (Fig. 6). As we reported previously (15,20), replacement of extracellular glucose with Na lactate or Na pyruvate did not maintain synaptic activity (Fig. 6*B*). However, in hippocampal slices recovered from transient hypoglycemia, subsequent replacement of glucose with 10 mmol/l Na lactate or 10 mmol/l Na pyruvate did result in the maintenance of synaptic activity (Fig. 6*B*). These results indicate that synaptic adaptation to repeated hypoglycemia depends on the utilization of monocarboxylates in guinea pig hippocampal neurons.

DISCUSSION

In the present study, we report *1*) a unique synaptic phenomenon whereby synaptic transmission becomes resistant to repeated hypoglycemia, i.e., hypoglycemic synaptic adaptation occurs; *2*) hypoglycemic synaptic adaptation is not dependent on glycogenolysis as a metabolic substrate; and *3*) synaptic utilization of monocarboxylates sustains synaptic function during repeated hypoglycemia. Adaptation of brain function is frequently postulated to explain the deteriorated neuroendocrine and neurobehavioral responses to recurrent hypoglycemia (3–5). To our knowledge, however, this is the first report showing that synaptic function is able to adapt to repeated hypoglycemia in hippocampus dissociated from cerebral vessels.

Physiological significance of synaptic adaptation to hypoglycemia in the hippocampus. In this study, we demonstrated hypoglycemic synaptic adaptation in the granule cell layer of hippocampal dentate gyrus from the guinea pig brain. The hippocampus is particularly sensitive to hypoglycemia, as is the neocortex and the striatum, which have not been thought to adapt to hypoglycemia. In contrast, the hypothalamus, which contains glucose-sensing areas, and the thalamus, brainstem, and cerebellum are more resistant to hypoglycemia. It should be noted that synaptic activity in hypoglycemia-sensitive tissues is served by a protective mechanism against repeated hypoglycemia. Similar observations were reported recently by Hasuo et al. (24), who used optical recordings. In rat brain sections containing lateral septum and hippocampal area

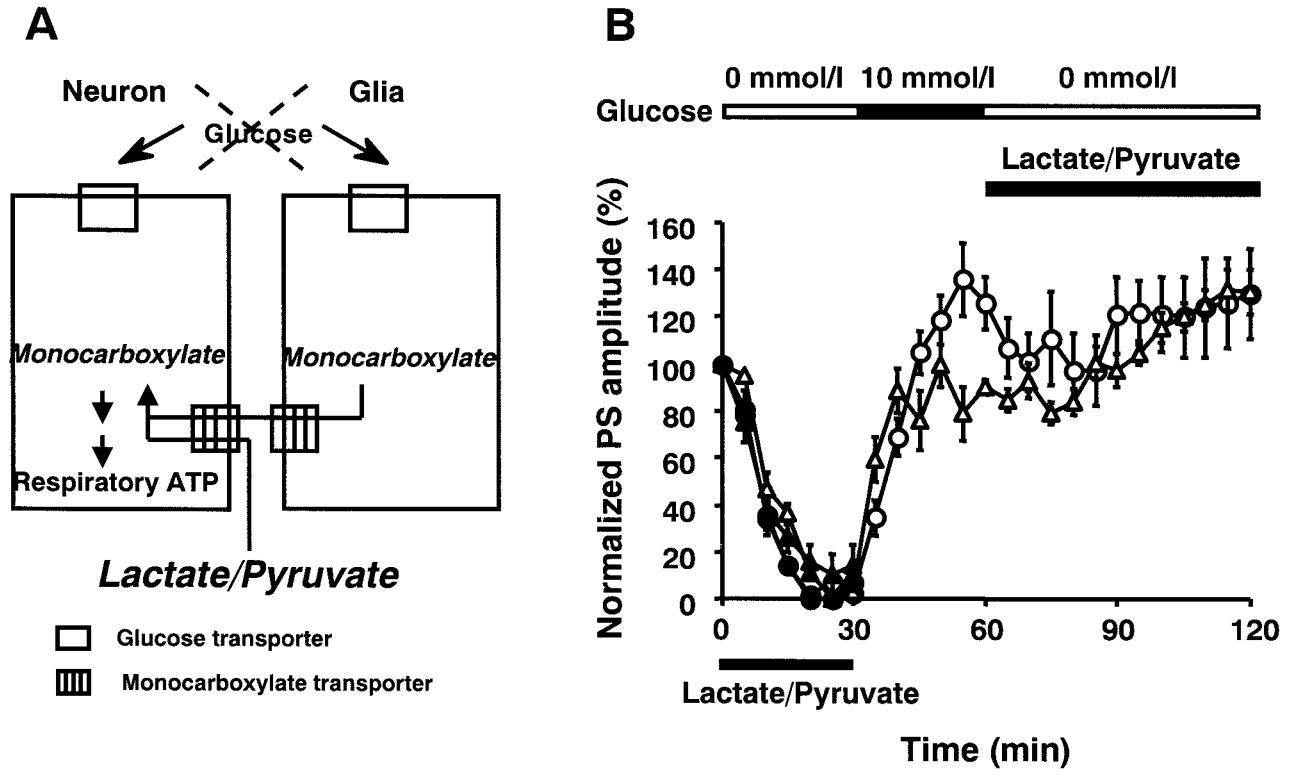

FIG. 6. Replacement of exogenous glucose with Na lactate or Na pyruvate maintains synaptic function after transient glucose deprivation. *A*: Schematic of glial-neuronal metabolic interactions after temporary glucose deprivation. Glial monocarboxylates and exogenous lactate or pyruvate may support synaptic activity. *B*: The effect of replacement of glucose with lactate or pyruvate on PS amplitude was tested. PS recordings were obtained as described in Fig. 1*A*. Replacement of glucose with 10 mmol/l Na lactate (●; $n = 10$) or 10 mmol/l Na pyruvate (▲; $n = 7$) depressed PS amplitude within 30 min. In contrast, after transient glucose deprivation, replacement of glucose with 10 mmol/l Na lactate (○; $n = 8$) or 10 mmol/l Na pyruvate (△; $n = 7$) resulted in maintained synaptic activity for >60 min.

