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FOOD INTAKE TAEES FACILIATES BRAIN PLASTICITY

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ПРИЕМ ПИЩИ СПОСОБСТВУЕТ ПЛАСТИЧНОСТИ ГОЛОВНОГО МОЗГА

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To define how extracellular glucose levels affect synaptic efficacy and long-term potentiation (LTP), we evaluated electrophysiological and neurochemical properties in hippocampal CA1 region following alterations in glucose levels in the ACSF with 3,5 mM glucose, fEPSPsgenerated by Schaffer collateral/commissural stimulation markedlyincreased when ACSF glucose levels were increased from 3,5 to 7,0 mM. The paired-pulse facilitation reflecting presynaptic transmitter release efficacy was significantly suppressed by elevation of 7,0 mM glucose indicating the increase of the presynaptic transmitter release. Single pulse stimulation of presynaptic terminals also shows the increase of fEPSP amplitudes. Prolonged potentiation of fEPSPs by elevation of 7 mM glucose coincided with increased autophosphorylation both Ca ions/calmodulin dependent protein kinase II(CaMKII) and protein kinase C (PKC α). The increased I/O relationship of fEPSPs was also associated with markedly increased synapsin Iphosphorylation by CaMKII. Transmitter-evoked postsynaptic currents were also measured in CA1 neurons by electrophoretic application of NMDA anAMPA by elevation to 7,0 mM. Notably high frequency stimulation of the Schaffer collateral/commissural pathway failed to induce LTP in the CA1region at 3,5 mM glucose but LTP was restored dose dependently by increasing glucose levels to 7,0 mM and l0mM. LTP induction in the presence of 7,0 mM glucose was closely associated with further increase in CaMKII autophosphorylation without changes in PKC α autophsphorylation. Taken together, CaMKII and PKC activation likely mediate potentiation of fEPSPsby elevated glucose levels, and CaMKII activity is also associated with LTP Induction in the hippocampal CAl region.
Key words: Glucose concentration in the brain before and after eating, NMDA, CaMKII, LTP (Long'— term potentiation), Plastic cognitive function.

С целью определить как уровень внеклеточной глюкозы влияет на эффективность синаптической передачи и ее долговременное потенцирование (ДВП) мы исследовали электрофизиологические и нейрохимические свойства области CA1 гиппокампа после изменения уровня глюкозы в искусственной цереброспинальной жидкости (ИЦСЖ) с концентрацией глюкозы 3,5 мМ. Возбуждающие постсинаптические потенциалы (ВПСП) при стимуляции коллатерали Шаффера/комиссурального пути значительно усиливались повышением уровня глюкозы с 3,5 до 7,0 мМ. Облегчение синаптической передачи спаренными импульсами, отражающее эффективность пресинаптического высвобождения трансмиттеров существенно подавлялось повышение уровня глюкозы до 7,0 мМ, что указывает на усиление пресинаптического высвобождения трансмиттеров. Стимуляция пресинаптических терминалей единичными импульсами продемонстрировало увеличение амплитуды ВПСП. Длительное потенцирование ВПСП повышением глюкозы до 7,0 мМ совпадало с повышением аутофосфорилирования как зависимой от ионов для Са и от кальмодулина протеинкиназы II (CaMKII), так и протеинкиназы С (PKCα). Увеличение соотношения сигнал/ответ (С/О) у ВПСП также было связано со значительным повышением фосфорилировапния синапсина I под действием CaMKII. Вызываемые трансмиттером постсинаптические токи в нейронах CA1 при электрофоретическом нанесении N-метил-D-аспартата (NMDA) и 2-амино-3-(5-метил-3-оксо-1,2-оксазол-4-ил) пропаноевой кислоты (AMPA) также усиливались при повышении глюкозы до 7,0 мМ. Примечательно, что высокочастотная стимуляция коллатерали Шаффера/комисурального пути не приводила к индукции ДВП в области CA1 при 3,5 мМ глюкозы, но индукция восстанавливалась в зависимости от уровня глюкозы при повышении до 7,0 и 10,0 мМ. Индукция ДВП в присутствии 7,0 мМ глюкозы было тесно связано с дальнейшим увеличением аутофосфорилирования без изменений в аутофосфорилировании PKCα. По совокупности полученных результатов, вероятно, что активация CaMKII и PKCα опосредует потенциирование ВПСП повышенными уровнями глюкозы и что активность CaMKII также связана и индукцией ДВП в области CA1 гиппокампа.
Ключевые слова: концентрация глюкозы в головном мозге до и после приема пищи, NMDA, CaMKII, долговременное потенцирование, пластичность когнитивных функций.

Introduction. It is widely recognized that glutamate acting via the N-methyl-D-aspartate receptor (NMDAR) and AMPAR accounts for excitatory j synaptic transmission in the mammalian brain. NMDAR is involved in various physiological processes including synaptic plasticity, learning, and memory [1, 2]. LTP induction is a state of increasing synaptic transmission following high frequency stimulation and is known to be a model for neuronal events underlying learning and memory [1, 3]. Long-term synaptic plasticity is regulated by NMDAR function in the CA1 region of the hippocampus [1, 4]. A critical role for activation of calcium/calmodulin-dependent protein kinase II (CaMKII) in LTP induction has been established [5–10]. CaMKII is highly enriched in postsynaptic densities of excitatory synapses and becomes constitutively active through autophosphorylation, thereby increasing synaptic efficacy [7, 10–14, Lledo et al., 1995; Wang and Kelly, 1995; Barria et al., 1997; Giese et al.] Facilitating synaptic efficacy by CaMKII reguires up-regulation of postsynaptic AMPAR function by direct phosphorylation [13, 15] and trafficking of AMPAR into postsynaptic membranes [16–18].

