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Neuronal Ca^{2+} disregulation in diabetes mellitus

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Abstract

The Ca^{2+} hypothesis of brain ageing and dementia may account for part of the available data on the pathogenesis of dementia and certain neurodegenerative disorders. The hypothesis proposes that disturbances in the homeostasis of neuronal cytosolic free Ca^{2+} are part of a final common pathway, ultimately leading to neuronal dysfunction and cell death. The hypothesis also proposes that a small change in cytosolic free Ca^{2+} sustained over a long period of time will result in similar damage as a large change over a short period. Diabetes mellitus is associated with neurological complications in the peripheral and central nervous system, as reflected in peripheral neuropathy, modest cognitive impairments and an increased risk of dementia. In animal models of diabetes, learning impairments are associated with alterations in Ca^{2+} -dependent forms of hippocampal synaptic plasticity. Disturbances in the homeostasis of cytosolic free Ca^{2+} may present a final common pathway in the multifactorial pathogenesis of neurological complications of diabetes, which involves vascular changes, oxidative stress, and non-enzymatic protein glycation. In line with the Ca^{2+} hypothesis of neurodegenerative disorders, a prolonged, small increase in basal cytosolic Ca^{2+} levels indeed exists in sensory neurones of diabetic animals. In addition, Ca^{2+} dynamics are affected. Ca^{2+} channel blockers, such as nimodipine, have been shown to improve experimental peripheral neuropathy, through a vascular mechanism, possibly in combination with direct neuronal effects. Preliminary studies indicate that nimodipine may also improve Ca^{2+}-dependent forms of synaptic plasticity in the hippocampus of diabetic rats.

Keywords: Diabetes mellitus; Nerve; Brain; Neuron; Ca^{2+}; Pathogenesis; Treatment; Encephalopathy; Neuropathy

1. Introduction: the Ca^{2+} rationale in ageing and neurodegenerative disorders

It is generally assumed that ageing is one of the most important and consistent risk factors for Alzheimer's disease. The Ca^{2+} hypothesis of brain ageing and dementia has been put forward to account for at least part of the available data on the pathogenesis of dementia (Khachaturian, 1994). Whether true or not, the hypothesis is most certainly of importance in understanding the ageing brain. The hypothesis proposes that the cellular mechanisms that regulate the homeostasis of cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) play a critical role in brain ageing, as Ca^{2+} mediated signal transduction cascades and Ca^{2+} homeostasis are part of the final common pathway for cellular changes leading to neuronal dysfunction and cell death. Furthermore, the hypothesis proposes that the extent of the perturbation in $[Ca^{2+}]_i$ and the duration of this disregulation is a constant (Khachaturian, 1994). In other words, a small change in free $[Ca^{2+}]_i$ sustained over a long period of time will result in similar cellular damage as will a large change over a short period. Indeed there is ample evidence for a close relation between Ca^{2+} homeostasis, the production of reactive oxygen species, ischaemia, and (brain) cell death (Finkel and Holbrook, 2000; Kristian and Siesjo, 1996).

This paper will review the pathogenesis of peripheral and central neurological complications of diabetes mellitus, focusing on the putative role of Ca^{2+}. In addition, the potential effects of Ca^{2+} channel blockers in the treatment of peripheral and central diabetic neuropathy will be discussed.

2. Neurological complications of diabetes

2.1. Clinical findings

Diabetes mellitus is a metabolic disorder characterised by hyperglycaemia resulting from defective insulin secretion, resistance to insulin action, or both (American Diabetes

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Association, 2002). Type 1 and type 2 diabetes, previously defined as insulin-dependent and non-insulin-dependent diabetes mellitus, are the most common forms. In type 1 diabetes, immune-mediated destruction of pancreatic β-cells leads to insulin deficiency, and patients require insulin treatment for survival. Type 2 diabetes is characterised by insulin resistance and relative, rather than absolute, insulin deficiency. Treatment aims to reduce insulin resistance (diet, exercise, and drug therapy) and to stimulate insulin secretion. With the current standards of medical care for diabetic patients blood glucose can be maintained within acceptable limits (American Diabetes Association, 2002). Despite this treatment, the development of diabetic complications remains a serious concern. In addition to the nervous system, these complications involve the cardiovascular system, the kidneys and the eyes. With regard to the neurological complications of diabetes, attention has long been focussed mainly on peripheral neuropathy. In recent years, however, there is an increasing awareness of the significance of the adverse effects of diabetes on the central nervous system.

Diabetic neuropathy is a leading cause of polyneuropathy in the Western world. Its development is related to the level of metabolic control, age and diabetes duration (Dyck et al., 1993; Tesfaye et al., 1996). It is a heterogeneous disorder, of which distal symmetric polyneuropathy is the most common form (Dyck et al., 1993; Ross, 1993). Distal symmetric polyneuropathy often occurs in association with autonomic neuropathy, which can lead to postural hypotension, gastroparesis, diarrhoea, constipation, neurogenic bladder, and male impotence (Spallone et al., 1995). Distal symmetric diabetic neuropathy is associated with reductions in motor and sensory nerve conduction velocity (Arezzo, 1997) and with myelinated nerve fibre loss (Yagihashi, 1995).

Diabetes can affect the central nervous system through both acute and chronic metabolic and vascular disturbances (Gispen and Biessels, 2000). The consequences of acute metabolic and vascular insults to the brain, such as hypoglycaemia and stroke, are well recognised and have been reviewed extensively (e.g. Bell, 1994; Cryer et al., 1994). We focus on alterations in the brain that develop more insidiously, a condition that may be referred to as diabetic encephalopathy. Diabetic encephalopathy may manifest itself by cognitive deficits, mostly affecting learning and memory and complex information processing (Ryan, 1988; Stewart and Liolitsa, 1999). In patients up to the age of 60, cognitive deficits are generally modest and not likely to affect day-to-day functioning to a significant extent (Gispen and Biessels, 2000; Ryan, 1988). In the elderly, however, cognitive deficits appear to be more pronounced and can be detected with relatively crude tests (Stewart and Liolitsa, 1999). Moreover, recent studies report an association between especially type 2 diabetes and dementia in old age (Leibson et al., 1997; Ott et al., 1999). Other manifestations of cerebral deficits in diabetes include alterations in signal conduction, as reflected in increases in the latencies of auditory, visual and sensory evoked potentials (Di Mario et al., 1995). In addition, structural cerebral changes have been noted, including subcortical (ischaemic) lesions and a moderate subcortical and cortical atrophy (Araki et al., 1994; Peress et al., 1973).

