

Abstracts of the International Diabetes Federation Congress Fifth Diabetic Neuropathy Satellite Symposium

October 31 – November 4, 2000
San José, Costa Rica

David R. Tomlinson, Chairman of Organizing Committee
Soroku Yagihashi, Chairman of Scientific Committee

FUTURE PROGRESS IN DIABETIC NEUROPATHY

Ward JD. Sheffield, UK.

Over three decades much knowledge regarding the pathogenesis of diabetic neuropathy has been gathered but sadly this has led to very little understanding. This final review lecture of the symposium will attempt to analyse why progress has been so slow and look to the future for signs of inventive improvement. Papers presented during the symposium will be reviewed to allow estimate of future success and where perhaps efforts should be focused. At present the best we can do for our patients is to control blood glucose as rigidly as possible for preventative therapy and logical interventional drugs are not available. Hopefully the symposium will provide new insights into future investigations and potential pathways.

ELECTROPHYSIOLOGICAL CHANGES IN DIABETIC NEUROPATHY: FROM SUBCLINICAL ALTERATIONS TO DISABLING ABNORMALITIES

Baba M. Department of Neurology, Hirosaki University School of Medicine, Hirosaki 036-8216, Japan.

Clinical spectrum of diabetic neuropathy is variable; it may be asymptomatic, but once established, it becomes irreversible and disabling. Some investigators suggested that earliest change in diabetic nerve function is alteration in axonal excitability due to alterations in ion conductance of axon membrane, although these functional changes of ion channels necessarily cause permanent damage or degeneration of nerve fibers. Among various parameter of nerve conduction study in diabetics, prolonged F-wave latency in the peroneal and tibial nerve seems the commonest abnormality in asymptomatic patients. Decrease in amplitude of compound sensory action potential of sural nerve is another earlier abnormality, which is, then, accompanied by a fall in motor amplitude of peroneal and tibial nerves in advanced patients. In disabled patients no motor response is often elicited in the legs. Previous electrophysiological studies could not make clear if central axons were involved or not in diabetic neuropathy. Recently, our group has demonstrated that somatosensory central conduction from the spinal

cord to the sensory cortex is delayed in diabetics as well as in the peripheral conduction, which might be partly responsible for the irreversible clinical presentation of diabetic neuropathy.

PAINFUL DIABETIC NEUROPATHY - A THERAPEUTIC CHALLENGE FOR THE NEW MILLENNIUM

Boulton AJM. Manchester Royal Infirmary, Manchester, UK.

Although less than 20% of patients with diabetic neuropathy experience severe symptoms, the management of painful diabetic neuropathy presents a major therapeutic challenge. Pain may occur as a feature not only of chronic or acute sensory neuropathy, but also the focal neuropathies, however, this lecture will focus on pain in symmetrical distal polyneuropathy. A number of theories exist to explain the mechanisms of pain in diabetic neuropathy: these include ectopic impulse formation by regenerating sprouting axons, various neuro structural theories and, more recently, ischaemia of peripheral nerve due to active epineurial arterio venous shunting. A number of scoring systems exist to assess neuropathic pain: which ever is used, it must rely upon the patient's description of the painful symptoms they are experiencing. A therapeutic approach to neuropathic pain should firstly exclude non-diabetic causes of neuropathy, and then aim to obtain optimal stable glycaemic control. A number of therapeutic agents exist that have been shown in controlled trials to alleviate painful neuropathic symptoms; these include the tricyclic drugs, newer anti convulsant agents such as Gabapentin and Topiramate, and also the centrally-acting drug Tramadol. Some evidence exists for the efficacy of the topical agent Capsaicin. Other promising new agents include alpha lipoic acid. A number of therapies may well be helpful although unproven in controlled trials, and these include acupuncture and electrical spinal cord stimulation.

ALPHA-LIPOIC ACID EFFECTS AND COMBINATION THERAPY WITH GAMMA-LINOLENIC ACID

Cameron NE, Cotter MA. Biomedical Sciences, University of Aberdeen, Scotland.

Alpha-lipoic acid (LPA) has marked effects in experimental diabetic neuropathy. Treatment for 2 weeks after 8 weeks of diabetes in rats corrected motor and sensory conduction velocity and endoneurial perfusion deficits with an ED₅₀ of approximately 40 mg/kg. There was no difference in efficacy between R- and S-enantiomers of LPA. When combined with the n-6 essential fatty acid, gamma-linolenic acid (GLA), either as a drug mixture or joint compound, there was a synergistic effect on neurovascular function, resulting in a 6-fold increase in treatment efficacy. LPA has wide-ranging actions on markers of endothelial damage, the coagulation system and lipids that are important risk factors for neuropathy and vascular disease in patients. Thus, 2 months of diabetes in rats caused 2.5- to 5.4-fold elevations of von Willebrand factor, factor VII, triglycerides and LDL cholesterol, which were 41-64% attenuated by 2 weeks of LPA treatment. Vascular effects are not restricted to endoneurial perfusion. Thus, the time taken for a fixed-volume tail-bleed was 4.2-fold greater with diabetes and this was 79% attenuated by LPA treatment. Nitric oxide and endothelium-derived hyperpolarizing factor dependent vasodilation were markedly reduced by diabetes in corpus cavernosum and mesenteric vasculature, LPA giving a high degree of protection. The function of small nerve fibres, exemplified by the nitroergic vasodilator innervation to corpus cavernosum, is greatly diminished by diabetes, high-dose LPA having a protective effect. When LPA was combined with GLA, as for large fibres, there was a marked synergy for both the innervation and the endothelium of corpus cavernosum. Thus LPA alone, and particularly combined with GLA, has a broad spectrum of action highly relevant for the treatment of diabetic neuropathy and micro and macrovascular disease.

SIGNAL TRANSDUCTION BY STRESS-ACTIVATED MAP KINASES

Davis RJ. Howard Hughes Medical Institute & Program in Molecular Medicine, University of Massachusetts Medical School, 373 Plantation Street, Worcester, MA 01605.

The JNK group of stress-activated MAP kinases consists of ten protein kinases that phosphorylate the NH₂-terminal activation domain of c-Jun on Ser-63 and Ser-73 causing increased transcriptional activity. JNK protein kinase activity is increased in response to treatment of cells with pro-inflammatory cytokines or exposure to environmental stress. Activated JNK is phosphorylated on Thr and Tyr within the tripeptide motif Thr-Pro-Tyr located in kinase subdomain VIII. Mutational analysis demonstrates that JNK activation requires the phosphorylation of both Thr and Tyr within this motif. This phosphorylation is mediated by dual specificity protein kinases, including MKK4 and MKK7. The function of the JNK signaling pathway has been studied using a combination of biochemical and genetic approaches. Genetic analysis of JNK signaling in *Drosophila* demonstrates that the JNK signaling pathway is required for early embryonic morphogenesis. Similarly, disruption of the JNK signaling pathway in mice using homologous recombination demonstrates that JNK signaling is required for embryonic viability. In contrast, mice with genetically engineered selective defects in JNK signaling are viable, but exhibit changes

in stress-induced gene expression and apoptosis. These studies provide insight into the role of the JNK stress-activated MAP kinase pathway in the cellular response to environmental stress, including radiation.

THE RISK FACTORS FOR DIABETIC COMPLICATIONS

Dyck PJ. Mayo Clinic, 200 First Street SW, Rochester, MN 55905.

The major late complications of diabetes mellitus can be classified into those attributable to atherosclerosis (heart attacks, stroke, and peripheral vascular disease) and to microvascular disease (diabetic polyneuropathy [DPN], retinopathy, and nephropathy). The risk factors for diabetic polyneuropathy had not previously been adequately assessed by: 1) assessing representative population-based cohorts; 2) assessing DPN comprehensively and quantitatively; 3) expressing nerve tests as a percentile and normal deviate value correcting for anthropometric characteristics as based on study of a randomly selected normal subject cohort from which neurologic disease had been excluded; 4) assessing risk factors serially over time; and 5) performing multivariate analysis of risk factors. For DPN we have found that the risk factors are: 1) markers of diabetic microvessel disease (diabetic retinopathy, 24-hour proteinuria, or 24-hour microalbuminuria); 2) chronic hyperglycemic exposure; and 3) type of DM. Excluding markers of microvessel disease and type of DM we found that the highest correlation coefficients were obtained by glycated hemoglobin (GH) \times duration of DM $1/2 \times 18/\text{age}$ of onset DM (lowest score = 1) \times total plasma antioxidant activity. Similarly, major determinants for diabetic retinopathy and nephropathy also are GH \times duration of DM. These results provide strong supportive evidence that the product of chronic hyperglycemic (especially GH) exposure \times duration of DM are the main determinants of the diabetic complications of retinopathy, nephropathy, and neuropathy.

NEUROTROPHINS IN DIABETIC NEUROPATHY

Fernyhough P. Division of Neuroscience, School of Biological Sciences, University of Manchester, UK.

The recent Genentech/Roche sponsored FDA phase III clinical trial of nerve growth factor (NGF) for the treatment of diabetic neuropathy failed. The aim of this talk is to discuss the reasons for this failure and based upon the lessons learned identify if there remain any other viable avenues for studies on neurotrophins in diabetic neuropathy. The presentation will briefly introduce the basic biology of the neurotrophins and their putative role in the aetiology and/or treatment of diabetic neuropathy. Some details of the NGF clinical trials will be presented and reasons for failure assessed. The main part of the talk will focus on future strategies for neurotrophin work with an emphasis on the possible roles of neurotrophin-3 and brain-derived neurotrophic factor. Alternative approaches based upon regulation of endogenous neurotrophin expression and use of neurotrophin trk receptor agonists will also be discussed. The aim of the talk is to show that lessons learned from the failed NGF trial should lead to a re-invigora-

tion of studies directed at determining alternative methods for optimising neurotrophic support of sensory neurones in diabetes.

THE MOLECULAR MECHANISM OF THE ORGANELLE TRANSPORTS IN NEURONS; KINESIN SUPERFAMILY PROTEINS (KIFs), STRUCTURE, GENE, AND FUNCTION

Hirokawa N. Department of Cell Biology and Anatomy, University of Tokyo, Graduate School of Medicine.