Like CaMKII, protein kinase C (PKC) is also essential for hippocampal LTP induction [19]. Phosphorylation of NR1 mediated by PKC accounts for up-regulation of NMDAR function and is likely required for CaMKII-dependent LTP enhancement [20]. Although extracellular glucose is essentialfor synaptic transmission to supply energy through ATP, there is little known regarding levels of glucose required for LTP induction.

Experimental methods

Animals. All animals were male Wistar rats obtained from Japan SLC, Inc. They were housed in controlled air and light conditions at a room temperature of 28±1° C. Lights were on from 7:00 to 19:00, and food and water were administered ad libitum. Animals and procedures used were approved by the Animal Care and Committees of Tohoku and Kyushu Universities.

Electrophysiology. Brains were rapidly removed from ether-anesthetized male Wistar rats (7–8 weeks old) and hippocampi were dissected out. Transverse hippocampal slices (400 pm thick) prepared using a vibratome (microslicer DTK-1000) were incubated for 2 h in continuously oxygenized (95% O_2, 5% CO_2) artificial cerebrospinal fluid (ACSF) containing 126 mM NaCl, 5 mM KC1, 26 mM $NaHCO_3$, 1,3 mM $MgSO_47H_2O$, 1,26 mM KH_2PO_4, 2,4 mM $CaCl_22H_2O$ and 3,5 mM glucose at room temperature. After a 2h recovery period, a slice was transferred to an interface recording chamber and perfused at a flow rate of 2 ml/min with ACSF warmed to 34° C. Field EPSPs were evoked by a 0,05 Hz test stimulus through a bipolar stimulating electrode placed on the Schaffer collateral/commissural pathway and recorded from the stratum radiatum of CA1 using a glass electrode filled with 3 M NaCl. Recording was performed using a single-electrode amplifier (CEZ-3100, Nihon Kohden, Tokyo, Japan), and the maximal value of the initial fEPSP slope was collected and averaged every 1 min (3 traces) using an A/D converter (PowerLab 200; AD Instruments, Castle Hill, Australia) and a personal computer. After a stable baseline was obtained, high frequency stimulation (HFS) of 100 Hz with a 1-s duration was applied twice with a 10-s interval, and test stimuli were continued for the indicated periods. Postsynaptic potentials recorded intracellularly from hippocampal CA1 neurons using a glass pipette electrode of 0,1 pm tip diameter (DC resistance,150 MO) filled with 1 M potassium acetate were evoked by separate microelectrophoretic applications of quisqualic acid (AMPA) (10 mM in 150 mM NaCl, pH 7,5) and NMDA (10 mM in 150 mM NaCl, pH 7,4) through a multi-barrel array to the distal apical dendrites of the same neuron. Slices were then transferred to a plastic plate on ice to dissect out the CA1 region under a microscope. CA1 regions were frozen in liquid nitrogen and stored at — 80° C until analysis.

Immunoblotting analysis. Hippocampal CA1 samples were homogenized in 70 pl of homogenizing buffer containing 50 mM Tris-HCl (pH 7,4), 0,5% Triton X-100, 4 mM EGTA, 10 mM EDTA 1 mM Na_3VO_4, 40 mM sodium pyrophosphate, 50 mM NaF, 100 nM calyculin A, 50 pg/ml leupeptin, 25 pg/ml pepstatin A, 50 pg/ml trypsin inhibitor and 1 mM dithiothreitol (DTT). Insoluble material was removed by a 10-min centrifugation at 15 000 rpm. After determining protein concentration in supernatants using Bradford's solution, samples were boiled 3 min in Laemmli's sample buffer (Laemmli, 1970). Samples containing equivalent amounts of protein were subjected to SDS-polyacrylamide gel electrophoresis. Proteins were transferred to an Immobilon PVDF membrane for 2 h at 70 V. After blocking with TTBS solution (50 mM Tris-HCl, pH 7,5, 150 mM NaCl, and 0,1% Tween 20) containing 2,5% bovine serum albumin for 1 h at room temperature, membranes were incubated overnight at 4° C with anti-phospho CaMKII, (1:5000, Fukunaga et al., 1988), anti-CaMKII, (1:5000, 6) anti-phospho-synapsin I (Ser-603) (1:2000, Chemicon, Temecula, CA, USA), anti-synapsin 1 (1:2000, Fukunaga et al., 1992), anti-phospho- GluRl (Ser-831) (1:1000, Upstate, Lake Placid, MA, USA), anti-GluRl (1:1000, Chemicon), anti-phospho-PKCα (1:2000, Upstate),

anti-phospho-MARCKS (Ser-152/156) (1:2000, Chemicon), anti-MARCKS (1:2000, Ohmitsu et al., 1999), anti-phospho-NRl (Ser-896) (1:2000, Upstate), anti-NRl (1:2000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-phospho-DARPP-32 (Thr-34) (1:2000, IMGENEX, San Diego, CA,USA), or anti-β-tubulin (1:2000, Sigma). Antibody signals were visualized using the enhanced chemiluminescence detection system (Amersham Life Science, Buckinghamshire, UK) and analyzed semi- quantitatively using the National Institutes of Health Image program.

