Effect of Acute Changes in Glucose Concentration on Neuronal Activity and Plasticity in the Rat Hippocampus

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ABSTRACT

Objectives: *Diabetes mellitus is a major public health concern in many regions of the world, including the Caribbean. Diabetes is associated with multi-system pathology and central nervous system complications have been receiving increasing attention (dementia, cognitive decline and memory loss). While such pathology has been shown to be associated with long term derangement in glucose metabolism, less is known about the effects of acute changes in glucose concentration on neuronal function. This study assesses the effects of acute changes in glucose concentration upon neuronal transmission and neuronal plasticity.*

Methods: *We made use of extracellular recordings from hippocampal slices of young adult rats and exposed them to changes in glucose concentration for 60 minutes before assessing synaptic plasticity. Experiments were carried out at both 30°C and 35°C.*

Results: *At 30°C, glucose concentrations of 30 mM and 4 mM had little effect upon population spike potentials (PSP). However, reducing glucose concentration to 2 mM, 1 mM and 0 mM respectively resulted in a progressive decrease in the size of PSP until they were completely abolished. Similar results were observed at 35°C except that 30 mM caused a significant increase in PSP size. Changes in glucose concentration had no effect upon synaptic plasticity at either 30°C or 35°C except below 2 mM glucose.*

Conclusion: *Acute changes in glucose concentration have a limited impact on neuronal transmission unless concentrations drop below 2 mM. However, there seems to be little impairment of synaptic plasticity even at very low concentrations of glucose. We suggest that short term acute changes in glucose concentrations may not contribute directly to the cognitive decline associated with diabetes unless extremely severe.*

Efecto de los Cambios Agudos en la Concentración de Glucosa sobre la Actividad Neuronal y la Plasticidad en el Hipocampo de las Ratas

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RESUMEN

Objetivos: *La diabetes mellitus es una de las principales preocupaciones de la salud pública en muchas regiones del mundo, incluyendo el Caribe. La diabetes se encuentra asociada con una patología multi-sistémica, y en tiempos recientes las complicaciones del sistema nervioso central han estado recibiendo cada vez mayor atención, incluyendo la demencia, el deterioro cognitivo y la pérdida de la memoria. Si bien se ha demostrado que esta patología se encuentra asociada con trastornos a largo plazo del metabolismo de la glucosa, poco se sabe de los efectos de los cambios agudos en la concentración de la glucosa en el funcionamiento neuronal. Este estudio evalúa los efectos de los cambios agudos en la concentración de glucosa, sobre la transmisión y la plasticidad neuronales.*

Métodos: *Se hizo uso de grabaciones extracelulares de segmentos del hipocampo de ratas adultas jóvenes. Las grabaciones fueron expuestas a cambios en la concentración de glucosa durante 60 minutos antes de evaluar la plasticidad sináptica. Los experimentos se llevaron a cabo a 30ºC y 35ºC.*

Resultados: *A 30ºC las concentraciones de glucosa de 30 mM y 4 mM tuvieron poco efecto sobre los*

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potenciales de punta PSP (Population Spike Potentials). Sin embargo, la reducción de la concentración de glucosa a 2 mM, 1 mM y 0 mM respectivamente trajo como resultado una progresiva disminución del tamaño de los PSP hasta llegar a su total anulación. Resultados similares que observaron a 35°C excepto en el caso de 30 mM, en el que se produjo un aumento significativo en la magnitud de los PSP. Los cambios en la concentración de glucosa no tuvieron efecto alguno sobre la plasticidad sináptica a 30°C ni a 35°C excepto por debajo de 2 mM de glucosa.

Conclusión: *Los cambios agudos en la concentración de la glucosa tienen un impacto limitado sobre la transmisión neuronal a menos que las concentraciones caigan por debajo de 2 mM. Sin embargo, al parecer hay poca afectación de la plasticidad sináptica, incluso a concentraciones muy bajas de glucosa. Sugerimos que los cambios agudos a corto plazo de las concentraciones de glucosa pueden no contribuir directamente al deterioro cognitivo asociado con la diabetes, a menos que sea extremadamente severa.*

Palabras claves: *glucosa, potenciación a largo plazo (PLP); plasticidad sináptica, diabetes mellitus; facilitación pareada de los impulsos.*

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INTRODUCTION

In the past twenty years, there has been a rapid rise in the numbers of overweight and clinically obese persons in the West, accompanied by a significant increase in associated disease conditions, in particular, Type II diabetes mellitus. According to the World Health Organization (WHO), 171 million persons were suffering from diabetes in 2000 and this was expected to be more than double by 2030 (*http://www.who.int/diabetes/facts/world_figures/en/index.html*). In the Caribbean, diabetes is of particular concern with high prevalence rates and concomitant mortality and morbidity (1).