2.2. Experimental models

Various rodent models of both type 1 and type 2 diabetes are available (Shafrir, 1997). In some models diabetes develops spontaneously, due to a genetic predisposition, in others diabetes is induced experimentally. A common method for diabetes induction is an intravenous or intraperitoneal injection with the β-cytotoxic agent streptozotocin (STZ). This glucosamine–nitrosourea compound is taken up into the cell via the GLUT-2 glucose-transporter, which is highly expressed by the insulin-producing β-cells of the islets of Langerhans (Schnedl et al., 1994). Direct effects on the brain do not occur following systemic administration, due to absence of the GLUT-2 glucose-transporter at the blood–brain barrier (Kumagai, 1999). Blood glucose levels typically are 20–25 mmol/l (normal 5 mmol/l). STZ-diabetic rodents are hypoinsulinaemic, but do not require insulin treatment to survive.

2.2.1. Peripheral and central neurological deficits in experimental diabetes

Neuropathy in rat models of diabetes mimics clinical diabetic neuropathy in several aspects (Sima et al., 2000; Yagihashi, 1995). Early peripheral nerve dysfunction, as shown by reductions in motor and sensory nerve conduction velocities, can be detected within weeks after onset of diabetes in models of type 1 (e.g. STZ-induced diabetic rats and BB/Wor rats; Kappelle et al., 1993, 1994) and type 2 dibetes (e.g. Zucker diabetic fatty rats; Sima et al., 2000). These early reductions in conduction velocity are mostly rapidly reversible (Arezzo, 1997). A further progressive, more persistent, impairment of nerve conduction velocities is related to morphological changes in the nerve, such as myelinated fibre loss and ultrastructural abnormalities in the paranodal axo-glial junction (Sima et al., 1986; Yagihashi, 1995).

Changes in signal conduction do also occur in the central nervous system (Biessels et al., 1999; Morano et al., 1996), albeit after a longer duration of diabetes. While peripheral impairments develop within weeks after diabetes induction, central impairments take months to develop (Biessels et al., 1999). For example, increases in the latencies of auditory and visual evoked potentials can be detected only after 3 months of untreated STZ-diabetes and signal conduction in the spinal cord is significantly reduced after 6, but not after 3 months of STZ-diabetes (Biessels et al., 1999). Like in diabetic patients, these central conduction deficits develop in association with modest impairments of learning and memory (Gispen and Biessels, 2000). While performance on relatively simple cognitive tasks is mostly unaffected

(Bellush and Rowland, 1989), the performance of diabetic rodents on more complex learning tasks, such as an active avoidance T-maze, or a Morris water maze is impaired (Biessels et al., 1996; Biessels et al., 1998; Flood et al., 1990). Control experiments showed that these performance deficits were not due to sensorimotor impairments (Biessels et al., 1996, 1998). The development of the learning deficits is related to diabetes duration and the severity of hyperglycaemia (Biessels et al., 1996). In senescent rats the learning deficits appear to be more pronounced (Kamal et al., 2000), which is suggestive for an interaction between the effects of diabetes and ageing on the brain.

2.2.2. Alterations in Ca^{2+}-dependent forms of synaptic plasticity

Plastic changes in synaptic strength are considered to form the neuronal basis of learning and memory (Bliss and Collingridge, 1993). Long-term potentiation (LTP) and depression (LTD) are two experimentally induced forms of activity-dependent synaptic plasticity that have been studied extensively (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999). In LTP, brief high-frequency afferent activity leads to a long-lasting increase in the strength of synaptic transmission, whereas in LTD prolonged low-frequency activity results in a persistent reduction in synaptic strength. Both processes are triggered by an increase in the level of post-synaptic intracellular Ca^{2+} concentration $[Ca^{2+}]_i$ (Bliss and Collingridge, 1993; Malenka and Nicoll, 1999; Yang et al., 1999). LTP is triggered by a brief increase of $[Ca^{2+}]_i$ with relatively high magnitude, whereas a prolonged modest rise of $[Ca^{2+}]_i$ induces LTD (Yang et al., 1999).

Several groups have studied the effects of experimental diabetes on synaptic plasticity in the hippocampus, a structure in the medial temporal lobe that plays an important role in certain types of learning and memory. Learning deficits in STZ-diabetic rats develop in association with distinct changes in synaptic plasticity in hippocampal slices, which appear to be dependent on diabetes duration and severity (Gispen and Biessels, 2000). Deficits in the expression of *N*-methyl-D-aspartate (NMDA)-dependent LTP in the CA1 field and dentate gyrus (Biessels et al., 1996; Chabot et al., 1997; Kamal et al., 1999), and in the expression of NMDA-independent LTP in the CA3 field have been noted (Kamal et al., 1999). Insulin treatment prevents the development of the changes in LTP, but is less effective against existing LTP deficits (Biessels et al., 1998). In contrast to LTP, expression of LTD is enhanced in the CA1 field in hippocampal slices from diabetic rats (Kamal et al., 1999). In line with the behavioural observations, the LTP deficit in diabetic rats is accentuated by ageing (Kamal et al., 2000).

Paired-pulse facilitation is a form of short-term synaptic plasticity that is characterised by an increase in a second excitatory post-synaptic potential when it is elicited shortly after a first (Zucker, 1989). This increase is considered to result from an increase in transmitter release, due to residual $[Ca^{2+}]_i$ remaining in the pre-synaptic terminal after the first action potential (Fisher et al., 1997; Zucker, 1989). After 12 weeks of STZ diabetes, paired pulse facilitation is unaffected with an inter-stimulus interval of 50 ms (Biessels et al., 1996). Although this indicates that there are no major changes in the Ca^{2+}-dependent pre-synaptic processes involved in neurotransmitter release, the effects of STZ-diabetes on this form of plasticity need further investigation. Another Ca^{2+}-dependent feature of synaptic transmission in the hippocampus is the so-called slow afterhyperpolarisation. This slow afterhyperpolarisation occurs following a train of action potentials, due to the activation of the voltage-independent, Ca^{2+}-dependent K^+ current (Landfield, 1994). The amplitude and duration of the slow afterhyperpolarisation is increased in CA1 neurones from aged rats (Landfield, 1994), which is considered to be a neurophysiological hallmark of ageing. Recent experiments in our laboratory demonstrated a similar increase in the amplitude of the slow afterhyperpolarisation in CA1 pyramidal cells in hippocampal slices from 12 weeks STZ-diabetic rats (Kamal and Ramakers, unpublished observations).