Results

Increased extracellular glucose enhances fEPSPs and presynaptic transmitter release in the hippocampal CA1 region. Hippocampal slices were prepared in the presence of 3,5 mM glucose and perfused in ACSF with 3,5 mM. When hippocampal slices were exposed to 7,0 or 10 mM glucose for 15 min, the slope of evoked fEPSPs in the CA1 region gradually increased and remained elevated (30 min: 367,4±62,8% of baseline at 7,0 mM; 60 min: 379,4±58,8% of baseline at 10 mM, n=4 from 4 rats) (Fig. 1*a*). This augmentation was maintained for more than 30 min, even after returning to 3,5 mM glucose in the perfusion medium. We next examined a potential presynaptic effect of increased extracellular glucose using a paired- pulse facilitation method in hippocampal CA1 region [22, 23]. As shown in Fig. 1*b*, the ratios of fEPSPs with various interpulse intervals (b/a in the figure) significantly decreased when slices were exposed to 7,0 mM glucose and were then partly restored after a return to 3,5 mM glucose at 30 min (Fig. 1*b*). Reduction in the ratio fEPSPs by glucose elevation is likely due to an increase in evoked fEPSP by the first stimulation. Thus, the ratio of the second to first fEPSPs was slightly decreased by 7,0 mM glucose exposure. To confirm changes in presynaptic parameters, the input-output relationship (I/O relationship) was assessed at 3,5 and 7,0 mM glucose. The amplitude of fEPSPs at 3,5 mM glucose was saturated by a stimulus intensity of more than 0,6 mA, whereas at 7,0 mM glucose the I/O ratio showed a linear increase up to 1,0 mA (Fig. 1*c*).

Increased extracellular glucose dose-dependently enhances LTP at Schaffer collateral CAI synapses. We next examined the effect of extracellular glucose on hippocampal LTP induction. High frequency stimulation (HFS) of Schaffer collateral/commissural pathways in the presence of 3,5 mM glucose failed to

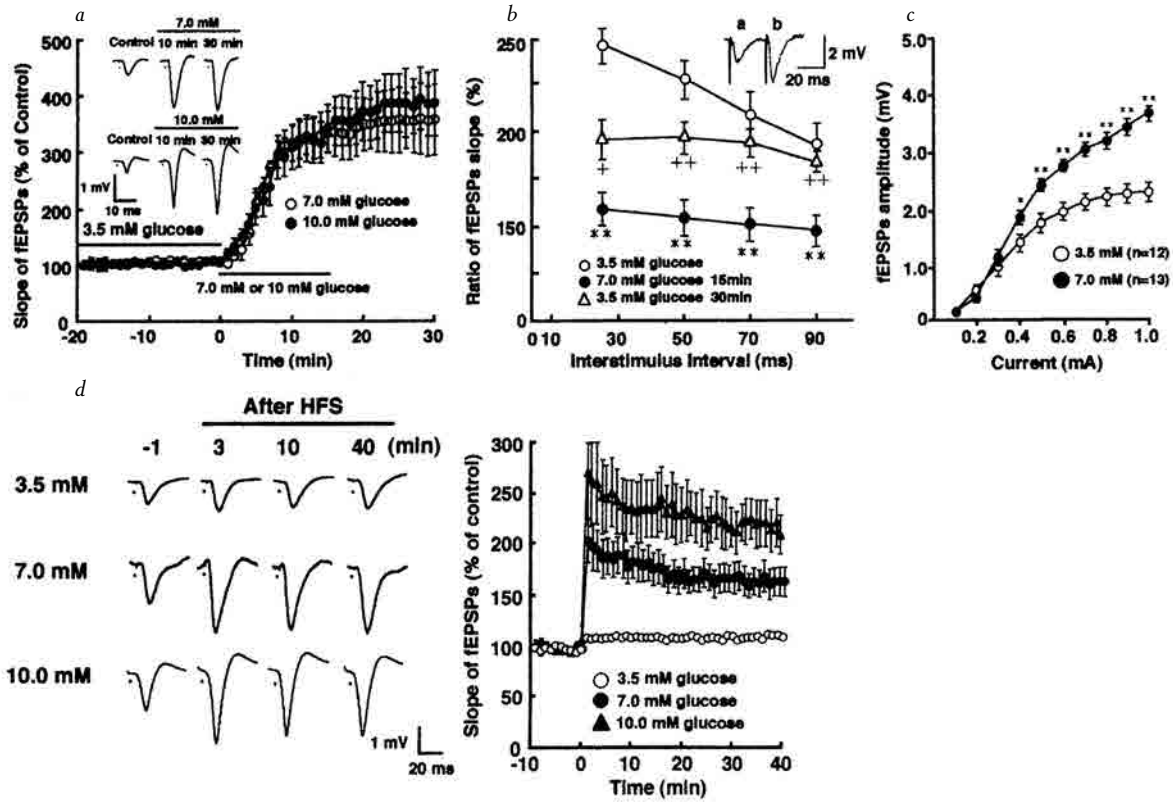

Fig. 1. Enhancement of*fEPSPs in the hippocampal CAl region by a two-fold increase in extracellular glucose and changes in the ratio of fEPSP slope foliowfing HFS. Aj changes in slope of fEPSPs by increasing extracellular glucose from 3,5 to 7 or 10 mM. *b* — presynaptic effect of extracellular glucose alterations evaluated by paired-pulse experiment. C3 in the 7,0 or 10,0 mM extracellular glucose and analysis of extracellular glucose level from 3,5 to 7 or 10,0 mM just prior to HPS for 15 min. E p 30,05, p 50,01 versus 3,5 mM glucose, +, p<0,05, ++, p<0,01 versus 7,0 mM glucose.