Many proteins are transported to their proper destination as membranous organelles or protein complexes after synthesis. Especially in highly polarized cells such as epithelial cells and neurons this transport is very important for the proper targeting of proteins to distinct parts of cells. Actually we visualized dynamics of membrane organelles carrying certain kinds of proteins in living cells using GFP technology. In order to understand the mechanism of this transport we identified 26 new members of microtubule based molecular motors, KIFs. Using multiple molecular cell biological and molecular genetical approaches we have characterized these new members. In this lecture I will focus on some of KIF members such as KIF1A, 1B, 3, C2 and 17. KIF1A is the fastest (1.5 $\mu\text{m}/\text{sec}$) anterograde monomeric motor for transport of precursor of synaptic vesicles and essential for neuronal function and survival while KIF1B is a unique monomeric anterograde motor (0.5 $\mu\text{m}/\text{sec}$) for transport of mitochondria. KIF3A and KIF3B, expressed ubiquitously, form a heterodimer associated with a protein, KAP3, and work as a new anterograde transporter for membranous organelles which are different from synaptic vesicle precursors and essential for neurite extension. KIF2 is a neuron specific C-terminal motor domain type KIF transporting multivesicular body-like organelles to dendrites. Very recently we have identified new KIF17 which is expressed specifically in neurons and transport NMDA receptors in nerve dendrites. Thus, our studies revealed that transport of important functional molecules as various kinds of membrane organelles and protein complexes is accomplished very precisely by these new molecular motors.

DIABETIC NEUROPATHY: PATHOGENETIC MECHANISMS AND TREATMENT IMPLICATIONS

Low PA¹, Nickander KK¹, Kihara M¹, Schmelzer JD¹, Nagamatsu M¹, Sasaki H¹, Tritschler H-J². ¹Dept of Neurology, Mayo Clinic, ²Rochester MN, USA, ²Asta Medica, FRG.

Chronic hyperglycemia underlies the pathogenesis of diabetic neuropathy (DN). Recent research emphasizes the roles of ischemia-hypoxia, oxidative stress, glycation, $\omega 6$ fatty acid metabolism and the interacting roles of excessive lipolysis, hyperactive polyol pathway and PKC activity and deficiencies of growth factors. In DN, oxidative stress is due primarily to nerve ischemia and auto-oxidation/glycation, but all the above mechanisms are interactive and all can cause oxidative stress. Oxidative stress appears to be a particularly important mechanism in the pathogenesis of DN. Antioxidant enzymes are reduced in peripheral nerve and are further

reduced in DN. That lipid peroxidation will cause neuropathy is supported by the development of neuropathy de novo when normal nerve is rendered α -tocopherol deficient. Recent studies emphasize the prominent pathological alterations in dorsal root ganglion (DRG) and nerve roots of experimental DN. DRG mitochondrial cytopathy is suggested to result. Histochemical studies of DRG shows defective respiratory enzyme activity, TUNEL staining and caspase-3 immunostaining, suggesting apoptosis, especially apoptosis lente, a process that results in impairment of function with modest and slow cellular loss. These pathogenetic observations have spawned clinical trials with aldose reductase inhibitors, growth factor, protein glycation, γ -linolenic acid, PKC- β , and α -lipoic acid. Clinical trials on human diabetic neuropathy have been underpowered, especially with the short duration of the studies. Treatment with antioxidants have been especially efficacious in experimental diabetes, mainly in a preventive role. In established human DN, a minimum of 2 years is likely required to demonstrate benefit. A 4 year study of α -lipoic acid in human DN is underway.

AUTONOMIC NEUROPATHY IN SORBITOL DEHYDROGENASE INHIBITOR (SDI)-TREATED STREPTOZOTOCIN-DIABETIC RATS

Schmidt RE. Washington University School of Medicine, Saint Louis, MO USA.

We have developed an animal model of diabetic autonomic neuropathy characterized by neuroaxonal dystrophy (NAD) involving ileal mesenteric nerves and prevertebral sympathetic superior mesenteric ganglia (SMG) in chronic streptozotocin-diabetic rats. We have examined the effect of SDI-158, which interrupts the conversion of sorbitol to fructose (and reactions dependent on the second step of the polyol pathway), on NAD in control and diabetic rats. SDI-treatment (SDI-Rx) did not produce NAD in control animals despite the fact that sciatic nerve sorbitol levels reached the same levels as untreated diabetic animals. SDI-Rx resulted in a dramatically increased frequency of NAD in ileal mesenteric nerves and SMG. SDI-Rx diabetic rats developed lesions prematurely, after only one month of diabetes, and in greater numbers than untreated diabetics. SDI-Rx for 1,2,3 and 6 months showed a statistically significant but progressively smaller effect on NAD. The ultrastructural appearance of SDI-Rx ganglia and mesenteric nerves were identical to that previously reported in long-term untreated diabetics; however, short term SDI-Rx resulted in less compacted tubulovesicular elements in the SMG. Three month SDI-Rx superior cervical ganglia failed to develop NAD in the same animals in which large numbers of lesions were found in the SMG. Dystrophic axons involved ileal mesenteric nerves while sparing those to the jejunum. Dystrophic tyrosine hydroxylase immunoreactive sympathetic axons were distributed to myenteric and submucosal ganglia within the gut wall but not to the mesenteric vasculature. Therefore, although SDI-Rx diabetic rats show an exaggerated severity and accelerated time course of NAD compared to untreated diabetics, lesion appearance, immunoreactivity and distribution are comparable to those found in untreated STZ-diabetic rats of much longer durations.

EVALUATION OF DIABETIC NEUROPATHY BY SKIN BIOPSY

Yasuda H. Third Department of Medicine, Shiga University of Medical Science, Otsu, Shiga 520-2192, Japan.

Instead of nerve biopsy which is most reliable method for diagnosing diabetic neuropathy but rather harmful, we have examined the usefulness of skin biopsy to evaluate the grade of diabetic neuropathy and therapeutic effects of given compounds. Nerve fibers immunostained by antibodies against protein gene product (PGP)9.5 and labeled with streptavidin fluorescein isothiocyanate and measured under confocal laser scanning microscopy were significantly shorter in the epidermis and dermis and around sweat glands in diabetic patients than in healthy subjects. Sural nerve conduction velocity was significantly correlated with dermal nerve fiber length (NFL) ($p < 0.05$) in diabetic patients. Patients with higher aldose reductase (AR) level of erythrocytes (>10.8 ng/Hg) showed shorter dermal NFL than those with lower AR level (<10.8) ($p < 0.05$). Using this technique, we evaluated the therapeutic effect of AR inhibitor (ARI) epalrestat during approximately 13 months in diabetic patients with neuropathy (19, epalrestat-treated; 12, control). The number of patients whose percent change of dermal NFL exceeded its mean \pm 2SD in the control group was significantly higher in the ARI group than in the control group ($p < 0.05$, 8/9 vs 1/12). These results suggest that quantitation of cutaneous nerves using biopsied skin samples may provide important information about the severity of diabetic neuropathy and that epalrestat can elongate dermal unmyelinated nerve fibers in at least part of diabetic patients.

CURRENT EVIDENCE FOR TREATING DIABETIC NEUROPATHY

Ziegler D. German Diabetes Research Institute at the Heinrich Heine University, Düsseldorf, Germany.

At least one of three diabetic patients is affected by polyneuropathy which represents a major health problem, as it may present with partly excruciating neuropathic pain and is responsible for substantial morbidity, increased mortality, and impaired quality of life. Treatment is based on four cornerstones: 1.) causal treatment aimed at (near)-normoglycaemia, 2.) treatment based on pathogenetic mechanisms, 3.) symptomatic treatment, and 4.) avoidance of risk factors and complications. Recent experimental studies suggest a multifactorial pathogenesis of diabetic neuropathy. Several pathogenetic mechanisms are being discussed which, however, in contrast to previous years are no longer regarded as separate hypotheses but in the first place as a complex interplay with multiple interactions between metabolic and vascular factors. From the clinical point of view it is important to note that, based on these pathogenetic mechanisms, therapeutic approaches could be derived, some of which are currently being evaluated in clinical studies. These treatments include the inhibition of the increased flux through the polyol pathway by aldose reductase inhibitors, correction of the deficits in essential fatty acid and prostanoid metabolism by substitution of γ -linolenic acid contained in evening primrose oil, administration of antioxidants (α -lipoic acid) to reduce the enhanced

formation of reactive oxygen species that induce increased oxidative stress, improvement in endoneurial blood flow and resulting hypoxia by vasodilating agents such as ACE inhibitors and protein kinase C (PKC) β inhibitors, neurotrophic support by administration of NGF, inhibition of non-enzymatic glycation and formation of AGEs, correction of C-peptide deficiency by substitution of C-peptide, and immunosuppressive treatment. Currently only α -lipoic acid is available for treatment in several countries. Randomized controlled clinical trials using this agent have demonstrated that 1.) short-term treatment for 3 weeks (600 mg/day i.v.) reduces the chief symptoms and deficits due to diabetic polyneuropathy. 2.) Oral treatment for 4-7 months tends to ameliorate neuropathic deficits and cardiac autonomic neuropathy. 3.) Preliminary data over 2 years indicate possible long-term improvement in motor and sensory nerve conduction in the lower limbs. Clinical and postmarketing surveillance studies have revealed a highly favorable safety profile of the drug. Despite the recently accelerating publication rate for controlled trials demonstrating significant pain relief with several agents, the pharmacologic treatment of chronic painful diabetic neuropathy remains a challenge for the physician. A simple measure of therapeutic efficacy (number needed to treat: NNT) permits to estimate the risk-benefit-ratio for each agent on the basis of the available controlled trials. In recent meta-analyses, the NNTs have been calculated for several drugs employed in the treatment of painful diabetic neuropathy which may serve the physician in deciding for the individual treatment. Epidemiological data indicate that not only increased alcohol consumption but also the traditional cardiovascular risk factors such as hypertension, smoking, and cholesterol play a role in development and progression of diabetic neuropathy and, hence, need to be prevented or treated.

METABOLIC VS. VASCULAR ABNORMALITIES IN DIABETIC NEUROPATHY

Greene D.

The DCCT clearly implicates hyperglycemia as an important contributory factor in the pathogenesis of diabetic polyneuropathy, a multifaceted set of clinical syndromes reflective of widespread damage to peripheral nerve fibers. The biochemical cascade stemming from hyperglycemia and responsible for this disorder remains controversial. Recently published clinical trials in diabetic neuropathy with aldose reductase inhibitors implicate the aldose reductase pathway as an important component in this process. Activation of the aldose reductase pathway by hyperglycemia produces secondary derangements in $\text{NAD(P)}^+:\text{NAD(P)H}$ ratios, depletion of alternative intracellular osmolytes such as taurine and myo-inositol, and accelerated protein glycation. These biochemical alterations in turn can lead to the formation of reactive oxygen species (ROS), as can glucose-related aldose-reductase-independent pathways such as auto-oxidation and protein glycation. Aldose reductase-dependent and aldose-reductase-independent ROS generation may directly damage end-organ tissues or their vascular support, producing ischemia. Mitochondria are damaged by ROS, mitochondrial ROS generation is increased by imbalances in substrate and oxygen availability, and mitochondrial defense against ROS-induced damage

requires adequate energy supply. Hence metabolic oxidative stress from glucose and ischemic injury both target the mitochondria. Mitochondrial damage is an important initiator of programmed cell death, or apoptosis, through the release of mitochondrial cytochromes, and activation of caspase enzymes. This cascade of events, stemming from hyperglycemia, operating through aldose-reductase-dependent and aldose-reductase-independent generation of ROS, involving mitochondrial damage and impaired vascular support and tissue ischemia, and resulting in apoptosis, appears to play a potentially important role in the pathogenesis of chronic diabetic complications such as peripheral neuropathy.