Alterations in neuronal Ca^{2+} homeostasis are likely to play an important role in the changes in cognition and synaptic plasticity that have been observed in experimental diabetes, together with alterations in other signal transduction cascade components such as protein kinase A, protein kinase C, cAMP, phospholipase C, phospholipase A2, diacylglycerol and inositol phosphate (Bhardwaj et al., 1999; Di Luca et al., 1999). Alterations in glutamatergic neurotransmission also appear to be involved (Di Luca et al., 1999; Gagne et al., 1997). After 12 weeks of STZ-diabetes, the level of the NR2B subunit of the NMDA receptor is decreased by 40%, whereas the levels of the NR1 and NR2A subunits are unchanged (Di Luca et al., 1999; Gardoni et al., 2002). Moreover, the phosphorylation of the NR2A/B subunits by Ca^{2+}/calmodulin-dependent protein kinase II is reduced in STZ-diabetes (Di Luca et al., 1999). These molecular changes in the NMDA receptor complex are accompanied by a reduction in NMDA receptor mediated currents in hippocampal Tpyramidal neurones (Gardoni et al., 2002). The course of development and response to insulin treatment of the alterations in the NMDA-receptor complex closely matches that of changes in behaviour and synaptic plasticity in this model (Gardoni et al., 2002).

3. Pathogenesis: various mechanisms, common pathways?

3.1. Metabolic and vascular factors

The pathogenesis of peripheral and central neurological complications of diabetes is likely to share many features. The pathogenesis of peripheral neuropathy, in particular, has been studied intensively (Cameron et al., 2001; Sima and Sugimoto, 1999). It is likely to be a multifactorial process

involving (i) metabolic alterations, such as increased polyol pathway flux (Greene et al., 1987), oxidative stress (Van Dam et al., 1995) and non-enzymatic protein glycation (Brownlee, 2001; Singh et al., 2001), (ii) vascular dysfunction, leading to decreases in nerve blood flow and hypoxia (Cameron et al., 2001) and (iii) alterations in neurotrophic support (Brewster et al., 1994). The pathogenesis of diabetic encephalopathy has not yet been studied in similar detail. However, increases in polyol pathway flux, leading to accumulation of sorbitol and fructose (Sredy et al., 1991), accumulation of advanced glycation end products (Vlassara et al., 1983), and increased concentrations of the by-products of lipid peroxidation (Kumar and Menon, 1993), have been demonstrated in the brain tissue of diabetic rats. Vascular changes, involving both large and small cerebral blood vessels, are likely to play an additional pathogenetic role (Mankovsky et al., 1997).

It is beyond the scope of this review to cover the complex pathogenesis of the neurological complications of diabetes to its full extent. What we like to emphasise here is that despite the fact that the various pathogenetic factors affect the nervous system through quite different mechanisms, many of them disturb neuronal Ca^{2+} homeostasis. Alterations in neuronal Ca^{2+} homeostasis have, for example, been implicated in the mechanisms of neuronal degeneration in response to oxidative stress or hypoxia (Kristian and Siesjo, 1998; Mattson et al., 2001; Sheen et al., 1992). Disturbances in neuronal Ca^{2+} homeostasis may thus present a final common pathway in the pathogenesis and a potential target for therapy.

3.2. Neuronal Ca^{2+} homeostasis

Data from animal models of diabetes and from diabetic patients reveal that the $[Ca^{2+}]_i$ is increased in most tissues, including the nervous system (Levy et al., 1994). Using electron probe X-ray microanalysis, Lowery et al. (1990) observed increased mitochondrial and axoplasmic Ca^{2+} levels in peripheral nerve of diabetic rats. This technique does, however, not allow differentiating between free and bound Ca^{2+}. In individual dorsal root ganglion (DRG) sensory neurones, obtained from BB/Wor rats and diabetic mice, voltage-dependent Ca^{2+} currents through L- and N-channels, but not T-channels, were shown to be enhanced (Hall et al., 1995; Kostyuk et al., 1995; Voitenko et al., 1999, 2000). Impaired inhibitory G-protein function may contribute to these increased Ca^{2+} currents (Hall et al., 2001). In addition, Ca^{2+} storage mechanisms are disturbed in neurones of experimentally induced diabetes mellitus in rats and mice (Kostyuk et al., 1999; Voitenko et al., 1999). Because the changes in Ca^{2+} homeostasis are present in DRG neurones and in secondary nociceptive neurones from substantia gelatinosa of spinal dorsal horn slices, it was suggested that a disturbed Ca^{2+} homeostasis could be the reason for the development of sensory polyneuropathy and its symptoms in the early stages of diabetes mellitus (Kostyuk et al., 2001). Likewise, Ohsawa and Kamei (1999) conclude that thermal allodynia and hyperalgesia in diabetic mice may be due, in part, to enhanced $[Ca^{2+}]_i$ levels in the spinal cord.

As the Ca^{2+} hypothesis of neurodegeneration suggests that small, prolonged changes in $[Ca^{2+}]_i$ can lead to similar neuronal degeneration as acute Ca^{2+} overloads, we examined $[Ca^{2+}]_i$ in intact DRGs of STZ-diabetic rats, using Ca^{2+} imaging techniques. DRGs were loaded in vivo with the fluorescent Ca^{2+} indicator fura-2-dextran by a newly developed method, in which we transected the sciatic nerve, and placed a cuff containing fura-2-dextran around the proximal stump, 7 h before removing the DRGs and performing the measurements (Fig. 1). With this technique, we obtained sufficient loading of the conjugated indicator in the somata of the DRG neurones to allow accurate, reproducible measurements of $[Ca^{2+}]_i$. A representative recording, obtained from a whole DRG of a control animal, is shown in Fig. 2A. In this figure, each point represents the Ca^{2+} concentration in the DRG at that particular moment in time, calculated from the 340:380 nm ratio at that time. From these traces basal Ca^{2+} concentrations and reactions to electric stimulation (area under the peak) were calculated. By using impermeable fura-2-dextran conjugates, the neurones of the DRG that have axons in the afferent bundle were