Diabetes results in multiple organ dysfunction including cardiovascular, renal and metabolic abnormalities but, increasingly, central nervous system (CNS) complications are being observed and investigated. These include vascular and non-vascular dementia (2, 3), memory loss (4, 5) and cognitive decline (6). Even early diabetic states are now being shown to be associated with CNS impairment (7). Mechanisms responsible for these changes are still to be fully elucidated, though the hippocampus, a region of the brain involved in learning and memory, has been shown to be particularly vulnerable to variations in glucose concentration (8–10).

Long term potentiation (LTP) is a form of synaptic plasticity which is widely believed to be involved in learning and memory though this claim still remains to be fully proven (11). Animal models have demonstrated that synaptic plasticity is impaired in diabetes (12, 13), that these effects can be reversed by insulin treatment (14) and that these and other deficits may be the result of glutamate receptor dysfunction (15).

Such studies have focussed upon the longer term effects of diabetes. However, one of the main problems associated with the management of diabetes is both the irregular dosing of medication and a lack of compliance. Such situations result in acute changes in blood glucose levels (both hyper and hypoglycaemia) that may also have effects upon neurological function and memory impairment. There has been much less work reported on the effects of acute changes in glucose levels on synaptic functioning.

In an early study, Izumi *et al,* noted that 30 minutes of hypoglycaemia had no effect upon basal synaptic transmission. However, when hippocampal slices were exposed to hypoglycaemia prior to induction, LTP was abolished; an effect that was dependent upon nitric oxide (16). A more recent report demonstrated that hypoglycaemia did not block the induction of LTP but it did attenuate its maintenance (17). Other studies have not observed this, in particular Kamal *et al* who showed no impairment of LTP or paired pulse facilitation unless glucose concentrations were reduced to 0 mM (18). Such differences in results may be due to the induction protocols used and the varying metabolic demands that the slices were exposed to as a result of the experimental design.

Given the various reports in the literature and the importance acute changes in glucose concentration may have upon the CNS, we have sought to assess the possible effects of acute changes in glucose concentration upon neuronal function in the submerged rat hippocampal slice. Both short term and long term neuronal plasticity in various glucose concentrations at both 30°C and 35°C were investigated.

Preparation of hippocampal slices

All experiments were carried out in accordance with procedures established by the Faculty Ethics Committee. Hippocampal slices were prepared from male Sprague-Dawley rats of age 5–8 weeks. The animals were anaesthetized with urethane (1.5 – 1.7 g/kg i.p.) and allowed to breathe 100% O_2. While residual effects of urethane on the slices cannot be ruled out, all animals were treated in the same manner and so any differences are unlikely to be attributable to this compound. The rats were then sacrificed by cervical dislocation and decapitated. The brain was rapidly removed into ice-cold artificial cerebrospinal fluid (aCSF) pre-gassed with 95%O_2/ 5%CO_2. The aCSF was made of (in mM): NaCl 115; KCl 2.0; KH_2PO_4 2.2; $NaHCO_3$ 25;

$MgSO_4$1.2 and $CaCl_2$ 2.5. Slices of hippocampus were produced 350 µm thick using a McIlwain Tissue Chopper and placed in an incubation chamber for at least 1 hour at 23–24°C before being transferred to the recording chamber.

Extracellular recording

A single slice was transferred to a recording chamber, fully submerged in pre-gassed (95%O_2/5%CO_2) aCSF and perfused at approximately 4 ml/min at 30°C or 35°C using a temperature regulator (Warner Instruments, USA). Glucose was added to or removed from the aCSF in order to produce the desired concentration. To maintain a constant osmolality throughout all experiments, sucrose was added to the aCSF such that the glucose concentration plus sucrose concentration always equalled 30 mM.

Orthodromic evoked population spike potentials (PSP) were recorded via a glass micro-electrode, tip diameter of approximately 5 µm, containing 3M sodium chloride placed in the stratum pyramidale and stratum radiatum respectively. The Schaffer collateral pathway in the stratum radiatum was stimulated every 15 seconds using a bipolar electrode (FHC, USA) delivering a constant current stimulus (120–350 µA), duration 100 µs. Potentials were amplified and stored digitally on a microcomputer via a CED micro1401 interface and CED Signal 3 software. The peak-to-peak amplitude of the PSP (in mV) was used as a marker of cellular function within the CA1 region.