induce LTP (at 40 min after stimulation: 110,0±1,6% of baseline, n=4 from4 rats) (Fig. 1*d*). By contrast, after exposure to 7,0 or 10 mM glucose, HFS dose-dependently induced stable LTP in the hippocampal CA1 region, which lasted more than 40 min (162,9±14,7% of baseline by 7,0 mM and 208,3±19,3% of baseline by 10 mM, n=4 from 4 rats) (Fig. 1*d*).

Increased extracellular glucose alters postsynaptic responses to NMDA and AMPA in hippocampal CA1 neurons. We next employed a whole-cell recording technique to determine which receptor-ion channels were affected by changing from 3,5 to 7,0 mM glucose. When NMDA and AMPA were applied to the apical dendrites of the same neuron in CA1, the NMDA (n=4 from 4 rats) and AMPA (n=4 from 4 rats) responses were clearly enhanced concomitant with increases in the repetitive firing rate after exposure to 7,0 mM glucose (NMDA: 131,7±5,6% of baseline by 7,0 mM; AMPA 142,6±10,9% of baseline by 7,0 mM) (Figs. 2*a* and *c*). The firing rate did not return to basal levels after re-exposure to 3,5 mM glucose (Figs. 2*a* and *c*). At the same time, both resting membrane potentials and resistance were not affected by increased glucose (Fig. 2*b*).

Autophosphorylation of CaMKII and PKCα in the hippocampal CA1 region is modulated by extracellular glucose levels. To test effects of extracellular glucose levels on protein kinase activities, hippocampal slices prepared in 3,5 mM glucose were exposed to 2,0, 7,0, or 10,0 mM glucose for 15 min. Lysates from slices were then prepared and evaluated for autophosphorylation of CaMKII or PKCα and phosphorylation of dopamine- and cyclic AMP-regulated phosphoprotein with molecular weight32 kDa (DARPP-32) (Thr-34) by immunoblot analyses using phospho-specific antibodies.We found that when extracellular glucose was increased from 3,5 mM to 7,0 or 10,0 mM, autophosphorylation of CaMKII and PKCα significantly increased (CaMKII: 144,5±8,6%, n=8 from 4 rats (7,0 mM); 150,4±9,1%, n=8 from 4 rats (10,0 mM)) (PKCα: 153,1±15,1%, n=8 from 4 rats (7,0 mM); 169,8±7,8%, n=8 from 4 rats (10,0 mM)) (Figs. 3*a* and *b*), whereas DARPP-32 (Thr-34) phosphorylation remained unchanged. On the other hand, when extracellular glucose was decreased from 3,5 mM to 2,0 mM, CaMKII and PKCα autophosphorylation still increased (CaMKII: 123,2±8,9%, n=8 from 4 rats; PKCα:119,6±7,2%, n=8 from 4 rats) (Figs. 3*a* and *b*) without changes in DARPP-32 (Thr-34) phosphorylation. Increased CaMKII and PKCα phosphorylation following exposure to 2 mM glucose is nkelygue ro elevation of intracellular calcium promoted by hypoglycemia. However, stable fEPSPs could not be induced under conditions of 2,0 mM glucose because of ATP depletion.