INCREASED CML DEPOSITION, RAGE EXPRESSION, AND NF- κ B ACTIVATION IN DIABETIC NEUROPATHY

Bierhaus A, Haslbeck KM², Morcos M¹, Schiekofer S, Andrassy M, Chen J, Humpert PM, Möller W³, Tritschler H³, Dehmer T, Neundörfer B², Heuss D², Schwaninger M¹, Häring H-U, Schleicher E, Nawroth PP. Sect. Vaskuläre Medizin, Med. Klinik IV und Inst. für Neuropharm. Univ. Tübingen; ¹Med. Klinik I und Neurolog. Klinik, Univ. Heidelberg; ²Neurol. Klinik Universität Erlangen-Nürnberg; ³ASTA-Medica³ GmbH Frankfurt.

There is growing evidence that upregulation of NF- κ B-controlled adhesion molecules and cytokines contributes to neuropathic pain and diabetic neuropathy. Since AGE/RAGE-interactions activate NF- κ B, we asked whether deposition of the defined AGE-adduct CML, upregulation of RAGE, and NF- κ B activation is evident in peripheral nerves in human and animal models of diabetes mellitus. When immunohistology was performed on sural nerve biopsies from diabetic patients, local accumulation of CML, prominent upregulation of RAGE and strong activation of NF- κ B was observed in the perineurium and the vascular endothelium. This colocalisation was not observed in control patients. Schwann cells also demonstrated increased RAGE expression, but no staining for CML and NF- κ B. In mouse models of diabetes mellitus, histological examination confirmed upregulation of NF- κ B in the perineurium, but was also evident in Schwann cells. In the mouse model, NF- κ B activation could be reversed by the antioxidant α -lipoic acid. These data demonstrate that NF- κ B activation occurs in hyperglycemia, but affects different neuronal cells in human and mice. Hyperglycemia dependent activation of NF- κ B results in enhanced NF- κ B dependent gene expression in transgenic mice, carrying a β -globin reporter transgene controlled by NF- κ B. Infusion of AGE-albumin also led to induction of the β -globin-gene and could be blocked in the presence of soluble RAGE, neutralizing anti-RAGE-antibodies and α -lipoic acid. These studies provide evidence that the AGE-RAGE-NF- κ B axis is upregulated in diabetic neuropathy. NF- κ B-activation and subsequent induction of NF- κ B-dependent gene expression can be reduced by treatment with α -lipoic acid.

EARLY DETECTION OF AUTONOMIC NEUROPATHY IN DIABETIC MINIPIGS BY BLOOD PRESSURE AND HEART RATE VARIABILITY: RELATIONSHIP WITH CHANGES IN VAGUS NERVE MORPHOMETRY

Mésangeau D¹, Laroche S¹, Laude D², Elghozi JL², Malik RA³, Walker D³, Siddique I³, Tomlinson DR³. ¹Merck-Lipha Research, France, ²CNRS UMR 8604, Paris, France, ³University of Manchester, U.K.

Early detection of cardiac autonomic neuropathy (CAN) is imperative for successful therapeutic intervention. The streptozotocin-diabetic minipig is a suitable model to study cardiac function and determine the earliest markers of the condition. Blood Pressure (BP) and Heart Rate (HR) variability, analysed with spectral analysis and performed in control animals exhibited a mostly unique peak synchronized with respiration. The Respiratory Peak (RP) of HR was dramatically reduced by atropine, suggesting a vagal control of this oscillation. A pharmacological baroreflex curve was also constructed, and the gain of this curve was found significantly related ($r = 0.70$, $p < 0.05$) with the gain of the cross-spectral analysis between SBP and HR. Diabetes significantly decreased BP at 3 and 6 months and increased HR at 6 months (77.4 ± 3.8 vs 66.6 ± 2.7 bpm). The RP of HR was significantly lowered at 3 months (3.2 ± 0.4 vs 5.6 ± 0.7 bpm) and was still decreased at 6 months (3.7 ± 0.5 vs 4.9 ± 0.7 bpm). The gain of the cross spectral analysis was also reduced at the early stage of the disease (1.1 ± 0.2 vs 1.6 ± 0.4 bpm/mmHg, $p < 0.05$). Morphometric analysis revealed abnormalities only in the proximal vagus nerve with a significant difference in the axon and fibre size frequency distribution between controls and diabetic animals ($p = 0.05$), due to a greater proportion of fibers with smaller axon sizes ($<3 \mu\text{m}^2$) in diabetic minipigs. In conclusion this model exhibited CAN at an early stage of diabetes with an impairment of the baroreflex sensitivity associated with structural changes of proximal vagus nerve.

ACTIVATION OF MITOGEN ACTIVATED PROTEIN KINASES (MAPKS) IN RESPONSE TO HIGH GLUCOSE IN PRIMARY SENSORY NEURONES

Purves T, Fernyhough P and Tomlinson DR. Neuroscience Division, School of Biological Sciences, Manchester University, UK.

In diabetes high glucose stresses cells as a prelude to complications. The MAPKs are serine-threonine kinases, which are putative glucose stress transducers, comprising extracellular signal regulated kinases (ERKs), p38 and c-Jun, n-terminal kinases (JNKs). In 10 week streptozotocin-induced diabetic rats JNK activation was increased when compared to age matched controls. This study aimed to determine the signaling pathways activated in response to high glucose in adult sensory neurones *in vitro*. Cultures of adult rat dorsal root ganglia (DRG) were treated with 10mM, 25mM and 50mM glucose for 16 hours. MAPK activation was examined in Western blots using antibodies raised against phosphorylated and non-phosphorylated epitopes (results expressed as a ratio of phosphorylated to non-phosphorylated kinase). Glucose caused a concentration-dependent increase in phospho-p38 with a 1.6 fold increase at 25mM (0.77 ± 1.04) and a 2.4 fold increase at 50mM (1.18 ± 1.44) when compared to 10mM (0.49 ± 0.60) glucose. Phosphorylation of the p56 JNK isoform increased 2.4 fold (4.37 ± 3.59) and the p46 isoform 2.2 fold (1.95 ± 1.35) at 50mM glucose when compared to 10mM (p56 1.80 ± 0.99 , p46 $0.88 \pm$

0.31). ERK phosphorylation remained unchanged in 3 different experiments. Immunocytochemistry located these changes to neurones, rather than the small percentage of non-neurones that remain in culture. Transcription factor activation as a result of MAPK activation is being investigated using electrophoretic mobility shift assays. We conclude that the activation of MAPK pathways is involved in the response of neuronal cells to high glucose stress.

GLUCOSE-INDUCED MAP KINASE ACTIVATION AND ALDOSE REDUCTASE EXPRESSION IN PRIMARY SCHWANN CELL CULTURE

Mouchot C, Tomlinson DR. Neuroscience Division, School of Biological Sciences, University of Manchester, UK.

Diabetic hyperglycaemia creates biochemical alterations in nerve that lead to Wallerian degeneration, resulting in the modification of the Schwann cell phenotype. This suggests that the peripheral neuroglia could play a crucial role in diabetes-induced peripheral neuropathy. Hyperglycaemia-induced cellular stresses result in up-regulation of aldose reductase (AR) protein expression. This study aimed to determine whether activation of mitogen-activated protein kinases (MAPK) in Schwann cells *in vitro* might represent an early step in the transduction of hyperglycaemia to diabetes-induced increased AR protein expression. We observed MAPK activation (Western blots for JNK and p38) in response to raised glucose and MAPK responses were expressed as the ratio of phosphorylated to total form. Neonatal rat Schwann cell cultures were treated with glucose (50, 200mM) for up to 24 hours. Mannitol (45, 195mM) was used as an osmotic control. Glucose for 1 hour (200mM) caused transient activation of both p38 (1.9 fold) and JNK ($p < 0.01$ for p56 and p46) versus controls (5.6mM glucose). Similar results were seen for JNK in response to mannitol. The mannitol-induced p38 activation was sustained from 1 to 8 hours of treatment ($p < 0.01$ versus controls and $p < 0.05$ versus glucose treatment). Similarly, we observed a significant increase of AR content ($p < 0.01$ and $p < 0.05$ in response to glucose and mannitol, respectively). However, the maximum response occurred at 24 hours of treatment. Immunocytochemistry studies showed that activated JNK was located both in cytoplasm and nucleus, while accumulation of AR was mainly restricted to the cytoplasm surrounding the nucleus. These results suggest that JNK and/or p38 could transduce increased AR expression in response to high glucose, at least under *in vitro* conditions.

AGE-INDUCED NEUROPATHY IN RATS

Yagihashi S, Nishizawa Y, Baba M, Takeuchi M. Department of Pathology, Hirosaki University School of Medicine, Hirosaki, Department of Biochemistry, Hokuriku University School of Pharmacy, Kanazawa, Japan.

We studied the effects of exogenously administered advanced glycation end-products (AGE) on the peripheral nerve function and structure in normal rats. Normal Wistar rats aged 6 weeks were injected intraperitoneally with puri-

fied AGE (20 mg/kg/day) produced by incubation of glucose with bovine serum albumin (BSA) for 12 weeks. Control rats were treated with BSA alone. One of AGE-treated groups was co-treated with 50 mg/kg aminoguanidine (AG). During the experimental period, body weight and blood glucose levels were not affected in AGE-treated rats. Serum AGE levels were elevated two fold in AGE-treated group whereas BSA treated rats maintained normal levels, whereas tissue AGE levels in sciatic nerve were not increased in treated group. AG did not alter the levels of serum AGE. AGE-treated rats exhibited significant delay of motor nerve conduction velocity by 30% and reduction of sciatic nerve in Na,K-ATPase activity by 25% in AGE-treated rats. AG treatment significantly inhibited these changes. Immunostains on the cross-sections of sciatic nerve demonstrated significant increase in cells positive for 8 hydroxy-deoxyguanosine, a marker of oxidative stress-induced DNA injury, in AGE-treated group. AG treatment significantly inhibited this reaction. There was no difference in morphometric data on myelinated fibers in sural nerve among the experimental groups. AGE-injected rats thus showed the neuropathic changes, similar to those found in experimentally-induced diabetic animals and it is therefore suggested that AGE have a pathogenetic role in the development of diabetic neuropathy through induction of excessive oxidative stress.