Once a maximum potential was established, using a supra-threshold stimulus, the stimulus strength was adjusted to yield a potential approximately 55% of the maximum. This allowed quantification of changes in potential size. Recordings were allowed to stabilize before a baseline was recorded for 15 mins. Slices were then superfused with aCSF containing a particular glucose concentration for 60 mins. Mean spike height was calculated at every 30 seconds and normalized with reference to the average spike amplitude during the initial 15 minute baseline period.

Paired pulse recording were made after perfusion with the test media for 30 mins at 20, 50, 80 and 150 ms pulse intervals. These were compared to paired pulse induced in control media. PS-LTP was induced via a theta burst paradigm (a train of four pulses at 100 Hz, repeated 10 times 200ms apart) at the same stimulus strength as utilized to achieve the baseline. This was repeated three times at 60-second intervals.

All the chemicals used in the study were purchased from Sigma-Aldrich, USA.

Data analysis

Values from several slices from different rats were pooled into groups and compared using Student's t-test or ANOVA where appropriate. Differences between means were assessed using Tukey's HSD test. Significance was noted at the level of $p < 0.05$. Data are presented as mean ± standard error of mean (SEM) and the 'n' values reflect the number of slices used.

1. RESULTS

Neuronal Transmission and Changes in Glucose Concentration

At 30°C and normal glucose concentrations of 10 mM (+20 mM sucrose) baseline studies showed no significant change in PSP size (103.7 ± 1.1%, n = 4) over the 75 minutes tested. Superfusion of slices with 30 mM glucose produced a small but non-significant increase in PSP size (108.3 ± 4.8%, n = 11). When glucose concentrations were reduced to 4 mM, there was a small reduction in potential size, 90.3 ± 4.0% (n = 7) but this was not significant. However, when concentrations were reduced to 2 mM and 1 mM, the decrease in potential size was 80.6 ± 8.0% (n = 6) and 61.0 ± 5.1% (n = 7) respectively. These decreases were significantly different from control experiments (Students *t*-test < 0.05) and the results are summarized in Fig. 1A. Perfusion with 0 mM

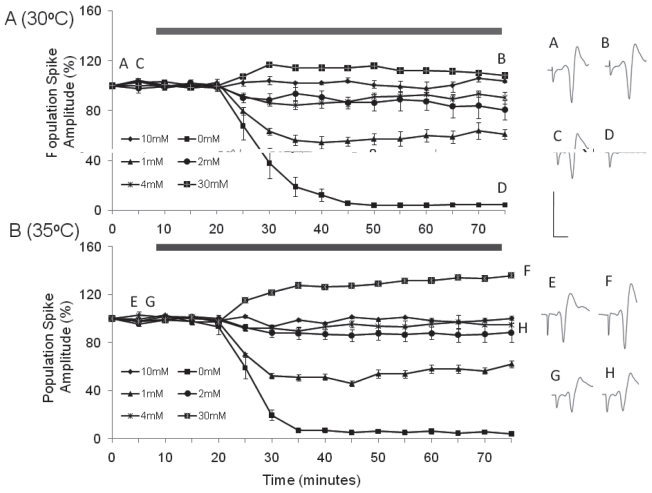

Fig. 1: Acute changes in glucose concentration have little effect upon neuronal transmission graphs show the variation in PSP amplitude with time during experiments in which slices were exposed to changes in glucose concentration for 60 mins (grey bar). Each point represents the mean and se of PSP amplitudes at 5 min intervals. The top graph (**A**) shows the changes that took place at 30°C. The bottom graph (**B**) shows the changes that took place at 35°C. Calibration bars: 3mV and 5ms.

glucose resulted in a decrease in PSP size that lead to complete abolition of PSP but the fibre volley was maintained (n = 8). When slices were returned to normal aCSF, there was recovery of PSP to 98.7 ± 3.5% (n = 8) after 30 min.