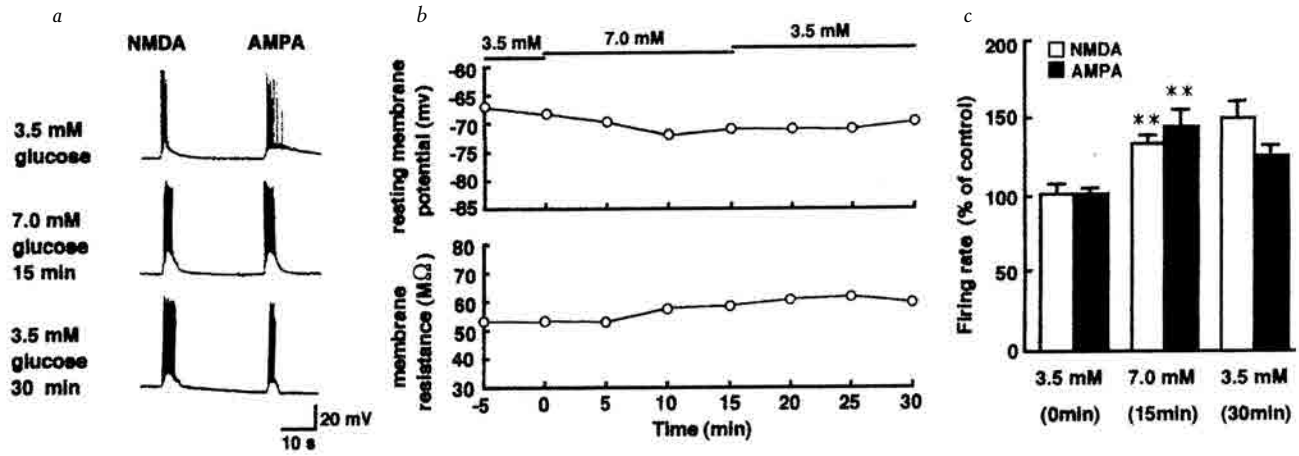

Fig. 2. Enhanced NMDA response evoked by a two-fold increase in extracellular glucose. *a* — typical postsynaptic responses to electrophoretic application of neurotransmitters to apical dendrites of hippocampal CAl neurons. Shown are responses to applied NMDA (10 nM, for 1 s) and AMPA (20 hM, for 1 s) in the presence of differing extracellular glucose levels and measured as postsynaptic response in the same neuron. *b* — recording of membrane potential (mean -67,8+5,0 mV, n=10) and membrane resistance (mean± 57,8±8,0 MQ, n=10) at various glucose levels in CA 1 neurons. *c*— quantitative analyses of firing rate responses to applied NMDA (10 nM, for 1 s) and AMPA (20 nM, for 1 s) in the presence of altered glucose levels and measured as postsynaptic responses in the same neuron (n=4),measured postsynaptic responses in the same neuron (n=4), ** p<0,01 versus 3,5 mM glucose.

Increased CaMKII but not PKC autophosphorylation is associated with LTP induction in hippocampal CA1 at 7,0 mM glucose. Since CaMKII and PKC activities are essential for LTP induction (Fukunaga et al., 1993; Collingridge et al., 2004) and PKCα is the majorPKC isoform expressed in hippocampus [24] weassessed CaMKII and PKCα autophosphorylations before or after LTP induction in 3,5 and 7,0 mM glucose. Under basal conditions at

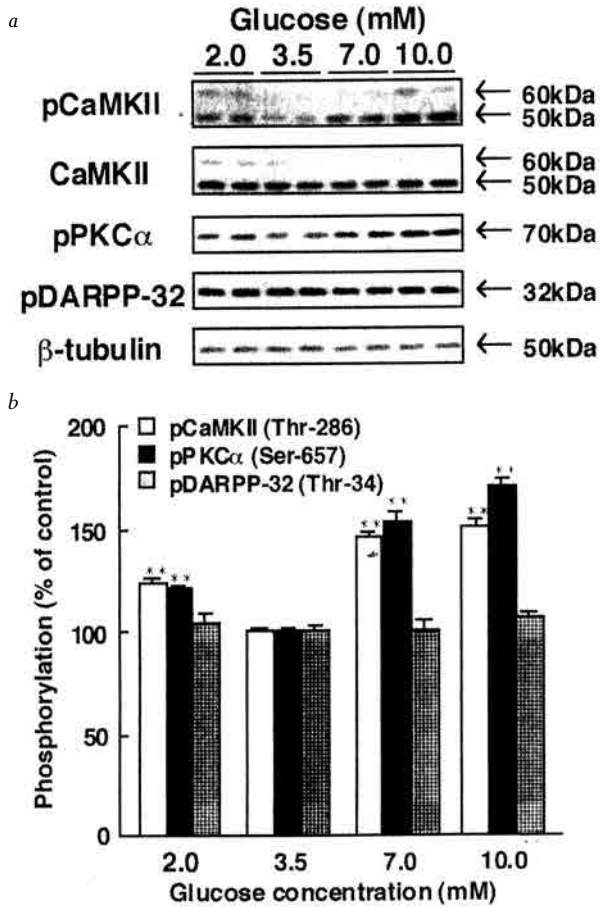

Fig. 3. Increased or decreased extracellular glucose significantly augments CaMKII and PKC activities. *a* — CAl slices were prepared in 3,5 mM glucose (control) and exposed to either 2,0, 7,0 or lOmM glucose nd then analyzed by immunoblotting with various antibodies. Representative immunoreactive signals are shown. CaMKII antibodies detected both a and ¡3 isoforms. *b* — summary of signals densities normalized for each antibody using values from CAl slices. Data were obtained from eight slices and represent means + SEM. ** p<0,01 in controls versus 3,5 mM glucose.

3,5 mM glucose, HFS failed to increase CaMKII and PKCα autophosphorylations, in agreement with the lack of LTP induction. After exposure to 7,0 mM glucose, CaMKII autophosphorylation significantly increased without changes in total CaMKII protein level (147,3±8,5% of baseline, n=9 from 4 rats) as shown in Fig. 3. LTP induction further enhanced CaMKII autophosphorylation (166,9±12,0% of the control, n=9 from 4 rats) at 7,0 mM glucose (Figs. 4*a* and *b*).

On the other hand, PKCα autophosphorylation significantly increased following exposure to 7,0 mM glucose (158,1±7,4% of 'baseline, n=9 from 4 rats) (Figs. 4*a* and *b*). However, a further. Increase in PKCα autophosphorylation was not apparent after HFS at 7,0 mM glucose.

Since protein kinase A (PKA) activity contributes to LTP induction [25], we examined phosphorylation of the PKA substrateDARPP-32. As shown in Figs. 4*a* and *b*, DARPP-32 phosphorylation at 3,5 mM glucose was unchanged at 7,0 mM glucose and remained unchanged after HFS in either condition. Taken together, these findings indicate that PKA does not mediate glucose-induced synaptic potentiation and LTP enhancement and that PKC likely mediates glucose-induced synaptic potentiation but not LTP enhancement, whereas CaMKII is required both for glucose-induced synaptic potentiation and LTP enhancement.