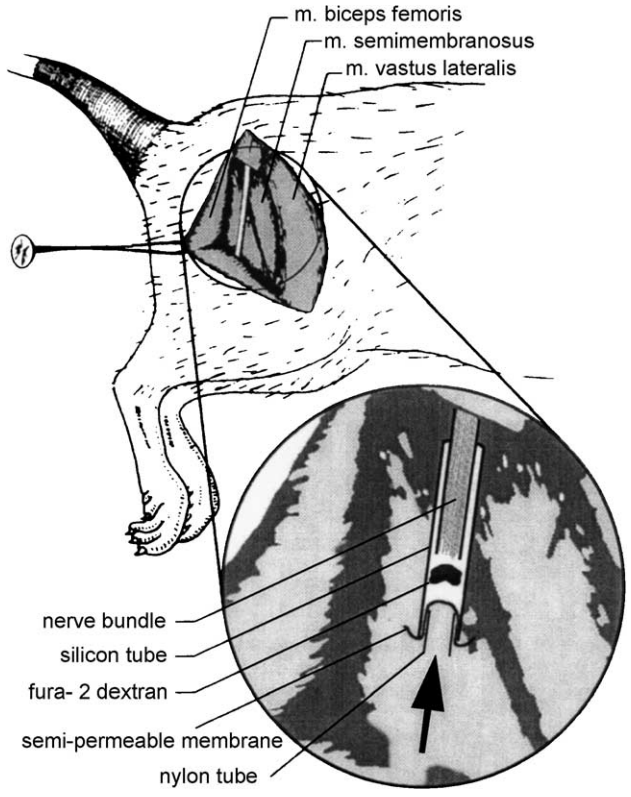

Fig. 1. Schematic representation of the placement of the cuff containing a conjugated Ca^{2+} indicator to allow retrograde transport of the indicator to the somata of dorsal root ganglion neurones. The arrow indicates flow of extracellular fluid into the transected axons of the afferent bundle, allowing uptake of the conjugated indicator into these axons.

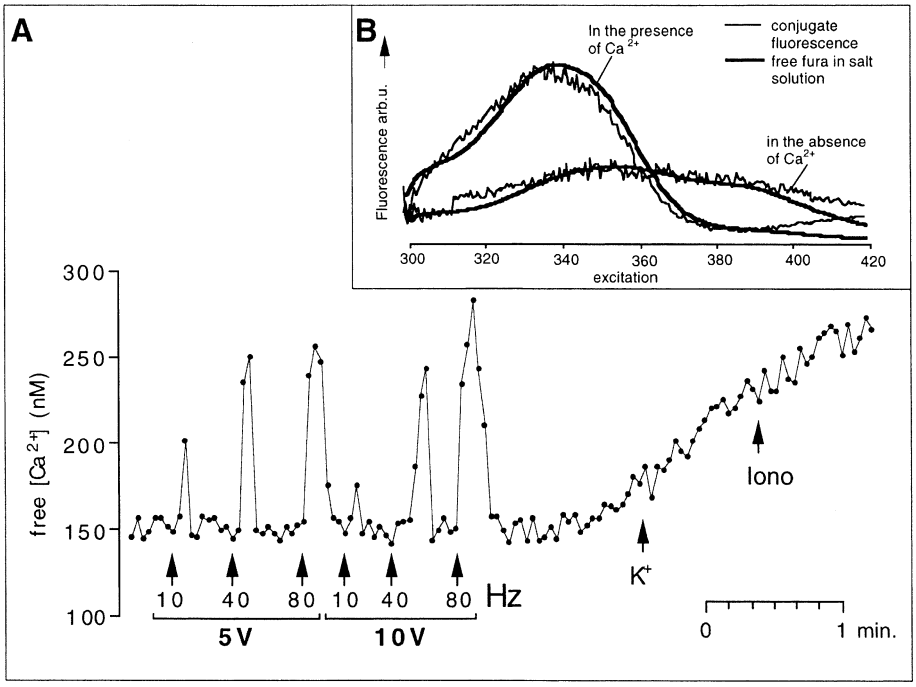

Fig. 2. (A) Typical data trace from $[Ca^{2+}]_i$ recordings from an intact dorsal root ganglion from a control animal. Each data point represents a calculated and calibrated ratio between emission at two wavelengths. Arrows indicate 1-s episodes of electrical stimulation to the afferent bundle with the indicated voltage and frequency and application of chemical agents: 50 nM KCl (K^+) and 5 μM ionomycin (Iono). (B) Excitation spectra for fura-2-dextran (thin line) and free fura-2 (thick line) in the presence and absence of Ca^{2+}, showing that fura-2 conjugated to the dextran tail has the same optical properties as the more commonly used free fura-2. Emission was collected at 510 nm.

loaded selectively, thus preventing contamination of the signal by $[Ca^{2+}]_i$ from for example glial cells and local neurones.

The experimental outline was as follows: 36 animals were randomly divided into three groups of 12 animals each. In two groups diabetes was induced, and one group served as age-matched controls. One diabetic group was treated with nimodipine in polyethylene glycol (every 48 h, intraperitoneally), the second diabetic group as well as the age-matched controls were treated with vehicle only. Sensory and motor nerve conduction velocities were measured in the left sciatic nerve in weeks 4, 6 and 9. In week 10, the cuff containing the fura-2-dextran was placed on the right sciatic nerve and Ca^{2+} concentrations were measured. Basal Ca^{2+} levels were calculated from the points (ratio-pairs) in the traces before the first stimulation. In the diabetic animals basal $[Ca^{2+}]_i$ was significantly increased (182.2 ± 2.25 nM) as compared to the controls (159.1 ± 1.87 nM) (Fig. 3). Upon electrical stimulation of the afferent bundle, $[Ca^{2+}]_i$ rose transiently in the neurones of the intact DRG in a frequency-dependent manner (see representative experiment in Fig. 2). These responses were smaller in diabetic animals as compared to the age-matched controls, albeit not statistically significant. The area under the peak after electrical stimulation is depicted in Fig. 4. With increasing frequencies the Ca^{2+} influx increased both at 5 V and at 10 V. At higher frequencies (40 and 80 Hz) controls showed a trend to react with a higher Ca^{2+} influx than diabetic rats did. The lowest electrical stimulation (5 V–10 Hz) elicited only a small response or no response at all, indicating that this was a threshold stimulation intensity.