Similar experiments were carried out at 35°C. Baseline recordings revealed no change in PSP in 10 mM glucose (+ 20 mM sucrose), 100.0 ± 5.9% (n = 5) but application of 30 mM glucose did result in a significant increase in PSP size (136.0 ± 7.3%; n = 8, Student's *t*-test $p < 0.05$). This was also significantly different from the response noted at

30°C (Students *t*-test, $p < 0.05$). Perfusion with 4 mM and 2 mM glucose yielded PSP of 95.1 ± 7.8% (n = 7) and 88.4 ± 2.9% (n = 8) respectively, results that were not significantly different (Students *t*-test > 0.05). However, perfusion with 1 mM glucose led to a decrease in potential size of 62.3 ± 6.6%, n = 7 which was significantly reduced when compared to controls (Student's *t*-test < 0.05). These results are summarized in Fig. 1B. Perfusion at 0 mM (n = 5) glucose

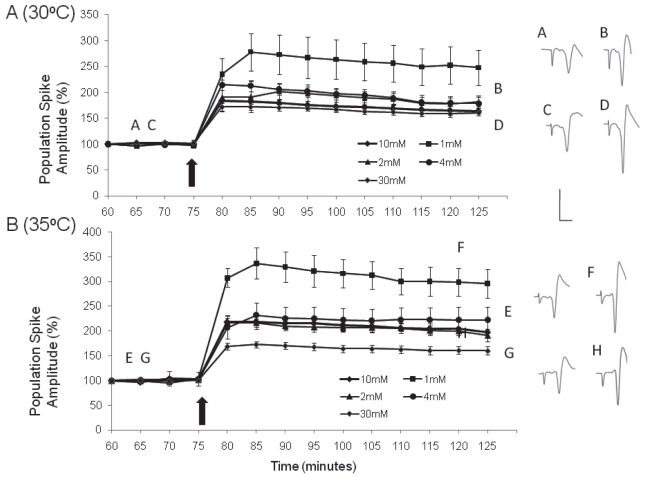

Fig. 2: Acute changes in glucose concentration do not alter the induction or maintenance of PS-LTP. Graph showing the change in PS amplitude with time during experiments in which PS-LTP was induced at time 75 min, 60 minutes after the change in glucose concentration (upward arrow) and recordings followed for a further 45 min in various concentrations of glucose. Values were normalized to the 5 min period prior to the induction of PS-LTP. Each point represents the mean and standard error of PSP amplitudes at 5 min intervals The top graph (**A**) shows the changes that took place at 30°C. The bottom graph (**B**) shows the changes that took place at 35°C. Calibration bars: 3 mV and 5 ms.

lead to a decrease in PSP size such that after 30 mins the potentials had been abolished and this remained so throughout the experiment. There was no recovery when slices were reperfused with a CSF containing normal glucose for 30 minutes.

Synaptic Plasticity and Changes in Glucose Concentration

After perfusion in different concentrations of glucose, synaptic plasticity was assessed by the induction of PS-LTP in the same slice. At 30°C, PS-LTP (163.6 ± 9.4%, n = 7) was induced in normal glucose (10 mM glucose + 20 mM sucrose). This represented a significant increase over baseline values (Student's *t*-test, $p < 0.05$).

To allow a comparison of the extent of PS-LTP, baselines were normalized to the average of the 5 minutes prior to induction of PS-LTP. For all concentrations tested 30 mM, 4 mM, 2 mM and 1 mM, PS-LTP was induced, yielding values of 158.4 ± 5.2% (n = 10), 178.5 ± 12.6% (n = 7), 181.8 ± 11.7% (n = 6) and 247.9 ± 34.2% (n = 7) respectively. All treatments yielded a significant increase in PSP as compared to baselines (Student's *t*-test $p < 0.05$) with 1 mM yielding a significantly larger increase in PS-LTP when compared to the other treatments between treatments (ANOVA). These results are summarized in Fig. 2A. Slices that were exposed to 0 mM glucose and then allowed to recover in normal aCSF also yielded PS-LTP, 185.6 ± 9.7% (n = 5). This was not significantly different from control PS-LTP (Student's *t*-test, $p > 0.05$)

At 35°C, PS-LTP was induced in normal glucose (198.0 ± 10.9%, n = 6) and this was significantly different from the baseline (Student's *t*-test, $p < 0.05$). This also represented a significant difference over the extent of PS-LTP induced at 30°C (Student's *t*-test, $p < 0.05$). At 30 mM, 4 mM and 2 mM glucose, the PS-LTP values recorded were as follows: 160.0 ± 8.4% (n = 8); 222.5 ± 26.4% (n = 6) and 199.6 ± 8.6%, (n = 8) respectively. At 1 mM, PS-LTP was 296.2 ± 25.5% (n = 7) but LTP could not be induced in slices exposed to 0 mM at this temperature. There was no significant difference between the extents of PS-LTP induced at the different concentrations of glucose (ANOVA) except 1 mM. These results are summarized in Fig. 2B.