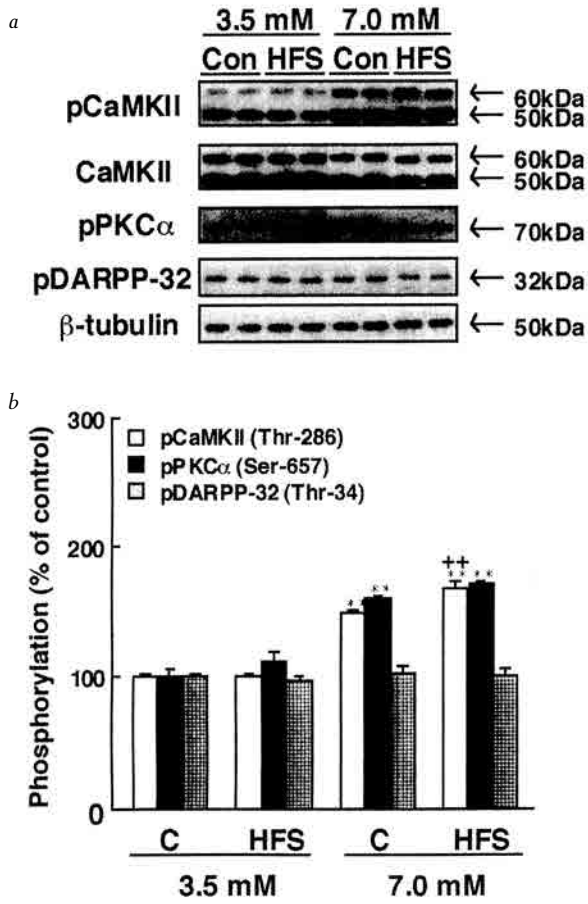

Fig. 4. Analysis of phosphoproteins in hippocampal CAl slices at 3,5 and 7 mM glucose. *a* — CAl slices were prepared before HFS (control) or 50 min after HPS and analyzed by rmmunoblotting of lysates. Representative immunoreactive signals are shown. CaMKII antibodies detected both a and /3 isoforms. *b* — summary of signal densities normalized for each antibody using values from CAl slices at 3,5 mM glucose without HFS treatment. Data were obtained from at least four slices and represent means ±SEM.

Increased CaMKII-regulated AMPAR phosphorylation is associated with LTP induction in hippocampal 'CAl at 7,0 mM glucose. To analyze enhanced synaptic efficacy and LTP by 7,0 mM glucose exposure in the hippocampal CAl region, we assessed phosphorylation of CaMKII and PKC substrates using phospho-specific antibodies. Consistent with the failure of LTP induction under these conditions, HFS did not sti-

mulate GluRl (Ser-831) or synapsin I (Ser-603) phosphorylation at 3,5 mM glucose. However, GluRl (Ser-831) and synapsin I (Ser-603) phosphorylations were increased by 7,0 mM glucose exposure (GluRl: 149,8±11,4% of the control; synapsin I: 200,0±12,3% of the control, n=9 from 4 rats; synapsin I (Ser-603), n=9 from 4 rats) (Figs. 5*a* and *b*). Importantly, GluRl phosphorylation was further enhanced by HFS (182,3±20,4% of the control, n=9 from 4 rats) with concomitant LTP enhancement (Figs. 5*a* and *b*), whereas synapsin I (Ser-603) phosphorylation showed no further increase following HFS at 7,0 mM glucose (209,7±12,6% of the control, n=9 from 4 rats).

and NR1 (Ser-896) at 7,0 mM glucose (Figs. 4*a*, *b*, 5*c* and *d*). Thus, increased GluRl (Ser-831) phosphorylation is relevant for LTP induction at 7,0 mM glucose, while PKCα but not PKA activity likely mediates enhanced synaptic efficacy seen in the presence of elevated glucose levels.

Effects of CaMKII, PKC and PKA inhibitors on glucose-induced enhancement of fEPSPs and LTP induction in the hippocampal CA1 region. To confirm a causal relationship between increased CaMKII and PKCα phosphorylations and enhancement of fEPSPs or LTP, we tested the effects of CaMKII, PKC or PKA inhibitors. Treatment of hippo-