Supported by Juvenile Diabetes Foundation International (1-2000-263), Japan Diabetes Foundation, Japanese Ministry of Science, Education, Sports and Culture.

MICROCIRCULATORY RESPONSES TO ELECTRICAL SPINAL CORD STIMULATION IN PAINFUL DIABETIC NEUROPATHY AND OTHER PAINFUL CONDITIONS

Harris ND¹, Eaton SEM¹, Selmi F¹, Patel KA¹, MacFarlane IA², Ward JD¹ and Tesfaye S.¹ ¹Royal Hallamshire Hospital, ²Sheffield and Walton Hospital, Liverpool, UK.

Electrical spinal cord stimulation (ESCS) has been used to provide pain relief in a number of conditions, including painful diabetic neuropathy (PDN). ESCS has also been shown to increase microvascular blood flow in peripheral vascular disease. If nerve hypoxia contributes to pain in PDN, ESCS may relieve this by increasing nerve blood flow. We have therefore investigated skin and sural nerve microvascular responses to ESCS. We studied subjects implanted with ESCS for pain relief, 4 had PDN and 7 were controls with other painful conditions. Blood flow, before and during stimulation, was assessed using Laser Doppler flowmetry. Only one (PDN) subject showed a statistically significant increase in skin blood flow during stimulation. The three remaining PDN subjects showed significant reductions in skin blood flow, as did 3/7 of controls. Sural nerve blood flow was measured on a separate occasion. During stimulation nerve blood flow increased in 1 (control) subject, decreased in 1 (PDN) subject and did not change in the other 5 tested (3 PDN and 2 control). In summary, ESCS did not produce any consistent increase in skin or nerve microvascular blood flow. ESCS reduces pain in a variety of different conditions, however this does not appear to be mediated by changes in blood flow. Until a thorough understanding of the patho-

genic mechanisms causing PDN is achieved, therapy will be limited to providing symptomatic relief.

CARDIOVASCULAR RISK FACTORS PREDICT THE DEVELOPMENT OF DIABETIC PERIPHERAL NEUROPATHY

Tesfaye S, Chaturvedi N, Eaton SEM, Ward JD and Fuller J. On behalf of the EURODIAB Prospective Study Group.

Hypertension has recently been shown to be an important determinant of diabetic retinopathy and nephropathy. The relationship between cardiovascular risk factors and the incidence of peripheral neuropathy (PN) was examined in type 1 diabetic subjects from 27 centres participating in the EURODIAB Prospective Complications Study. PN was assessed at baseline and follow-up using a standardised protocol involving combinations of neuropathic symptoms, tendon reflexes, age related vibration perception thresholds and autonomic function tests. Serum lipids/lipoproteins, HbA_{1c} and albumin excretion rate (AER) were measured in a central laboratory. Of 986 subjects with no PN at baseline (mean age 31 years; mean duration 13 years), 24.6% developed PN over the follow up period (average 7.3 years). The incidence of PN was significantly positively associated with age, duration of diabetes and HbA_{1c} at baseline. After statistical adjustment for these three factors the following baseline variables were significantly predictive of the development of PN; BMI, AER, triglyceride ($p < 0.001$), cholesterol and systolic BP ($p < 0.01$). This prospective study shows that over a 7-year period, about one quarter of type 1 diabetic patients will develop peripheral neuropathy; age, duration of diabetes and poor glycaemic control being major determinants. The development of PN is also associated with potentially modifiable cardiovascular risk factors such as serum lipids, BP, BMI and AER supporting risk reduction strategies in its prevention. Furthermore, these findings support the role of vascular factors in pathogenesis of PN.

SURAL NERVE HAEMODYNAMICS IN PAINFUL AND PAINLESS NEUROPATHY: CLUES TO THE CAUSE OF PAIN?

Eaton SEM, Ibrahim S, Harris ND, Selmi F, Patel KA, Tesfaye S and Ward JD. Royal Hallamshire Hospital, Sheffield, S10 2JF, UK.

Despite extensive research the cause of painful diabetic neuropathy remains elusive. Vascular factors and nerve hypoxia are known to be important in the pathogenesis of diabetic neuropathy although their contribution to painful neuropathy has not been fully determined. Using a combination of microlightguide spectrophotometry and fluorescein angiography we have recently confirmed that sural nerve intracapillary oxygen saturation and blood flow are reduced in diabetic neuropathy. We have applied these techniques to examine differences between painful and painless diabetic neuropathy by comparing 11 patients with pain to 8 patients without. There were no significant differences in neurophysiological parameters in each group. Intracapillary oxygen saturation (%HbO₂) was significantly higher in those subjects with painful symptoms compared to those without (median val-

ues 73.8% vs 67.7%, $p < 0.05$). Fluorescein rise time (FRT) was also significantly faster in those with painful neuropathy (18.3 vs 53.6 seconds, $p < 0.05$). These results indicate that there are distinct differences in sural nerve haemodynamics between painful and painless neuropathy. The higher %HbO₂ and faster FRT suggest the nerve is not hypoxic in painful neuropathy. These results would suggest that haemodynamic factors might have an important role in the pathogenesis of neuropathic pain.

EVIDENCE OF SPINAL CORD ATROPHY IN DIABETIC PERIPHERAL NEUROPATHY

Eaton SEM, Harris ND, Greenwood P, Wilkinson I, Rajbhandari SM, Ward JD, Griffiths PD and Tesfaye S. Diabetes Research Unit, Royal Hallamshire Hospital, Sheffield, UK.

Diabetic neuropathy (DN) has hitherto been considered to be a disease of the peripheral nerve, involvement of the spinal cord having been largely overlooked. We have assessed the spinal cord in DN using magnetic resonance imaging. T2-weighted axial images were taken at three anatomical levels (C4/5, T3/4 and T9/10) in 19 subjects with DN (9 with chronic pain and 10 without), 10 subjects with diabetes and no neuropathy and 10 normal healthy controls. Cord cross-sectional area was significantly lower in DN compared to controls at both C4/5 (86.9 vs 99.9 mm², $p < 0.01$) and T3/4 (51.9 vs 57.3 mm², $p < 0.05$). No significant differences were seen at T9/10 or between the painful and painless neuropathy subgroups. Spinal cord atrophy (defined as area less than 2SD below the mean of controls) was found in 9/19 (47%) of neuropathic subjects indicating significant, and potentially irreversible, disease in these subjects. This is the first time spinal cord atrophy has been demonstrated in diabetic peripheral neuropathy. These results may have implications to both the pathogenesis and treatment of this condition.

IMPAIRED MOTOR NERVE CONDUCTION VELOCITY (MNCV), ENDONEURIAL BLOOD FLOW (EBF), AND ACETYLCHOLINE-INDUCED VASODILATION IN ARTERIOLES THAT OVERLIE THE SCIATIC NERVE IN DIABETIC RATS IS PREVENTED BY ANTIOXIDANT THERAPY

Yorek M, Coppey L, Davidson E, Dunlap J, Lund D. University of Iowa and Veterans Affairs Medical Center, Iowa City, IA.

Diabetes mellitus produces marked abnormalities in motor nerve conduction, but the mechanism is not clear. In the present study we hypothesized that in the streptozotocin-induced diabetic rat impaired vasodilator function in arterioles that provide circulation to the sciatic nerve is associated with reduced EBF and that these defects precede slowing of MNCV, and thereby may contribute to nerve dysfunction. Three days after the induction of diabetes, EBF was reduced in the diabetic rat. After 1 week of diabetes, acetylcholine-induced vasodilation was found to be impaired and was accompanied by an increase in the superoxide level in these arterioles. These changes preceded the slowing of

MNCV. Treating diabetic rats weekly with hydroxyethyl starch-conjugated deferoxamine (Biomedical Frontiers, MPLS, MN), an iron chelator and inhibitor of hydroxyl radical formation, partially prevented the formation of superoxide in these arterioles and reduced the diabetes-induced deficits in MNCV, EBF and acetylcholine-induced vasodilation. We conclude that the development of diabetic neuropathy in this rat model is due in part to the production of oxygen free radicals and impairment of vascular function.

IS THERE A RELATIONSHIP BETWEEN BAROREFLEX SENSITIVITY, CARDIAC AUTONOMIC FUNCTION AND CARDIAC REPOLARISATION IN TYPE 1 PATIENTS?

Harris ND², Lee SPC¹, Yeoh LS¹, Leathard A², Heller SR¹.

¹Diabetes Centre, Northern General Hospital, Sheffield and ²Dept. of Medical Physics, Royal Hallamshire Hospital, Sheffield, UK.

Established diabetic autonomic neuropathy (DAN) carries a poor prognosis, with an increased risk of sudden death. Recently, baroreflex sensitivity (BRS) has been proposed as a sensitive marker of autonomic function in diabetes (DM). An association between DAN and abnormal cardiac repolarisation, manifest by increases in QT interval and/or QT dispersion on the ECG, has also been described. The aim of this study was to investigate the relationship between BRS and QT parameters. We have studied 28 Type 1 DM subjects (age 39 ± 10 yrs (mean \pm SD), DM duration 14 ± 11 yrs, HbA_{1c} $8.5 \pm 1.3\%$) with normal cardiac autonomic function tests (DAN-) and 8 subjects (age 49 ± 13 yrs, duration 22 ± 9 yrs, HbA_{1c} $9.5 \pm 1.1\%$) with abnormal tests using standard criteria (DAN+). We measured supine BRS using sequence analysis of systolic blood pressure and pulse interval. Rate-corrected QT (QTc) interval was measured using a high-resolution ECG system, and QT dispersion from a 12-lead ECG recorded at 50mm/s. Results are summarised below.

	BRS ms/mmHg	QTc interval	QT dispersion
DAN-	12.7 ± 6.6 (n=28)	411 ± 22 ms (n=28)	38 ± 12 ms (n=28)
DAN+	3.9 ± 1.9 (n=8)	409 ± 22 ms (n=8)	54 ± 12 ms (n=6)

Regression analysis revealed no correlation between BRS and QTc interval or QT. In summary, DAN+ patients had significantly lower BRS ($p < 0.001$) and increased QT dispersion ($p < 0.01$) compared to DAN- subjects, but no difference in QTc. In contrast to BRS, changes in QTc and QT dispersion may occur relatively late in the disease.

ACUTE INFLAMMATORY RESPONSES IN WALLERIAN DEGENERATION IN STZ-INDUCED DIABETIC RATS

Kamijo M*, Iwama Y*, Hasegawa K* and Baba M.** Aichi Medical University* and Hirosaki University School of Medicine**, JAPAN.