The study described above employed a novel method that enables measurements of $[Ca^{2+}]_i$ in intact DRGs in vitro, by pre-labelling with the fluorescent Ca^{2+} indicator fura-2-dextran in vivo. In earlier in vitro studies (Hall et al., 1995; Kostyuk et al., 1995), DRGs were dissociated

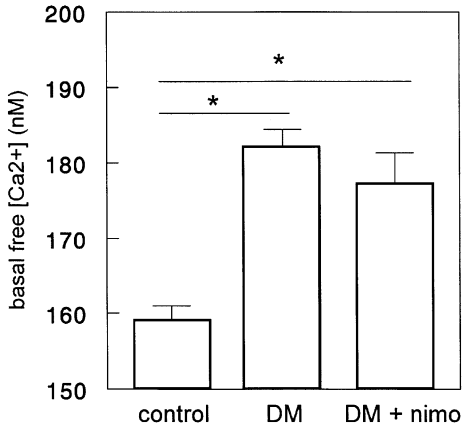

Fig. 3. Basal levels of $[Ca^{2+}]_i$ in neurones of intact fura-2-dextran loaded dorsal root ganglia from diabetic animals treated with nimodipine and placebo and from age-matched controls. Data represent averages of 16 or more traces from at least nine animals in each group ± S.E.M. Asterisk indicates differences with $P < 0.05$.

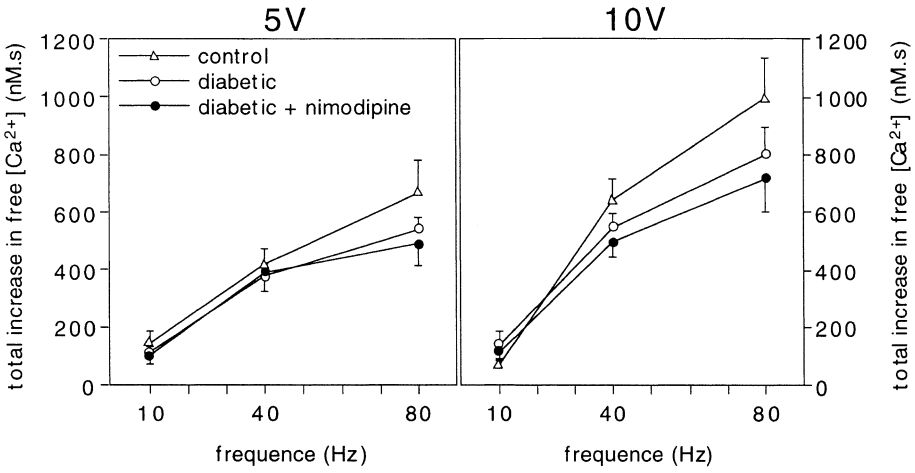

Fig. 4. $[Ca^{2+}]_i$-increase upon electrical stimulation in neurones in the intact dorsal root ganglia from diabetic animals treated with nimodipine (filled circles) or placebo (open circles) and from age-matched controls (triangles). Afferent bundles were stimulated with bipolar electrodes firstly with 5 V at three different frequencies and subsequently with 10 V and the same frequencies. At higher frequencies (40 and 80 Hz) Ca^{2+} influx in dorsal root ganglia from diabetic rats was reduced compared to controls, but this effect was not statistically significant. Data represent average of at least eight responses in each group ± S.E.M.

mechanically prior to the measurements. This approach in itself is likely to affect Ca^{2+} homeostasis, complicating the estimation of the original endogenous Ca^{2+} regulation. Obviously, our technique still required transection of the nerve and eventually dissection of the DRGs. An excessive rise in $[Ca^{2+}]_i$ is usually the first parameter to be affected in emergency responses during cell stress, thus inducing emergency gene responses and eventually cell death programs. During our recordings, several hours after the dissection of the DRGs, the $[Ca^{2+}]_i$ was still surprisingly low and the neurones responded with a strong and transient rise in intracellular free Ca^{2+} upon depolarisation. Hence, there was no sign of deterioration of the condition of the cell bodies of the DRG neurones in our experimental set-up. Thus, by manipulating the DRG as little as possible and measuring in intact DRG, we closely approached the in vivo situation.

The observed increase in the basal $[Ca^{2+}]_i$ in DRGs from diabetic animals as compared to age-matched controls is of particular interest with regard to the Ca^{2+} hypothesis of neurodegenerative disorders, which states that prolonged small increases in $[Ca^{2+}]_i$ could be as detrimental as acute overloads (Khachaturian, 1994). Although the relatively small increase in $[Ca^{2+}]_i$ in the diabetic rats may not directly trigger Ca^{2+}-dependent cell death programs, the chronic character of the increase may, over time, cumulate until detectable degeneration occurs. Such gradual processes correspond well with the slow development of diabetic neuropathy (and other neurodegenerative processes, especially in brain ageing) as compared to the acute damage of excitotoxicity, ischaemia or strong convulsions (Kristian and Siesjo, 1998). Since we analysed the whole intact DRG, we cannot determine if the increase in $[Ca^{2+}]_i$ in the diabetic DRGs was homogenous, or if specific subpopulations of neurones were particularly affected. Evidence from other studies, however, indicates that large DRG neurones are able to maintain proper resting levels of $[Ca^{2+}]_i$ when small neurones already show a significant elevation (Kostyuk et al., 1995, 1999).

4. Effect of Ca^{2+} channel blockers

L-type Ca^{2+} channel blockers are known to have both cardiovascular and neuropharmacological effects (Hoffmeister et al., 1985; Luiten et al., 1994; Scriabine et al., 1989). Nimodipine is an L-type Ca^{2+} channel blocker that is of particular interest, since it penetrates the blood–brain barrier relatively well (Hoffmeister et al., 1985). Nimodipine affects Ca^{2+} homeostasis in spinal cord neurones in culture (Bär et al., 1993) and appears to provide protection against the effects of hypoxia (Bär et al., 1990) and oxidative stress (Sheen et al., 1992) at the cellular level. Based on these characteristics, nimodipine might be expected to protect neurones against the adverse effects of diabetes.