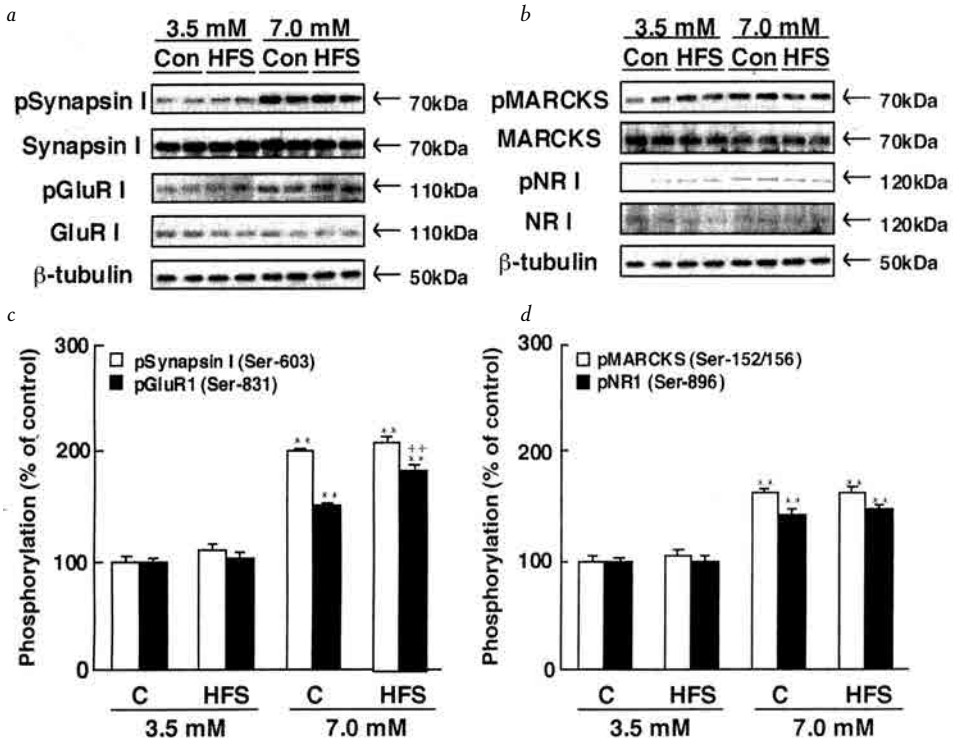

Fig. 5. Analysis of downstream targets of phosphoproteins in hippocampal CA1 slices at 3,5 and 7,0 mM glucose. *a* — CA1 slices were prepared before HPS (control) or 50 min after HPS and analyzed as described above. Representative immunoblots are shown. *b* — KN-93 complsummary of signal densities normalized for each antibody using values from CA1 slices at 3,5 mM glucose without HFS treatment. *c* — CA1 slices were prepared before HFS (control) or 50 min after HFS and analyzed as described. *d* — summary of signal densities normalized as described Data were obtained from at least four slices and represent mean±SEM. ** p<0. 01 in controls versus 3,5 mM glucose without HFS treatment; $^{++}$ p<0,01 versus 7,0 mM glucose without HFS treatment.

We next examined phosphorylation of MARCKS (Ser-152/156) and NR1 (Ser-896), which are PKC-regulated phosphoproteins, following 7,0 mM glucose -exposure and HFS. Phosphorylation of both increased after exposure to 7,0 mM glucose without changes in respective protein levels, as shown in Figs. 5*c* and *d* (MARCKS, 161,5±11,2% of the control, n=9 from 4 rats; NR1,142,5±12,4% of baseline, n=9 from 4 rats). However, HFS sufficient to induce LTP failed to further increase PKCα autophosphorylation and phosphorylation of MARCKS (Ser-152/156)

campal slices with a combination of KN-93 (1 pM), a CaMKII inhibitor, and chelerythrine (3 pM), a PKC inhibitor, totally abolished 7,0 mM glucose-induced fEPSP enhancement (30 min: 97,6±3,6%;of baseline, n=4 from 4 rats), whereas treatment with Rp-cAMPs (100 pM), a pp/V inhibitor, failed to block enhancement of fEPSPs in the hippocampal CA1 region (30 min: 344,7±40,9% of baseline, n=4 from 4 rats) (Fig. 6*a*). Likewise, pretreatment of hippocamphl slices with KN-93 (lpM) 10 min before HFS completely blocked LTP induction in the presence of 10 mM glucose in

CA1 (at 40 min after stimulation: 103,4±4,7% of the baseline, n=4 from 4 rats) (Fig. 6*b*).

Discussion

Experiments in humans and rodents [26–29] demonstrate that brain glucose levels play a critical role in memory function. Although neuronal intracellular glucose levels are tightly controlled by glucose transport through surrounding astrocytes, extracellular glucose levels fluctuate with blood levels. In human subjects undergoing surgery, extracellular glucose levels fluctuate from 0,8 mmol/1 at normal blood glucose (5,5 mmol/1), to 1,6 mmol/1 at high blood glucose (11,5 mmol/1) and 0,3 mmol/1 at low blood glucose (3,0 mmol/1) [30]. More dramatic changes in extracellular glucose levels from 2,1 to 8,7 mmol/1 occur in the brains of Sprague-Dawley rats in conditions ranging from normal to hyperglycemic [31]. Interestingly, in Sprague-Dawley rats, investigators have observed a rapid, 30% reduction in hippocampal extracellular glucose within 5 min of the start of an alternation task in a four-arm maze, and glucose levels rapidly return to the basal values at the end of behavioral task [32]. However, the pathophysiological relevance of such glucose level fluctuations has not been documented. In addition, neuronal mechanisms underlying cognitive enhancement by elevated brain glucose remain unclear. Therefore, we closely analyzed the effects of increased glucose on synaptic plasticity-related protein kinase activity and LTP induction in the hippocampal CA1 region.

Our findings demonstrate that enhanced synaptic efficacy occurs in the presence of increased extracellular glucose and that glucose- induced synaptic potentiation is associated with CaMKII and PKC activation in the rat hippocampal CA1 region. Furthermore, we found that enhanced CaMKII activity likely promotes transmitter release from presynaptic terminals through enhanced phosphorylation of synapsin I (Ser-603), a presynaptic CaMKII substrate [33]). This observation is consistent witn a decrease inthetano onpaired-pulse facilitation in the hippocampal CA1 region. As described previously, the decrease in the ratio of paired-pulse facilitation was due to increased fEPSPs induced by first triggered stimulation [34]. Thus, increased CaMKII-induced synapsin I phosphorylation possibly mediates increased transmitter release from CA1 presynaptic terminals [22]. Kamal et al. [34] also reported dose-dependent facilitation of fEPSPs by increased extracellular glucose. In this context, facilitation of transmitter release is positively correlated with improved learning and memory processes [22, 23].