Recently, chronic inflammatory cell responses including the upregulation of soluble adhesion molecules have been focused as a pathogenesis of chronic diabetic complications. However the response associated with acute Wallerian

degeneration (WD) in diabetic nerve has not been evaluated. In this study, we investigated the pathological profiles of acute inflammation and its role in the development of WD in axotomized sciatic nerves in both control and streptozotocin induced diabetic rats. Activated macrophages were observed in the epineurial and the endoneurial area in both control (C) and diabetes (D) at 3 and 14 days after axotomy. However, the density of macrophages in diabetes was significantly reduced in the endoneurial area (3 days: C 74 ± 8 /mm² vs. D 28 ± 12 /mm², Mean \pm SD, $P < 0.01$). CD8⁺ lymphocytes were rich in migration on the epineurial area at 3 days after axotomy in control. In contrast, density of CD8⁺ cell was significantly reduced in diabetes (C 132 ± 19 /mm² vs. D 75 ± 8 /mm², $P < 0.005$). At 14 days after axotomy, numerous lymphocytes filtrated in the endoneurial area in distal transected stump in control, although the epineurial CD8⁺ cell accumulation has already diminished. In diabetes, on the other hand, endoneurial lymphocytes were significantly fewer (D 31 ± 8 /mm² vs. C 82 ± 16 /mm², $P < 0.001$). On the pathological evaluation, a progress of nerve fiber degeneration was suppressed in diabetes. Our results suggested that the inflammatory response during acute WD is reduced in experimental diabetes, which might be a reason for delayed WD in diabetic nerve.

PERIPHERAL AND CENTRAL CONDUCTION ABNORMALITIES IN DIABETES MELLITUS

Suzuki C, Ozaki I*, Tanosaki M, Baba M. Department of Neurology, Hirosaki University School of Medicine. *Department of Physical Therapy, Faculty of Health Science, Aomori University of Health and Welfare.

Objectives: To investigate peripheral and central somatosensory conduction in diabetic patients. Methods: We recorded sensory nerve action potentials and 5-channel somatosensory evoked potentials (SEPs) with non-cephalic reference after median nerve stimulation in 55 diabetic patients and 41 age/height matched normal subjects. We determined onset latencies of the spinal N13-P13 and the cortical N20-P20 components, and obtained the central conduction time (CCT). Results: Onset latencies of all SEP components were prolonged in diabetic patients. The mean onset CCT in the diabetic group was 6.3 ± 0.5 ms (mean \pm SD), being significantly longer than that in the control group (6.1 ± 0.2 ms). The peripheral sensory conduction velocity was also decreased in the diabetic group, but there was no significant correlation between peripheral conduction slowing and the onset CCT prolongation. Conclusions: Diabetes affects conductive function in the central as well as peripheral somatosensory pathways. The CCT abnormality does not coincide with lowering of the peripheral sensory conduction. The present results do not favor the hypothesis that a central-peripheral distal axonopathy plays an important role in development of diabetic polyneuropathy.

ELEVATION OF ELECTRIC GUSTATORY THRESHOLD IN TYPE 2 DIABETIC PATIENTS WITH NEUROPATHY

Sakamoto Y, Akaboshi M, Mokuda O. 3rd Department of Internal Medicine, Teikyo Univ., Ichihara-City, Japan 299-0111.

To specify the diabetic complications related to taste function in type 2 diabetes mellitus, electrogustometry was performed in 49 patients and 48 normal subjects. The results showed that the electric gustatory threshold (EGT) rose with aging. The EGT in normal subjects was, on average, not changed compared to the diabetics without complications (6.29 dB vs 6.3 dB), but the EGT was significantly higher in diabetic patients with neuropathy compared to the normal (13.3 dB vs 6.29 dB $p < 0.05$). The EGT was significantly higher in diabetic patients with both neuropathy and retinopathy (18.1 dB vs 6.29 dB $p < 0.05$). In the patients with chronic renal failure due to chronic glomerulonephritis treated with hemodialysis, the EGT was not changed compared to the normal (3.8 dB vs 6.29 dB), but the EGT in the diabetic renal failure treated with hemodialysis was much higher compared to the CGN patients (19.1 dB vs 3.0 dB). Thus, the electric gustatory threshold is a sensitive indicator of diabetic complications.

SURAL NERVE PATHOLOGY IN ASYMPTOMATIC MINIMALLY NEUROPATHIC DIABETIC PATIENTS

Malik RA¹, Tesfaye S², Walker D¹, Newrick PG², Bandhari R², Siddique I¹, Boulton AJM¹, Ward JD². Manchester Royal Infirmary¹, Royal Hallamshire Hospital,² UK.

12 diabetic patients aged 47.5 ± 9.4 yr., duration of diabetes (14.6 ± 10.3 yr.) and 15 control subjects were studied. In diabetic patients neuropathy symptom score = 0, neuropathy deficit score = $4.5 + 0.7/30$, vibration = $12.0 + 1.8$ V, thermal perception ($2.0 + 0.8^\circ\text{C}$), heart rate variation during deep breathing ($17.8 + 2.3$), 30:15 ratio ($1.31 + 0.07$) was normal. Baseline ($n=12$) and repeat neurophysiology ($n=10$) performed $8.7 + 0.6$ years after sural nerve biopsy demonstrated normal values at baseline, with progression of neuropathy (peroneal motor nerve conduction velocity (ms^{-1}) ($42.3 + 2.9$ v $39.4 + 2.0$), sural nerve conduction velocity ($45.4 + 3.7$ v $43.6 + 1.7$). Myelinated fibre density, fibre and axonal area and g-ratio were not significantly reduced. Teased fibre studies showed paranodal abnormalities ($p < 0.001$), segmental demyelination ($P < 0.01$) with remyelination ($P < 0.01$) without axonal degeneration. Unassociated Schwann cell profile density ($p < 0.04$) and axon density ($P < 0.001$) were increased and axon diameter was decreased ($P < 0.007$) with a shift of the size frequency distribution to the left (skewness- 0.89 v 0.64 , $P < 0.03$) suggestive of unmyelinated axonal atrophy/regeneration. Endoneurial capillary basement membrane thickening ($P < 0.006$), endothelial cell hyperplasia ($P < 0.004$) and luminal narrowing ($P < 0.007$) occurred. Current measures of neuropathy are too insensitive to detect significant nerve fibre pathology. The presence of microangiopathy provides support for a microvascular basis of diabetic neuropathy.

C-PEPTIDE DEFICIENCY: AN IMPORTANT PATHOGENETIC FACTOR IN TYPE 1 DIABETIC NEUROPATHY

Sima AAF*, Sugimoto K, Murakawa Y, Zhang W-X. Wayne State University, Detroit, Michigan.

Background: C-peptide has insulin-like effects and ame-

liorates the acute nerve conduction defect (NCD) in experimental and human type 1 diabetic neuropathy (DN). Methods: In this study, diabetic BB/Wor-rats were treated with rat C-peptide (75 ng/kg) from onset of diabetes for 8 months (prevention-group, PG). In a separate experiment, 5-mo untreated diabetic BB/Wor-rats were started on the same C-peptide treatment continued to 8 mo of diabetes (intervention group, IG). Results: In the PG, the NCD was significantly decreased ($p < 0.001$) compared to untreated BB/Wor-rats and was similar to that of normo-C-peptidemic and isohyperglycemic type 2 BBZ rats. This effect was associated with significant preventions of nodal changes ($p < 0.001$) including axo-glial dysjunction ($p < 0.001$), which was not different from non-diabetic control rats. Axonal atrophy and Wallerian degeneration were significantly prevented (both $p < 0.05$). In the IG, the NCD decreased significantly ($p < 0.01$) during the 3 mo treatment period. Associated with the functional improvement, nodal changes improved significantly ($p < 0.001$) as did axonal degenerative changes ($p < 0.01$). C-peptide treatment in the IG resulted in a significant increase in the frequency of regenerating fibers ($p < 0.001$) compared with untreated 5 mo diabetic rats. Conclusion: These studies demonstrate that C-peptide replacement in type 1 diabetes prevents the chronic NCD and structural changes. Furthermore, C-peptide treatment significantly improves the already established functional and structural abnormalities of DN. This is the first demonstration of a therapeutic improvement of established neuropathy in experimental diabetes. We conclude that C-peptide deficiency in type 1 diabetes is an important pathogenetic component of DN and that its replacement may provide a valuable adjunct to intensive insulin treatment.

DELAYED IMMEDIATE EARLY GENE RESPONSES PRECEDE DELAYED INITIATION OF REGENERATION IN DIABETIC NERVE

Sima AAF*, Jiang H, Xu G. Wayne State University, Detroit, Michigan.

Nerve fiber regeneration is impaired in diabetic nerve and contributes to the relentless nerve fiber loss characterizing this disorder. Immediate early gene responses constitute the initial response to nerve injury and include upregulation of NGF and IGF-1 primarily by Schwann cells. These responses are believed to initiate macrophage recruitment necessary for initiation of axonal regeneration. We examined NGF, IGF-1 and CNTF mRNA in sciatic nerve at 10 timepoints (0.5hr to 24d) following sciatic nerve crush in diabetic BB/W-rats. The peak of the immediate upregulation of IGF-1 and NGF occurred at 0.5 and 6 hrs respectively in control nerves and was delayed to 24 hrs and 2d for IGF-1 and NGF respectively in diabetic nerve. Also the expression of NGF p75 receptor was significantly attenuated in diabetic nerve. CNTF mRNA showed an immediate downregulation following nerve crush with no significant differences between control and diabetic rats. These findings suggest that attenuations of the immediate gene responses of para- and autocrine IGF-1 and NGF in diabetic nerve may be responsible for the earlier reported defect in macrophage recruitment and delayed initiation of nerve fiber regeneration.

HIPPOCAMPAL APOPTOSIS IS AMELIORATED BY C-PEPTIDE REPLACEMENT IN TYPE 1 BB/W-RAT

Sima AAF*, Li Z-G, Zhang W-X. Wayne State University, Detroit, Michigan.

It has been suggested that perturbed insulin-like growth factors (IGFs) may be involved in CNS complications of diabetes. We examined IGF messages, hippocampal apoptosis and the effect of C-peptide replacement on encephalopathy in type-1 diabetic BB/Wor-rats. By Northern blot analysis, we found decreased (~50%) mRNA expression of IGF-I, IGF-II and IGF-I receptor (IGF-IR) in 2-month diabetic rats. In 6-month diabetic rats, these changes were associated with positive TUNEL staining, nucleosomal DNA fragmentation and an elevated (~2.5-fold) ratio of Bax/Bcl-xL in hippocampus as assessed by Western blotting and LM-PCR assays. C-peptide replacement (75 ng/kg body weight/day) partially corrected the expression of IGF-I, IGF-II and IGF-IR and eliminated hippocampal apoptosis. To investigate the mechanisms by which C-peptide exerts its antiapoptotic effect, human neuroblastoma SH-SY5Y cells were incubated in high concentrations of glucose (300 mM), which caused significant apoptosis of SH-SY5Y cells as shown by nuclear shrinkage, positive TUNEL staining and DNA laddering. This increased apoptotic activity was delayed in SH-SY5Y cells to which 10 nM C-peptide had been added. In the same cell system, the insulin-induced effect on PI-3 kinase activity was enhanced by C-peptide, in keeping with the notion that PI-kinase plays a role in antiapoptotic signaling. We therefore conclude that insulin and C-peptide deficiencies in type-1 diabetic BB/Wor-rats are associated with hippocampal apoptosis, which can be prevented by C-peptide replacement.