Paired Pulse Studies

At 30°C, paired pulse studies demonstrated facilitation of the second potential at all inter-pulse intervals tested, *ie* 20 ms, 50 ms, 80 ms and 150 ms for all glucose concentrations, 10 mM (n = 8), 1 mM (n = 8) 2 mM (n = 8), 4 mM (n = 8) and 30 mM (n = 8). There was no difference between PPF for all concentrations tested (ANOVA) except at 1 mM which was significantly increased.

At 35°C, paired pulse studies also demonstrated facilitation of the second potential at all inter-pulse intervals tested, for all glucose concentrations, 10 mM (n = 12), 1 mM (n = 8), 2 mM (n = 7), 4 mM (n = 11) and 30 mM (n = 7). There was no difference between PPF for all concentrations tested (ANOVA) except at 1 mM which was significantly increased. All paired pulse results are summarized in Fig. 3.

DISCUSSION

By using an *in-vitro* slice model, it was demonstrated that changes in extracellular glucose concentrations have minimal effect upon neuronal transmission and baseline function unless reduced to below 2 mM. This was so, both at 30°C and 35°C, despite the increased metabolic demands associated with a temperature increase to 35°C. Other studies utilizing comparable preparations have detailed similar findings (16) though some reports indicate decreases in basal neuronal transmission below 5 mM of glucose (17, 18).

Normal fasting blood glucose is approximately 5–7 mM but this can increase to over 20 mM during diabetes. While plasma glucose levels are readily available, much less is known about changes within the cerebrospinal fluid and therefore the micro-environment of neuronal tissue. Studies using microdialysis have attempted to define such levels and noted that glucose levels tend to be approximately 60–70%

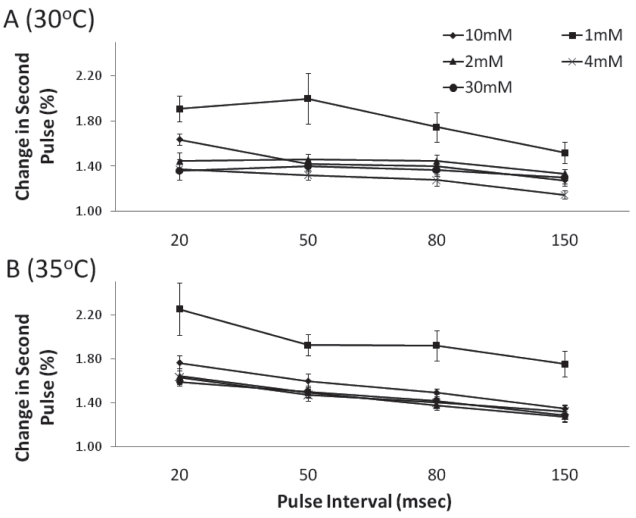

Fig. 3: The effect of changes in glucose concentration on paired pulse facilitation at 30°C and 35°C. Graph shows that for all concentrations tested and at both temperatures tested there was no difference in the extent of paired pulse facilitation at 20 msec, 50 msec, 80 msec and 150 msecs except in experiments carried out with 1 mM glucose where there was a significant increase in facilitation.

lower within the brain (19–21). Cognitive dysfunction tends to only occur when plasma glucose levels fall below 3mM (22, 23) which would correspond with brain ECF levels of less than 1 mM consistent with results of the present study.

While neuronal tissue was able to withstand severe hypoglycaemia, neuronal function was rapidly depressed if glucose levels were reduced below 2 mM. At 30°C, this did not appear to have a permanent deleterious effect as a return to normal aSCF rapidly resulted in normal transmission. However, at 35°C, total glucose deprivation (0 mM) appeared to abolish neuronal function and is suggestive of cell death. This may have implications for long term cognitive decline in diabetic patients. Iatrogenic hypoglycaemia is often the result of aggressive attempts to maintain 'normal' plasma glucose levels and therefore may lead to an erosion of functioning neurons over time.

We also demonstrated that acute hyperglycaemia (30 mM) resulted in a significant increase in PSP size but only at higher temperatures. One possible explanation for this is that neuronal function, at the more physiological temperature of 35°C, and one at which there is increased metabolic demand may be limited by glucose availability. Thus, by increasing the availability of glucose, we were able to increase PSP amplitude suggestive of improved neuronal transmission.