We previously reported that increased CaMKII autophosphorylation and synapsin I phosphorylation are closely associated with LTP induction and maintenance in the CA1 region [17, 18]. Likewise, GluRl (Ser-831) phosphorylation significantly increased following LTP induction and persisted for at least 60 min without changes in protein level [35]. Thus increased CaMKII activity primarily accounts for glucose-induced facilitation of excitatory synaptic transmission and LTP induction in the hippocampal CA1 region. Indeed, CaMKII autophosphorylation contributes to increased synaptic efficacy through diverse median isms, such as receptor and cytoskeletal protein phosphorylation [7, 8, 11, 12, 14, 15] and trafficking of AMP receptors into the postsynaptic membrane [16–18].

We also found increased NMDA and AMPA responses following 7,0 mM glucose exposure. The magnitudes of the membrane potential and resistance remained unchanged in CA1 neurons between 3,5 and 7,0 mM

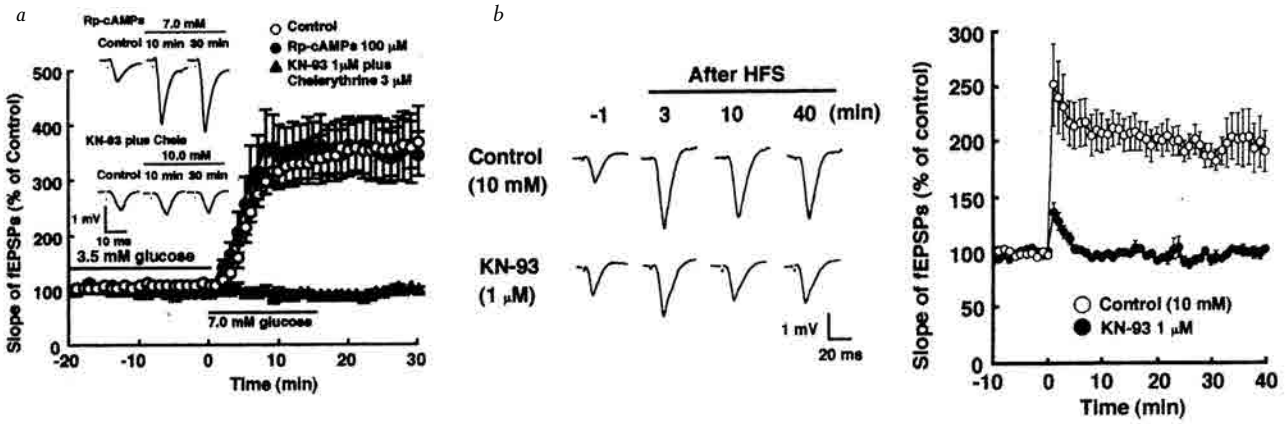

Fig. 6. Inhibition of CaMKII and PKC, but not PKA, block enhanced fEPSPs in CA1 seen following increased extracellular glucose, and CaMKII inhibitors block LTP in the presence of elevated glucose. *a* — KN-93, a CaMKII inhibitor, at 1 uM, combined with chelerythrme, a PKC inhibitor, at 3 pM, completely block fEPSPs seen following increasing extracellular glucose from 3,5 to 7,0 mM in the CA1. Treatment with Rp- cAMPs, a PKA inhibitor, at 100 pM, had no effect. *b* — typical example of effect of 1 uM KN-93 on fEPSPs in the CA1 region following HPS at 3,5 and 10,0 mM extracellular glucose. KN-93 completely blocks LTP in the CA1 region following HFS when extracellular glucose is increased from 3,5 to 10,0 mM.

glucose exposure. Since NMDA receptor activity is required for LTP induction [1, 4, 36] the increased NMDA response seen following 7,0 mM glucose exposure likely mediates enhanced LTP induction. Thus, both presynaptic and postsynaptic activation through CaMKII induced by increased glucose levels could enhance LTP induction. However, further studies are needed to define mechanisms underlying the increased NMDA response observed following glucose elevation.

In addition to CaMKII, PKC is also essential tor Hippocampal LTP induction (Collingridge et al., 2004). When extracellular glucose levels were increased from 3,5 to 7,0 mM, PKCa autophosphorylation significantly increased in the CAl region. Since NR1 phosphorylation by PKC up-regulates NMDA receptor function [20, 21], that activity likely accounts for LTP enhancement. However, PKCα activity was notfurther increased by HFS capable of inducing LTP. Thus PKCα activation by 7,0 mM glucose exposure is likely sufficient to produce LTP in the hippocampal CAl region. We assessed DARPP-32 phosphorylation as a downstream PKA target (Edwards et al., 2002). DARPP-32 phosphorylation was unchanged by high glucose levels, suggesting that PKA is not required for enhancement of synaptic activity in CAl by elevated glucose. In support of this idea, Rp-cAMPs, a specific PKA inhibitor, had no effect on enhancement of fEPSPs by elevating extracellular glucose from 3,5 to 7,0 mM in CAl (Fig. 6*a*).

In summary, we conclude that increased brain glucose levels directly modulate both presynaptic and postsynaptic activities through activation of CaMKII and PKC but not PKA in the hippocampal CAl region. Our findings support the idea that CaMKII but not PKC is essential for LTP induction in the hippocampal CAl region.

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