Our lab has consistently shown beneficial effects of nimodipine on sensory and motor nerve conduction velocities in both STZ-diabetic rats and BB/Wor diabetic rats (Kappelle et al., 1993, 1994). In the Ca^{2+} imaging experiment reported above, nimodipine treatment also enhanced motor and sensory nerve conduction velocity in STZ diabetic rats. However, nimodipine treatment had no obvious protective effect on either the increase in basal $[Ca^{2+}]_i$ levels or on the reduced Ca^{2+} dynamics (Figs 3, 4). A combined neuronal/vascular, rather than an exclusive neuronal effect of nimodipine on the sciatic nerve, may explain this lack of effect on neuronal parameters. Improvement of nerve conduction velocity in diabetic neuropathy is closely correlated with an increase in sciatic nerve blood flow (Kappelle et al., 1993, 1994). Moreover, nifedipine, a Ca^{2+} channel blocker that does not readily crosses the blood–nerve barrier, has also been shown to improve nerve conduction velocity in

diabetic rats (Robertson et al., 1992), again pointing to a vascular/neuronal mechanism.

5. Conclusion

Diabetes mellitus is associated with neurological complications in the peripheral and central nervous system. In line with the Ca^{2+} hypothesis of neurodegenerative disorders, we show that a prolonged, small increase in basal $[Ca^{2+}]_i$ indeed exists in DRG neurones of diabetic animals. In addition, Ca^{2+} dynamics are reduced in diabetic animals. Thus far, less is known about effects of diabetes on neuronal Ca^{2+} homeostasis in the central nervous system. The finding that diabetes affects Ca^{2+}-dependent forms of synaptic plasticity in the brain does, however, provide indirect evidence for central disturbances in neuronal Ca^{2+} homeostasis. As alterations in neuronal Ca^{2+} homeostasis may play a pivotal role in the impairment of learning and plasticity in diabetes, in particular the disturbed balance between LTP and LTD and the increase in the amplitude of the slow afterhyperpolarisation, this subject warrants further investigations.

Ca^{2+} channel blockers, such as nimodipine, have been shown to improve experimental peripheral neuropathy, through a vascular mechanism, possibly in combination with direct neuronal effects. Preliminary studies indicate that nimodipine may also improve Ca^{2+}-dependent forms of synaptic plasticity in the hippocampus of diabetic rats (Biessels et al., 2002).

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References

American Diabetes Association, 2002. Standards of medical care for patients with diabetes mellitus. Diabetes Care 25, 213–229.

Araki, Y., Nomura, M., Tanaka, H., Yamamoto, H., Yamamoto, T., Tsukaguchi, I., 1994. MRI of the brain in diabetes mellitus. Neuroradiology 36, 101–103.

Arezzo, J.C., 1997. The use of electrophysiology for the assessment of diabetic neuropathy. Neurosci. Res. Commun. 21, 13–23.

Bär, P.R., Traber, J., Schuurman, T., Gispen, W.H., 1990. CNS and PNS effects of nimodipine. J. Neural Transm., Suppl. 31, 55–71.

Bär, P.R., Renkema, G.H., Veraart, C.M., Hol, E.M., Gispen, W.H., 1993. Nimodipine protects cultured spinal cord neurones from depolarization-induced inhibition of neurite outgrowth. Cell. Calcium 14, 293–299.

Bell, D.S., 1994. Stroke in the diabetic patient. Diabetes Care 17, 213–219.

Bellush, L.L., Rowland, N.E., 1989. Stress and behavior in streptozotocin diabetic rats: biochemical correlates of passive avoidance learning. Behav. Neurosci. 103, 144–150.

Bhardwaj, S.K., Sandhu, S.K., Sharma, P., Kaur, G., 1999. Impact of diabetes on CNS: role of signal transduction cascade. Brain Res. Bull. 49, 155–162.

Biessels, G.J., Kamal, A., Ramakers, G.M., Urban, I.J., Spruijt, B.M., Erkelens, D.W., Gispen, W.H., 1996. Place learning and hippocampal synaptic plasticity in streptozotocin-induced diabetic rats. Diabetes 45, 1259–1266.

Biessels, G.J., Kamal, A., Urban, I.J., Spruijt, B.M., Erkelens, D.W., Gispen, W.H., 1998. Water maze learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: effects of insulin treatment. Brain Res. 800, 125–135.

Biessels, G.J., Cristino, N.A., Rutten, G., Hamers, F.P.T., Erkelens, D.W., Gispen, W.H., 1999. Neurophysiological changes in the central and peripheral nervous system of streptozotocin-diabetic rats: course of development and effects of insulin treatment. Brain 122, 757–768.

Biessels, G.J., Van der Heide, L.P., Kamal, A., Bleys, R.L., Gispen, W.H., 2002. Ageing and diabetes: implications for brain function. Eur. J. Pharmacol. 441, 1–14.

Bliss, T.V., Collingridge, G.L., 1993. A synaptic model of memory: long-term potentiation in the hippocampus. Nature 361, 31–39.

Brewster, W.J., Fernyhough, P., Diemel, L.T., Mohiuddin, L., Tomlinson, D.R., 1994. Diabetic neuropathy, nerve growth factor and other neurotrophic factors. Trends Neurosci. 17, 321–325.

Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. Nature 414, 813–820.

Cameron, N.E., Eaton, S.E., Cotter, M.A., Tesfaye, S., 2001. Vascular factors and metabolic interactions in the pathogenesis of diabetic neuropathy. Diabetologia 44, 1973–1988.

Chabot, C., Massicotte, G., Milot, M., Trudeau, F., Gagne, J., 1997. Impaired modulation of AMPA receptors by calcium-dependent processes in streptozotocin-induced diabetic rats. Brain Res. 768, 249–256.

Cryer, P.E., Fisher, J.N., Shamoon, H., 1994. Hypoglycemia. Diabetes Care 17, 734–755.

Di Luca, M., Ruts, L., Gardoni, F., Cattabeni, F., Biessels, G.J., Gispen, W.H., 1999. NMDA receptor subunits are modified transcriptionally and post-translationally in the brain of streptozotocin-diabetic rats. Diabetologia 42, 693–701.

Di Mario, U., Morano, S., Valle, E., Pozzessere, G., 1995. Electrophysiological alterations of the central nervous system in diabetes mellitus. Diabetes Metab. Rev. 11, 259–278.