It has been argued that normal neuronal transmission, as evidenced by PSP size, does not necessarily equate to normal neuronal function and that other aspects of functioning including synaptic plasticity must be assessed (24). Therefore, we assessed if changes in glucose concentration might affect changes in paired pulse facilitation and induction and maintenance of PS-LTP. Long term potentiation is believed to be a molecular correlate of learning and memory though this is still somewhat controversial (11).

At both temperatures tested and at glucose concentrations greater than or equal to 1 mM, we were able to both induce PS-LTP and demonstrate robust maintenance up to 45 minutes. Using normal glucose concentrations, PS-LTP induced at 35°C was significantly increased when compared to 30°C; this may be due to increased metabolic activity associated with the higher temperature.

There was no significant difference between the extent of PS-LTP induction and prior exposure (except at 1 mM glucose) to reduced glucose suggesting that moderate reductions in glucose levels did not impair normal cellular function. The large PS-LTP associated with 1 mM glucose is probably a result of the small size of the potential prior to the induction of LTP and suggests that the efficacy of PS-LTP is influenced by the relative size of the PSP prior to induction.

At 35°C, exposure to 30 mM glucose prior to PS-LTP induction resulted in a reduction in the extent of PS-LTP. In like manner, as to the large increase seen at 1mM, the increase in PSP size of approximately 36% prior to induction due to the presence of 30 mM glucose is probably responsible for this observation. Given that starting potentials were 50–60% of maximum, such an increase would have already taken the PSP close to their maximum and thus limited further potentiation induced by PS-LTP. In fact, the PS-LTP in these experiments actually showed a 218% increase when compared to the original baseline prior to the application of 30 mM glucose. This is further supported by the fact that within our laboratory, PS-LTP saturation occurs at a maximum of about 220% (data not reported).

Most studies (13, 14, 25) have demonstrated that diabetes attenuates LTP but Tekkok *et al* observed that LTP was preserved in middle-aged diabetic animals (26). They argued that moderate hyperglycaemia or 'normal' age related hyperglycaemia may help reduce the cognitive decline associated with ageing. Our studies suggest that acute hyperglycaemia in and of itself may not be responsible for decreased LTP and it may simply be a result of the larger starting baseline due to increased glucose concentration. Belanger *et al* who noted similar preservation of LTP in a Type II diabetes model suggested that hyperglycaemia might be accompanied by hyper-insulinaemia and it is this that offers the neuroprotection (27). Other associated complications of diabetes including impaired energy metabolism, glycosylation of lipids and proteins and vascular complications may ultimately be more important in impaired LTP and cognitive decline.

Results of the present study are similar to other reports including Kamal *et al* who also observed no change in the induction and maintenance of LTP both with reduced and increased concentrations of glucose (18, 25). Of note, is the induction paradigm for PS-LTP used in the present study, theta-burst model was utilized and this is considered more

physiologically relevant than the high frequency stimulus paradigm employed by others (28, 29). Other studies have not demonstrated normal LTP in reduced glucose media (16, 17). The recent study by Sadgrove *et al* was able to successfully induce LTP but unable to demonstrate LTP maintenance. They attributed the difference in their findings to the different chamber (interface) used to house their slices and the markedly impaired synaptic transmission they observed associated with low glucose concentrations.

The experiments in this report sought to assess short term plasticity by measuring paired pulse facilitation at various inter-pulse intervals. Paired pulse facilitation is believed to be a presynaptic phenomena associated with an increase in intracellular Ca^{2+} within the pre-synaptic terminal. In all cases 20, 50, 80 and 150 ms inter-pulse intervals resulted in facilitation and we found no differences in PPF for all glucose concentrations tested except at 1mM where again there was increased plasticity. We suggest that this also was due to the small size of the first pulse associated with the decreased in PS size seen at 1mM. Experiments carried out at 35°C showed slightly increased facilitation (though not statistically so) consistent with the results described for PS-LTP induction above. No change in PPF suggests that changes in glucose concentration do not affect the pre-synaptic terminus and neurotransmitter release.

In conclusion, we have demonstrated that acute exposure to reduced and elevated glucose concentrations have minor effects upon normal neuronal transmission at both 30°C and 35°C unless markedly reduced to below 2 mM. In addition, changing glucose concentration does not impair either, PPF or PS-LTP induction and maintenance; however, increases in temperature do appear to increase synaptic plasticity as evidenced by the increase in LTP magnitude. These results may offer insight into one of the mechanisms responsible for the cognitive decline associated with diabetes.

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