HYPOXIA IS NOT A CAUSE OF OXIDATIVE STRESS IN DIABETIC PERIPHERAL NERVE

Obrosova IG, Van Huysen C, Fathallah L, Stevens MJ. University of Michigan, Ann Arbor, MI, USA.

Severe ischemia and hypoxia in the neural tissues are associated with free radical production. The purpose of our study was to explore the contribution of endoneurial hypoxia vs hyperglycemia to oxidative stress in early experimental diabetic neuropathy. This was achieved by evaluating α_1 -adrenoceptor antagonist prazosin (5 mg/kg in the drinking water, for 3 wks from induction of diabetes), on diabetes-induced changes in endoneurial vascular conductance (VC), biochemical markers of nerve hypoxia, lipid peroxidation and antioxidative defense. Diabetes-induced decrease in VC as well as free NAD⁺/NADH ratios in mitochondrial cristae, matrix and cytosol, were completely (VC, free NAD⁺/NADH in mitochondrial matrix) or partially prevented by prazosin. Accumulation of lipid peroxidation products, depletion of GSH, ascorbate and taurine, increase in GSSG/GSH and DHAA/AA ratios were not affected by vasodilator therapy. Antioxidative defense enzyme activities were similar in control and diabetic rats treated with/without prazosin. Blood and nerve glucose concentrations were similarly elevated in prazosin-treated and untreated diabetic rats. In conclusion, hyperglycemia, but not hypoxia, is a major cause of oxidative stress in diabetic peripheral nerve. Free radical production in

PNS is dependent on the degree of hypoxia, and is not initiated by relatively mild hypoxia in early diabetes.

RELATIONSHIP BETWEEN ALDOSE REDUCTASE AND OXIDATIVE STRESS IN DIABETIC PERIPHERAL NERVE

Obrosova IG, Stevens MJ, Fathallah L. University of Michigan, Ann Arbor, MI, USA.

The relationship between aldose reductase (AR) activity and oxidative stress in tissue sites for diabetic complications remains controversial. We evaluated the ARI sorbinil and three antioxidants, i.e. DL- α -lipoic acid (LA), taurine (T) and vitamin E (VE), on indices of oxidative stress and sorbitol pathway intermediates in diabetic peripheral nerve. The groups included control (C) and streptozotocin-diabetic (D) rats treated with/without: 1.ARI (65 mg/kg, for 2 wks after 4 wks of untreated diabetes); 2.T (1% of the diet); 3.VE (1% of the diet); or 4.LA(100 mg/kg i.p.). T, VE and LA were administered for 6 wks from induction of diabetes. Malondialdehyde plus 4-hydroxyalkenal levels were increased in D vs C ($p < 0.01$) and this increase was corrected in D+ARI ($p < 0.01$ vs D). GSH and ascorbate levels as well as superoxide dismutase activity were decreased in D vs C, and this decrease was completely (GSH) or partially corrected by ARI. Three antioxidants counteracted oxidative stress, but not accumulation of the sorbitol pathway intermediates in the diabetic nerve. In conclusion, increased AR activity contributes to rather than protects from oxidative stress in the diabetic nerve. Oxidative stress is not a cause of sorbitol pathway intermediate accumulation in experimental diabetic neuropathy.

ANTIOXIDANT PROTECTION MECHANISMS AND ARACHIDONIC ACID SYNTHESIS ARE ALTERED IN SCHWANN CELLS GROWN IN ELEVATED GLUCOSE

Miinea C and Eichberg J. Dept. of Biology & Biochemistry, University of Houston, Houston, TX 77204 USA.

Accumulating evidence points to oxidative stress as an important factor in the onset of diabetic neuropathy. We have investigated the status of antioxidant protection mechanisms in immortalized rat Schwann cells cultured in high (30 and 50 mM) concentrations of glucose. As compared to growth in 5 mM glucose, the cells contained 40% less reduced glutathione ($n = 8$, $p < 0.01$). Total superoxide dismutase activity was diminished by more than 50% ($n=3$; $p < 0.001$), whereas catalase activity was unchanged. The cellular NADH/NAD⁺ ratio was progressively increased with increasing medium glucose concentrations. Our previous findings have established that upon exposure of cultured cells to elevated glucose, the proportions of arachidonic acid-containing molecular species (ACMS) in phospholipids are decreased in a pattern similar to alterations exhibited by diabetic nerve. To examine whether biosynthesis of arachidonic acid might be perturbed, confluent cells maintained in either high or low glucose were incubated with either [¹⁴C]linoleic acid (18:2) or [¹⁴C]dihomo- γ -linolenic acid (20:3) and radioactivity incorporated into molecular species of major phospholipid classes was measured. The incorpora-

tion of 18:2 either as unchanged fatty acid or into ACMS did not differ as a function of glucose concentration. Negligible labeled 18:3 or 20:3 molecular species were detected. In contrast, the uptake of 20:3 into 18:1/20:4 and 16:0/20:4 phosphatidylcholine and 18:1/20:4 phosphatidylethanolamine, but not into 20:3-containing molecular species, was significantly reduced in cells cultured in 30 mM glucose. These data imply that $\Delta 5$ desaturase activity is decreased in cells exposed to elevated glucose. This reduced enzyme activity could adversely affect polyunsaturated fatty acid metabolism and might arise as a consequence of impaired scavenging of reactive oxygen species. (Supported by NIH grant DK30577)

INCREASED DEPOLARIZATION-INDUCED CYTOSOLIC Ca^{2+} SIGNAL IN RAT DORSAL ROOT GANGLION NEURONS UNDER HIGH GLUCOSE WITH SUPPRESSED Na^+/K^+ PUMP ACTIVITY

Sanada M^{1,2}, Yasuda H¹, Omatsu-Kanbe M², Matsuura H², and Kikkawa R.¹ Department of Medicine¹ and Department of Physiology², Shiga University of Medical Science, Otsu, Shiga 520-2192, JAPAN.

Hyperglycemia and its associated Na^+/K^+ pump activity has been implicated in the development of diabetic neuropathy. We recently reported that high glucose in the presence of ouabain induced a progressive increase in the delayed K^+ current which was suppressed by a blocker of Ca^{2+} -activated K^+ channels and blockers of Ca^{2+} channels in rat single myelinated nerve fibers, suggesting an increase of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$). However, the influences of high glucose with ouabain on $[Ca^{2+}]_i$ in sensory neurons remain to be elucidated. The present study was undertaken to examine the modulation of depolarization-induced Ca^{2+} transients by high glucose and ouabain in isolated adult rat dorsal root ganglion (DRG) neurons using the fluorescent Ca^{2+} indicator fura-2. Bath application of KCl (50 mM) evoked a rapid increase in $[Ca^{2+}]_i$ through voltage dependent Ca^{2+} channels ($[Ca^{2+}]_i$: 154.2 ± 22.5 nM). This increase was enhanced under high glucose (30 mM D-glucose) in the presence of ouabain (100 M) ($[Ca^{2+}]_i$: 764.8 ± 210.1 nM). We conclude that a combination of high glucose and decreased Na^+/K^+ pump activity leads to an increase in $[Ca^{2+}]_i$ in rat DRG neurons, thereby resulting in nerve dysfunction.

SKIN SYMPATHETIC RESPONSE IN SUBCLINICAL DIABETIC NEUROPATHY

Kucera P,¹ Krahulec B,² Strbová, L.² 1st Department of Neurology and 2nd Department of Internal Medicine, Comenius University School of Medicine, Bratislava, Slovakia.

Skin sympathetic response (SSR)—a method of assessing sudomotor sympathetic fibres dysfunction—is absent in 9–83% of diabetic patients with distal symmetrical sensorimotor neuropathy (1,2). There are only few reports in the literature about SSR in subclinical stages of diabetic neuropathy. We therefore measured SSR in 73 diabetic patients (49 patients with clinically evident diabetic neuropathy/assessed with pin prick test, light touch, 128 Hz tuning fork, ankle reflexes and 10 g monofilament/ and 24 patients without clinical signs of diabetic neuropathy, but with abnormal con-

duction studies of nervus suralis and peroneus) and 32 normal subjects at both feet and hands. We considered the SSR abnormal only when it was absent in two or more extremities. SSR was present in all normal subjects in all extremities (100%). SSR was absent in 47% of the whole group of diabetic patients. It was absent in 34.7% of the subgroup of patients with clinically evident diabetic neuropathy, and 20.8% in the subgroup without clinical signs of neuropathy. These differences were not statistically significant. Our results support the observations of higher vulnerability and early involvement of small unmyelinated peripheral sympathetic fibres in diabetes mellitus. We conclude, that measuring of SSR can be helpful in diagnosis of subclinical diabetic neuropathy and can extend spectrum of electrophysiological diagnostic methods.

1. Soliven B, Maselli R, Jaspan J, Green A, Graziano H, Petersen M, Spire J-P: Sympathetic skin response in diabetic neuropathy. *Muscle Nerve* 10, 1987: 711–716.

2. Niakan E, Harati Y: Sympathetic skin response in diabetic peripheral neuropathy. *Muscle Nerve* 2, 1988: 261–264.

TAURINE REPLACEMENT PREVENTS APOPTOSIS IN EXPERIMENTAL DIABETIC NEUROPATHY

Pop-Busui R, Sullivan K, Russel J, Feldman E, Stockert C, Larkin D, Greene D, Stevens M. University of Michigan, Ann Arbor, MI USA.