Dyck, P.J., Kratz, K.M., Karnes, J.L., Litchy, W.J., Klein, R., Pach, J.M., Wilson, D.M., O'Brien, P.C., Melton, L.J., 1993. The prevalence by staged severity of various types of diabetic neuropathy, retinopathy, and nephropathy in a population-based cohort: the Rochester diabetic neuropathy study. Neurology 43, 817–824.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Fisher, S.A., Fischer, T.M., Carew, T.J., 1997. Multiple overlapping processes underlying short-term synaptic enhancement. Trends Neurosci. 20, 170–177.

Flood, J.F., Mooradian, A.D., Morley, J.E., 1990. Characteristics of learning and memory in streptozocin-induced diabetic mice. Diabetes 39, 1391–1398.

Gagne, J., Milot, M., Gelinas, S., Lahsaini, A., Trudeau, F., Martinoli, M.G., Massicotte, G., 1997. Binding properties of glutamate receptors in streptozotocin-induced diabetes in rats. Diabetes 46, 841–846.

Gardoni, F., Kamal, A., Bellone, C., Biessels, G.J., Ramakers, G.M., Cattabeni, F., Gispen, W.H., Di Luca, M., 2002. Effects of streptozotocin-diabetes on the hippocampal NMDA receptor complex in rats. J. Neurochem. 80, 438–447.

Gispen, W.H., Biessels, G.J., 2000. Cognition and synaptic plasticity in diabetes mellitus. Trends Neurosci. 23, 542–549.

Greene, D.A., Lattimer, S.A., Sima, A.A., 1987. Sorbitol, phosphoinositides, and sodium–potassium–ATPase in the pathogenesis of diabetic complications. N. Engl. J. Med. 316, 599–606.

Hall, K.E., Sima, A.A.F., Wiley, J.W., 1995. Voltage-dependent calcium currents are enhanced in dorsal root ganglion neurones from the Bio Bred/Worchester diabetic rat. J. Physiol. (Lond.) 486, 313–322.

Hall, K.E., Liu, J., Sima, A.A., Wiley, J.W., 2001. Impaired inhibitory G-protein function contributes to increased calcium currents in rats with diabetic neuropathy. J. Neurophysiol. 86, 760–770.

Hoffmeister, F., Belleman, H.P., Benz, U., Van den Kerckhof, W., 1985. Psychotrophic actions of nimodipine. In: Betz, E., Deck, K., Hoffmeister, F. (Eds.), Nimodipine: Pharmacological and Clinical Properties. F.K. Schattauer Verlag, Stuttgart, pp. 77–99.

Kamal, A., Biessels, G.J., Urban, I.J.A., Gispen, W.H., 1999. Hippocampal synaptic plasticity in streptozotocin-diabetic rats: impairment of long-term potentiation and facilitation of long-term depression. Neuroscience 90, 737–745.

Kamal, A., Biessels, G.J., Duis, S.E.J., Gispen, W.H., 2000. Learning and hippocampal synaptic plasticity in streptozotocin-diabetic rats: interaction of diabetes and ageing. Diabetologia 43, 500–506.

Kappelle, A.C., Biessels, G.J., Van Buren, T., Erkelens, D.W., de Wildt, D.J., Gispen, W.H., 1993. Effects of nimodipine on sciatic nerve blood flow and vasa nervorum responsiveness in the diabetic rat. Eur. J. Pharmacol. 250, 43–49.

Kappelle, A.C., Biessels, G.J., Bravenboer, B., Van Buren, T., Traber, J., de Wildt, D.J., Gispen, W.H., 1994. Beneficial effect of the Ca^{2+} antagonist, nimodipine, on existing diabetic neuropathy in the BB/Wor rat. Br. J. Pharmacol. 111, 887–893.

Khachaturian, Z.S., 1994. Calcium hypothesis of Alzheimer's disease and brain aging. Ann. N.Y. Acad. Sci. 747, 1–11.

Kostyuk, E., Pronchuk, N., Shmigol, A., 1995. Calcium signal prolongation in sensory neurones of mice with experimental diabetes. NeuroReport 6, 1010–1012.

Kostyuk, E., Svichar, N., Shishkin, V., Kostyuk, P., 1999. Role of mitochondrial dysfunction in calcium signalling alterations in dorsal root ganglion neurons of mice with experimentally-induced diabetes. Neuroscience 90, 535–541.

Kostyuk, E., Voitenko, N., Kruglikov, I., Shmigol, A., Shishkin, V., Efimov, A., Kostyuk, P., 2001. Diabetes-induced changes in calcium homeostasis and the effects of calcium channel blockers in rat and mice nociceptive neurons. Diabetologia 44, 1302–1309.

Kristian, T., Siesjo, B.K., 1996. Calcium-related damage in ischemia. Life Sci. 59, 357–367.

Kristian, T., Siesjo, B.K., 1998. Calcium in ischemic cell death. Stroke 29, 705–718.

Kumagai, A.K., 1999. Glucose transport in brain and retina: implications in the management and complications of diabetes. Diabetes Metab. Res. Rev. 15, 261–273.

Kumar, J.S., Menon, V.P., 1993. Effect of diabetes on levels of lipid peroxides and glycolipids in rat brain. Metabolism 42, 1435–1439.

Landfield, P.W., 1994. Increased hippocampal Ca2+ channel activity in brain aging and dementia. Hormonal and pharmacologic modulation. Ann. N.Y. Acad. Sci. 747, 351–364.

Leibson, C.L., Rocca, W.A., Hanson, V.A., Cha, R., Kokmen, E., O'Brien, P.C., Palumbo, P.J., 1997. Risk of dementia among persons with diabetes mellitus: a population-based cohort study. Am. J. Epidemiol. 145, 301–308.

Levy, J., Gavin, J.R., Sowers, J.R., 1994. Diabetes mellitus: a disease of abnormal cellular calcium metabolism? Am. J. Med. 96, 260–273.

Lowery, J.M., Eichberg, J., Saubermann, A.J., LoPachin, R.M.J., 1990. Distribution of elements and water in peripheral nerve of streptozocin-induced diabetic rats. Diabetes 39, 1498–1503.

Luiten, P.G.M., de-Jong, G.I., Schuurman, T., 1994. Cerebrovascular, neuronal, and behavioral effects of long-term Ca^{2+} channel blockade in aging normotensive and hypertensive rat strains. Ann. N.Y. Acad. Sci. 747, 431–451.