CA1, the initial exposure to exogenous glucose depletion suppressed synaptic activity, whereas activity remained unchanged in response to repeated hypoglycemia. Our findings, together with the observations of Hasuo et al., indicate that hypoglycemic synaptic adaptation is not a phenomenon specific to the hippocampal dentate gyrus, but rather is a synaptic phenomenon distributed perhaps throughout the central nervous system.

Preservation of high-energy phosphates during synaptic adaptation to hypoglycemia. Previous in vitro experiments have shown that hypoxia-induced inhibition of synaptic transmission is associated with a 15–25% decline in ATP in brain slices (25,26). In our experiments, initial glucose deprivation decreased ATP and PCr concentrations by ~15%, together with a complete decay of synaptic activity (Fig. 1). Our data indicate that synaptic function in the hippocampal dentate gyrus usually depends on exogenous glucose as an energetic substrate. However, in response to secondary glucose deprivation, high-energy phosphates of ATP and PCr were preserved during >120 min of observation, indicating that hippocampal neurons switch to an alternative endogenous fuel source during hypoglycemia. Glycogen or glucose does not seem to be used as an endogenous substrate, as demonstrated in Fig. 3. Other readily available substrates could be provided by citric acid cycle intermediates or free amino acids. However, the majority of these substrate pools are used during the first 5 min of severe hypoglycemia (27,28). Previous studies provided evidence that severe hypoglycemia induces a significant breakdown of phospholipids, concomitant with an increase in free fatty acids (28,29). The brain contains the enzyme systems required for metabolism of a range of different carbohydrate and lipid substrates (28). Substrates for these systems may serve as yet-undiscovered metabolic fuels during hypoglycemic synaptic adaptation.

Despite the sustained level of high-energy phosphates, synaptic activity was gradually eliminated during secondary glucose deprivation. In contrast, addition of 0.5 mmol/l glucose in the circulating medium after transient glucose deprivation sustained synaptic function and preserved high-energy phosphate levels (Fig. 2*B*). Alternative endogenous substrates were presumably used for production of high-energy phosphates. However, alteration of structural components, in addition to the absence of exogenous glucose, may result in the loss of synaptic activity.

Involvement of an astrocyte-neuron monocarboxylate shuttle in synaptic adaptation to hypoglycemia. Our results indicate that neurons use monocarboxylate as a main energetic substrate to sustain synaptic function during repeated hypoglycemia. We and others have reported that replacement of exogenous glucose with lactate, pyruvate, or β-hydroxybutyrate fails to maintain synaptic function (14,15,19,20,30,31). However, monocarboxylate (lactate or pyruvate) was able to maintain synaptic activity during secondary hypoglycemia (Figs. 4–6). These results suggest that temporary glucose deprivation triggers the utilization of monocarboxylates to sustain synaptic activity during hypoglycemic synaptic adaptation.

The concept of metabolic coupling between astrocytes

and neurons has been investigated for decades and explains the metabolic compartmentation that occurs between astrocytes and neurons. In the honeybee retina, it has been demonstrated that glucose taken up by astrocytes from capillaries is converted glycolytically to lactate, which is then released into the extracellular space to be utilized by photoreceptor neurons during photostimulation (32). In mammalian neuronal tissues, there is accumulating evidence to support the hypothesis of metabolic compartmentation (21,32,33). The specific subcellular distribution of MCTs and lactate dehydrogenase (LDH) isoforms (LDH_1 and LDH_5) between neurons and astrocytes supports the molecular aspects of the astrocyte-neuron monocarboxylate shuttle (32). In the adult rat brain, eight MCT isoforms have been identified with differential affinities for monocarboxylates (21). LDH_5, which is present in tissues that produce lactate glycolytically, is localized predominantly to astrocytes, and LDH_1, which is enriched in tissues that use lactate as a substrate, is distributed mainly in neurons. These observations are consistent with the concept of lactate production by astrocytes and lactate utilization by neurons. The physiological prevalence of the astrocyte-neuron monocarboxylate shuttle may contribute to hypoglycemic synaptic adaptation.

Clinically, hypoglycemic unawareness is a complication of diabetes, especially in aged patients with cognitive deficits (1,2,34). In hypoglycemia unawareness, previous episodes of hypoglycemia cause delayed and diminished symptomatic and hormonal responses to subsequent hypoglycemia. Similar defects of neurohormonal responses to hypoglycemia are induced by intravenous infusion of lactate during hypoglycemia in nondiabetic individuals (35,36). These results suggest the possible involvement of lactate in hypoglycemic unawareness. However, the neural basis of brain adaptation to recurrent hypoglycemia is still unclear. The possible contribution of hypoglycemic synaptic adaptation to brain adaptation in response to recurrent hypoglycemia and the molecular mechanism underlying the activation of an astrocyte-neuron monocarboxylate shuttle after transient glucose deprivation will be addressed in future studies.

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