The pathophysiology of diabetic neuropathy (DN) is complex involving both metabolic and vascular deficits. Recent data have implicated a potential role for programmed cell death (PCD) in the development of experimental DN (EDN). Oxidative stress has emerged as a leading candidate in the development of nerve blood flow (NBF) and nerve conduction deficits. We have previously shown that replacement of the endogenous antioxidant taurine in the nerve of streptozotocin-diabetic (STZ-D) rats corrects neuro-vascular dysfunction, prevents deficits in Na,K -ATPase activity and attenuates motor and sensory NCV slowing. The aims of this study were to examine the hypothesis that oxidative stress-mediated mitochondrial dysfunction may promote the activation of PCD pathways in STZ-D rats and to determine whether these effects could be attenuated by taurine. Dorsal root ganglion (DRG) neurons were extracted from perfused control (C), STZ-D and STZ-D rats given a 1% taurine supplemented diet for 6 weeks. STZ-D induced a significant decrease in sensory nerve conduction velocity (SNCV) and NBF vs controls and these deficits were corrected by taurine. Lumbar and sacral DRG were sectioned at 10 mm intervals and slides were prepared from two different sampling sites. Apoptosis was detected by TUNEL staining. 150 or more DRG nuclei were counted per animal. In STZ-D rats $21.3 \pm 4.9\%$ of DRG nuclei were found to be TUNEL positive as compared with $2.9 \pm 2.1\%$ in controls ($p < 0.05$). Taurine replacement markedly attenuated apoptosis since only $6.5 \pm 2.9\%$ neurons were TUNEL positive. In summary, 6 weeks of STZ-D increased apoptosis in DRG neurons of STZ-D rats. This increase in apoptosis could be prevented by replacement of the endogenous antioxidant taurine, implicating a role of taurine depletion in the development of sensory neuron degeneration.

VIBRATION PERCEPTION THRESHOLDS: COMPARISON OF CASE IV AND NEUROTHESIOMETER MEASUREMENTS

Bril V and Perkins BA. Toronto, Canada & Boston, USA.

Purpose: Vibration perception thresholds (VPT) are used frequently to assess somatosensory pathways in clinical trials. Different equipment, testing paradigms, and stimulation sites produce varying results which make comparisons between trials and patient populations challenging. Information comparing the VPT obtained with the Neurothesiometer to that with the Vibratron is available, but not for a similar comparison with the CASE IV¹. Methods: 478 subjects including reference, non-neuropathic subjects with diabetes mellitus (DM), and diabetic patients with mild, moderate and severe neuropathy had VPT measured with the CASE IV and Neurothesiometer, as well as the criterion standard sural nerve conduction studies all on the same day. The dorsum of the foot was stimulated for the CASE IV determination and the distal phalanx of the first toe for the Neurothesiometer. Results: VPT by the CASE IV correlate with the Neurothesiometer values ($R^2 = 0.547$, $p < 0.0001$). VPT determined by both Neurothesiometer and CASE IV correlate with the sural nerve amplitude ($R^2 = 0.456$ and 0.461 , $P < 0.0001$ for both). Conclusions: Results demonstrate a significant correlation of VPT values obtained by the two methods. Similar correlations between VPT and electrophysiological parameters are observed indicating that both methods are valid, and thus the Neurothesiometer is favored due to the ease and rapidity of testing by this method.

¹ Bril V, Kojic J, Ngo M, Clark K. (1997). Comparison of the Neurothesiometer and Vibratron in Measuring Vibration Perception Threshold and Relationship to Nerve Conduction Studies. *Diabetes Care*, 20:1360–1362.

CARPAL TUNNEL SYNDROME IN PATIENTS WITH DIABETIC POLYNEUROPATHY

Bril V and Perkins BA. Toronto, Canada & Boston, USA.

Diagnosis of carpal tunnel syndrome (CTS) in patients with diabetic polyneuropathy (DPN) is important as therapeutic interventions directed towards relief of CTS may be effective irrespective of DPN. The frequency of clinical CTS and the best electrodiagnostic discriminator of CTS from diffuse neuropathy are uncertain. 478 subjects including reference, non-neuropathic subjects with diabetes mellitus (DM), and diabetic patients with mild, moderate and severe neuropathy were evaluated for clinical features of CTS. All subjects had routine determinations of median nerve distal motor and sensory latencies, sensory and motor potential amplitudes and sensory conduction velocities. Other parameters tested were: ratios of median to ulnar nerve distal motor and sensory latencies, distal motor and sensory amplitudes, and distal conduction velocities. Similar median to sural nerve ratios for sensory latencies, amplitudes and conduction velocities were determined as were ratios of median nerve motor amplitudes and latencies to sural nerve parameters. Segmental median sensory nerve conduction velocities were evaluated. The frequency of clinical CTS was

2% in the reference population, 14% in diabetic patients without DPN, and 30% in those with DPN. We did not find any reliable electrodiagnostic discriminator for CTS in patients with DM +/- DPN. Some of the parameters worsened with severity of neuropathy, but none reliably distinguished diabetic patients with and without CTS. Given that CTS is frequent in patients with DPN, but electrodiagnostic criteria cannot distinguish those with clinical CTS, a trial of therapy may be indicated in these patients regardless of the electrodiagnostic findings.

POSSIBLE ROLE OF UNCOUPLING PROTEINS AS PROTECTORS FROM HYPERGLYCEMIA-INDUCED NEUROPATHY

Gustafsson H, Söderdahl T, Forsby A. Department of Neurochemistry and Neurotoxicology, Stockholm University, Stockholm, Sweden.

Diabetic neuropathy may be induced by oxidative stress, possibly as a consequence of the hyperglycemic situation. Uncoupling proteins (UCPs) have been reported to function as anti-oxidants by decreasing the production of reactive oxygen species (ROS). These mitochondrial carrier proteins are located in the inner membrane of mitochondria and upon activation, they dissipate proton gradients, which generates heat instead of ATP. In humans, UCP2 and UCP3 are believed to play a role as energy dissipaters and aberrant function could underlie metabolic defects seen in both obesity and non-insulin dependent diabetes (NIDDM). In this study we have shown that human neuroblastoma SH-SY5Y cells expressed UCP2 and UCP3 natively and that the expression was upregulated by insulin and IGF-I via the IGF-I receptor. In highly differentiated SH-SY5Y cells, which were cultured in hyperglycemic N2-medium (30 mM and 60 mM glucose) containing 8.6 nM insulin, the number of neurites per cell and total cellular protein levels/dish were significantly decreased, as compared to cells grown in N2-medium containing 17 mM glucose. This effect was abolished when the cells were grown with 10 nM IGF-I at 30 mM glucose and decreased at 60 mM glucose. Furthermore, in hyperglycemic cells, the IGF-I-induced increase in UCP3 protein levels was inhibited. Non-differentiated cells responded to hyperglycemic situations by increased rate of proliferation, leaving cell morphology intact. We conclude that differentiated SH-SY5Y cells can serve as an *in vitro* model for hyperglycemic neurons and that IGF-I protects the cells from hyperglycemia-induced neuropathy, suggestively by the involvement of UCP3.

ASSESSING QUALITY OF LIFE IN DIABETIC NEUROPATHY: CONDITION-SPECIFIC OR GENERIC APPROACH?

Vileikyte L, Boulton AJM. Manchester Royal Infirmary, UK.

Studies that assessed Quality of Life (QoL) in patients with diabetic peripheral neuropathy (DPN) used generic measures, focusing on extremes of DPN. This study compared the performance of the neuropathy-specific QoL mea-

sure (NeuroQoL) with the generic SF36, in diabetic patients with varying severity of DPN. 120 DPN patients were included, mean age 61 years, diabetes duration 12 years. DPN severity was assessed by neuropathy disability score (NDS) and vibration perception threshold (VPT): symptom severity by neuropathy symptom score (NSS). All NeuroQoL domains showed significant differences (eg., social; interpersonal relationships $p < 0.01$) between patients subdivided into those with moderate/severe symptoms ($NSS \geq 6$) compared to those with mild ($NSS < 6$), in contrast to only 2 SF36 domains (mental health; bodily pain $p < 0.05$). Similarly, when patients were subdivided according to DPN severity (moderate/severe: $VPT \geq 25$ and $NDS \geq 6$; mild: $VPT < 25$; $NDS < 6$), NeuroQoL domains (eg., negative symptoms; sexual problems $p < 0.05$) demonstrated differences between the groups whereas virtually no differences were seen in SF36. Furthermore, NeuroQoL maintained its ability to differentiate between those with mild ($VPT < 25$); moderate ($VPT: 25$ to 35) and severe ($VPT > 35$) DPN, whereas SF36's discriminatory power was lost. All significant differences remained after accounting for confounding variables. Thus, NeuroQoL detects more subtle differences between DPN patients and should therefore be more sensitive to change over time than the SF36.

EFFECT OF PROTEIN KINASE C- β INHIBITION ON DIABETIC NEUROPATHY IN OTSUKA LONG-EVANS TOKUSHIMA FATTY RATS

Kato K, Nakamura J, Kasuya Y, Kamiya H, Akiyama N, Watanabe G, Kawamura T, Hotta N. The Third Department of Internal Medicine, Nagoya University School of Medicine, Nagoya, Japan.

Although increased protein kinase C (PKC) activity has been invoked in the pathogenesis of diabetic retinopathy, nephropathy and macroangiopathy, the role of PKC in neuropathy remains unclear. We reported that total PKC activity in sciatic nerves of STZ-induced diabetic rats was not altered compared with that of normal rats, and that PKC- β inhibitor, LY-333531 (LY) ameliorated neural dysfunction and reduced endoneurial blood flow without affecting PKC activity, suggesting that this effect of LY would be mediated through its action on endoneurial microvasculature. In this study, effect of LY on diabetic neuropathy was investigated in Otsuka Long-Evans Tokushima fatty (OLETF) rats, an animal model of type 2 diabetes. Three-month-old male OLETF rats and Long-Evans Tokushima Otsuka (LETO) rats as normal control rats were divided into 5 groups as follows: 1) LETO control, 2) OLETF control, 3) LY (1 mg/kg/day)-treated OLETF, 4) sucrose-fed OLETF, and 5) sucrose-fed OLETF treated with LY. After 21-month treatment, motor nerve conduction velocity (MNCV) in tail nerves, sciatic nerve blood flow (SNBF) and protein expression of phosphorylated PKC in sciatic nerves were measured. 1) MNCV: OLETF rats demonstrated a significant delay in MNCV, which was enhanced by sucrose feeding, and these deficits were prevented by LY. 2) SNBF: reduced SNBF in OLETF and sucrose-fed OLETF rats was ameliorated by LY. 3) PKC: phosphorylated PKC expression was decreased in OLETF rats, which was more prominent in

sucrose-fed OLETF rats. Treatment with LY increased phosphorylated PKC expression in both groups of OLETF rats. These observations suggest that diabetes with long duration and poor glycemic control decreases phosphorylated PKC in nerve tissues, which would contribute to the development of diabetic neuropathy, and that PKC- β inhibition would be beneficial for the prevention of this neuropathy.

A SONIC HEDGEHOG (SH) FUSION PROTEIN CORRECTS MULTIFOCAL DEFECTS IN EXPERIMENTAL DIABETIC NEUROPATHY

Tomlinson DR¹, Delcroix J-D¹, Taylor F², Strauch K², Engber T², Galdes A.² ¹Division of Neuroscience, School of Biological Sciences, University of Manchester, UK and ²Bigen Inc., Boston, USA.