Malenka, R.C., Nicoll, R.A., 1999. Long-term potentiation—a decade of progress? Science 285, 1870–1874.

Mankovsky, B.N., Metzger, B.E., Molitch, M.E., Biller, J., 1997. Cerebrovascular disorders in patients with diabetes mellitus. J. Diabetes Its Complicat. 10, 228–242.

Mattson, M.P., Duan, W., Pedersen, W.A., Culmsee, C., 2001. Neurodegenerative disorders and ischemic brain diseases. Apoptosis 6, 69–81.

Morano, S., Sensi, M., Di Gregorio, S., Pozzessere, G., Petrucci, A.F.G., Valle, E., Pugliese, G., Caltabiano, V., Vetri, M., Di Mario, U., Purrello, F., 1996. Peripheral, but not central, nervous system abnormalities are reversed by pancreatic islet transplantation in diabetic Lewis rats. Eur. J. Neurosci. 8, 1117–1123.

Ohsawa, M., Kamei, J., 1999. Role of intracellular calcium in thermal allodynia and hyperalgesia in diabetic mice. Brain Res. 833, 278–281.

Ott, A., Stolk, R.P., Van Harskamp, F., Pols, H.A., Hofman, A., Breteler, M.M., 1999. Diabetes mellitus and the risk of dementia: the Rotterdam Study. Neurology 53, 1937–1942.

Peress, N.S., Kane, W.C., Aronson, S.M., 1973. Central nervous system findings in a tenth decade autopsy population. Prog. Brain Res. 40, 473–483.

Robertson, S., Cameron, N.E., Cotter, M.A., 1992. The effect of the calcium antagonist nifedipine on peripheral nerve function in streptozotocin-diabetic rats. Diabetologia 35, 1113–1117.

Ross, M.A., 1993. Neuropathies associated with diabetes. Med. Clin. N. Am. 77, 111–124.

Ryan, C.M., 1988. Neurobehavioral complications of type I diabetes. Examination of possible risk factors. Diabetes Care 11, 86–93.

Schnedl, W.J., Ferber, S., Johnson, J.H., Newgard, C.B., 1994. STZ transport and cytotoxicity. Specific enhancement in GLUT2-expressing cells. Diabetes 43, 1326–1333.

Scriabine, A., Schuurman, T., Traber, J., 1989. Pharmacological basis for the use of nimodipine in central nervous system disorders. FASEB J. 3, 1799–1806.

Shafrir, E., 1997. Diabetes in animals: contribution to the understanding of diabetes by study of its etiopathology in animal models. In: Porte, Jr., D., Sherwin, R.S. (Eds.), Ellenberg and Rifkin's Diabetes Mellitus; Theory and Practice. Appleton & Lange, Stamford, pp. 301–348.

Sheen, V.L., Dreyer, E.B., Macklis, J.D., 1992. Calcium-mediated neuronal degeneration following singlet oxygen production. NeuroReport 3, 705–708.

Sima, A.A., Sugimoto, K., 1999. Experimental diabetic neuropathy: an update. Diabetologia 42, 773–788.

Sima, A.A., Lattimer, S.A., Yagihashi, S., Greene, D.A., 1986. Axo-glial dysjunction. A novel structural lesion that accounts for poorly reversible slowing of nerve conduction in the spontaneously diabetic bio-breeding rat. J. Clin. Invest. 77, 474–484.

Sima, A.A., Zhang, W., Xu, G., Sugimoto, K., Guberski, D., Yorek, M.A., 2000. A comparison of diabetic polyneuropathy in type II diabetic BBZDR/Wor rats and in type I diabetic BB/Wor rats. Diabetologia 43, 786–793.

Singh, R., Barden, A., Mori, T., Beilin, L., 2001. Advanced glycation end-products: a review. Diabetologia 44, 129–146.

Spallone, V., Uccioli, L., Menzinger, G., 1995. Diabetic autonomic neuropathy. Diabetes Metab. Rev. 11, 227–258.

Sredy, J., Sawicki, D.R., Notvest, R.R., 1991. Polyol pathway activity in nervous tissues of diabetic and galactose-fed rats: effect of dietary galactose withdrawal or tolrestat intervention therapy. J. Diabetes Its Complicat. 5, 42–47.

Stewart, R., Liolitsa, D., 1999. Type 2 diabetes mellitus, cognitive impairment and dementia. Diabet. Med. 16, 93–112.

Tesfaye, S., Stevens, L.K., Stephenson, J.M., Fuller, J.H., Plater, M., Ionescu-Tirgoviste, C., Nuber, A., Pozza, G., Ward, J.D., 1996. Prevalence of diabetic peripheral neuropathy and its relation to glycaemic control and potential risk factors: the EURODIAB IDDM complications study. Diabetologia 39, 1377–1384.

Van Dam, P.S., Van Asbeck, B.S., Erkelens, D.W., Marx, J.J.M., Gispen, W.H., Bravenboer, B., 1995. The role of oxidative stress in neuropathy and other diabetic complications. Diabetes Metab. Rev. 11, 181–192.

Vlassara, H., Brownlee, M., Cerami, A., 1983. Excessive non enzymatic glycosylation of peripheral and central nervous system myelin components in diabetic rats. Diabetes 32, 670–674.

Voitenko, N.V., Kostyuk, E.P., Kruglikov, I.A., Kostyuk, P.G., 1999. Changes in calcium signalling in dorsal horn neurons in rats with streptozotocin-induced diabetes. Neuroscience 94, 887–890.

Voitenko, N.V., Kruglikov, I.A., Kostyuk, E.P., Kostyuk, P.G., 2000. Effect of streptozotocin-induced diabetes on the activity of calcium channels in rat dorsal horn neurons. Neuroscience 95, 519–524.

Yagihashi, S., 1995. Pathology and pathogenetic mechanisms of diabetic neuropathy. Diabetes Metab. Rev. 11, 193–225.

Yang, S.N., Tang, Y.G., Zucker, R.S., 1999. Selective induction of LTP and LTD by postsynaptic [Ca2+]i elevation. J. Neurophysiol. 81, 781–787.

Zucker, R.S., 1989. Short-term synaptic plasticity. Annu. Rev. Neurosci. 12, 13–31.