Diabetic neuropathy develops from defective interactions between nerve axons and other cells in the endoneurium; such interactions are influenced in development by hedgehog proteins. This study explored the possibility that this might be maintained in the adult and form a basis for therapy in diabetic neuropathies. Streptozotocin-diabetic rats were treated (final 5 weeks of 10 weeks diabetes) with a SH-IgG fusion protein (either 0.3mg/kg or 3.0mg/kg s.c. 3 times per week); control diabetic and non-diabetic rats received vehicle. Conduction velocity (MNCV, SNCV) data and sciatic nerve levels of nerve growth factor (NGF) and neuropeptide Y (NPY) are presented below.

	MNCV (m/s)	SNCV (m/s)	NGF (pg/cm)	NPY (fmol/mg)
Controls	60.4±2.4	58.7±3.7	50.9±22.8	168.6±28.2
Db vehicle	45.0±3.9	42.3±3.4	26.6±13.0	124.8±22.2
Db SH 0.3mg/kg	54.9±8.6	55.1±4.2	49.7±21.8	156.6±15.6
Db SH 3.0mg/kg	62.3±6.7	61.5±4.4	31.3±11.9	160.1±24.7

Diabetes caused significant ($p < 0.05$ by ANOVA with SNK tests) reductions in all variables and treatment with SH-IgG either attenuated or prevented ($p < 0.05$) these reductions. Since it is well-established that the conduction deficits are unrelated to neurotrophic deficits (NGF depletion) and that NPY depletion derives from a neurotrophic defect distinct from NGF, this treatment clearly acts at multiple components of the aetiology of diabetic neuropathy.

REDUCED NERVE BLOOD FLOW IN DIABETIC RATS IS A REFLECTION OF HINDLIMB MUSCLE WASTING

Tomlinson DR and Riaz S. Division of Neuroscience, School of Biological Sciences, University of Manchester, Stopford Building 1.124, Oxford Road, Manchester M13 9PT, UK.

We examined the influence of muscle wasting, as a result of streptozotocin-induced diabetes, on sciatic nerve laser Doppler flux (SNLDF), as an index of nerve blood flow, and conduction velocity (NCV). We compared dietary-restricted weight-reduced non-diabetic rats with controls and with diabetic rats and we studied the effects of clen-

buterol, an anabolic β -adrenoceptor agonist, in control and diabetic rats. Dietary restriction reduced the weights of hindlimb muscles—extensor digitorum longus, soleus and gastrocnemius—half as much as did streptozotocin-diabetes and clenbuterol increased muscle weights in control and diabetic rats. This gave a hierarchy of muscle weights in the order—clenbuterol-controls, untreated controls, weight-reduced non-diabetics, clenbuterol-diabetics and untreated diabetics. Diabetes without treatment reduced SNLDF by 51% ($p < 0.01$); dietary restriction by 25% ($p < 0.01$) and there were proportional increases associated with clenbuterol treatment. Combined muscle weights regressed closely with SNLDF ($r^2=0.69$; $p < 0.001$) and, when the latter was expressed relative to muscle weights, a similar value was obtained for all five groups—there were no significant differences. Thus, sciatic nerve blood flow is closely related to hindlimb muscle weight and the effect of diabetes on nerve blood flow may be secondary to muscle wasting. Sciatic/tibialis motor and sensory conduction velocities were also reduced by muscle wasting in the dietary restricted group of non-diabetic rats, but, unlike nerve Doppler flux, it was unaffected by clenbuterol.

MAP KINASES p38 AND JNK CONSTITUTE RETROGRADE STRESS SIGNALS IN DIABETIC NEUROPATHY?

Fernyhough P, Delcroix J-D, Jude EB, Boulton AJM, Tomlinson DR. Division of Neuroscience, Biological Sciences, and Department of Medicine, Manchester University, UK.

Stress-activated protein kinases (SAPKs—p38 MAPK and JNK) are activated by high glucose or experimental diabetes. They are also carried from the periphery to the soma by retrograde axonal transport in sensory neurones. This study examined SAPK activation in nerve from diabetic patients and retrograde transport of activated SAPKs in diabetic rats. Sural nerve specimens were obtained from diabetic and roughly matched non-diabetic individuals at amputation. Proteins were extracted for western blotting and blots exposed to antibodies raised against activated (phosphorylated on Ser/Thr) or all forms of JNK or p38, plus their common substrate, the transcription factor, ATF2. Retrograde transported SAPKs were collected distal to a 6h sciatic nerve crush in control and 8 wk streptozotocin diabetic rats. These were exposed to antibodies as described for human tissues. In human tissues, phosphorylation of JNK, p38 and ATF2 was increased between 2- to 4-fold compared to controls. In rats, retrograde transport of phospho-p38 (arbitrary units \pm SD) was massively increased in diabetic rats (60.8 ± 11.8 versus controls, 18.8 ± 1.9) as was transport of ATF2 (20.9 ± 3.8 versus controls, 6.7 ± 0.8). One of the isoforms of phospho—JNK-p46—showed a modest increase in retrograde transport in diabetic rats (13.7 ± 0.9 versus 8.1 ± 0.4), though the other isoforms (p54 and p56) were unaffected. These observations indicate a powerful retrograde signal in sensory neurones of diabetic rats in the form of exaggerated phosphorylation, and hence activation, of the SAPKs and one of their substrates, the transcription factor ATF2. This signal comes from some influence on the peripheral projection of these neurones and is a viable candi-

date for an early transcription-dependent change in neuronal phenotype—perhaps a forerunner of diabetic neuropathy.

THE ROLE OF C-JUN N-TERMINAL KINASE 3 (JNK3) IN SENSORY NERVE REGENERATION

Lockwood MF,^{1,*} Averill S,² Priestley JV,² Tomlinson DR,¹ Fernyhough P¹. Div of Neuroscience¹, School of Biological Sciences, University of Manchester and Neuroscience Section², Div of Biomedical Sciences, Queen Mary and Westfield College, United Kingdom.

The c-Jun N-terminal protein kinase (JNK or SAPK1) signals downstream from the small GTPases Rac/Rho/Cdc42 that are known to regulate neurite outgrowth. JNK may also control alterations in sensory neuron phenotype induced by axotomy via activation of transcription factors such as AP-1 and ATF2. The aim of this study was to use JNK3 knockout mice to determine the role of JNK3 in sensory neuron regeneration. Dissociated adult mouse and rat cultures were used and JNK levels probed by Western blot and immunohistochemistry using antibodies directed against total JNK, phospho-JNK and JNK3. Total JNK was located in the nucleus, cytoplasm and axons. Phospho-JNK was located primarily in the nucleus, with some axonal staining, and JNK3 displayed heavy staining in the cytoplasm. *In vivo*, DRG of adult rats exhibited JNK staining in neuronal soma, however, following axotomy of 2-3 weeks duration there was enhanced staining for phospho-JNK in satellite cells. *In vitro*, neurite outgrowth studies on dissociated DRG cultures from JNK3 knockout mice showed increased outgrowth by 1.75-2.54-fold at concentrations <0.1 ng/ml of nerve growth factor (NGF) vs wild-type (C57BL6). Increased neurite outgrowth was also observed in mouse DRG organ cultures of JNK3 knockout mice in the presence of NGF. The results suggest that JNK3 is a negative regulator of axonal outgrowth, possibly as part of the Rho pathway (Funded by a BBSRC PhD Studentship and Pfizer).

EARLY DETECTION OF DIMINISHED BAROREFLEX SENSITIVITY IN DIABETIC PATIENTS WITHOUT EVIDENCE OF CARDIOVASCULAR AUTONOMIC NEUROPATHY

Ziegler D¹, Laude D², Akila F¹, Elghozi J-L.² ¹German Diabetes Research Institute, Düsseldorf, Germany, ²Laboratoire de Pharmacologie, Faculté de Médecine Necker, Paris, France.

Diabetic cardiovascular autonomic neuropathy (CAN) carries an increased risk of mortality. Decreased baroreflex sensitivity (BRS) has been identified as a predictor of increased mortality following myocardial infarction. We evaluated spontaneous BRS in 39 healthy control subjects (C: age (mean \pm SEM): 41.5 ± 1.9 years) and 116 diabetic patients (64% Type 1, 36% Type 2; age: 45.8 ± 1.4 years; diabetes duration: 16.9 ± 1.0 years; HbA_{1c} : $9.2 \pm 0.2\%$) using cross-spectral analysis between systolic blood pressure and heart rate in the low-frequency (LF) and high-frequency (HF) bands as well as time domain (sequence) analysis in the supine and standing positions over 10 min. According to previously suggested definitions based on

autonomic function tests (AFTs), 36 patients had definite CAN (CAN+: ≥ 3 of 7 indices abnormal), 13 had borderline CAN (CAN[+]: 2 of 7 indices abnormal), and 64 had no evidence of CAN (CAN-: ≤ 1 of 7 indices abnormal). Maximum gain in cross-spectral LF band (standing) was significantly reduced in CAN- as compared with C (5.2 ± 0.4 vs. 7.2 ± 0.8 ms/mmHg, $p < 0.05$). Moreover, maximum gain in cross-spectral HF band was significantly lower in CAN- than in C (supine: 12.0 ± 1.2 vs. 17.9 ± 2.5 ms/mmHg, $p < 0.05$; standing: 4.9 ± 0.5 vs. 8.7 ± 1.0 ms/mmHg, $p < 0.05$). The slope of the regression line between defined increases or reductions in systolic blood pressure and R-R intervals was significantly reduced in CAN- compared to C (supine: 10.6 ± 0.7 vs. 14.2 ± 1.6 ms/mmHg, $p < 0.05$; standing: 5.6 ± 0.4 vs. 8.1 ± 0.7 ms/mmHg, $p < 0.05$). Similar differences were obtained when comparing the CAN- and CAN[+] groups, the latter showing significantly reduced BRS by both techniques ($p < 0.05$). In contrast, no such differences were noted when comparing the CAN[+] and CAN+ groups. In conclusion, reduced spontaneous baroreflex sensitivity is an early marker of autonomic dysfunction at a stage when autonomic function tests do not yet indicate the presence of CAN, while cases with borderline CAN show a degree of BRS abnormality that is comparable to the level seen in definite CAN. Prospective studies are needed to evaluate whether reduced BRS is a predictor of mortality in diabetic patients.

SORBITOL ACCUMULATION IN ASCORBIC ACID TREATED SCHWANN CELLS CULTURED IN HIGH GLUCOSE MEDIUM

Suzuki T¹, Yashima S¹, Taniko K¹, Mizuno K¹, Suzuki T¹, Yabe-Nishimura C.² ¹R&D Department, Sanwa Kagaku Kenkyusho Co., Ltd., Mie, Japan. ²Department of Pharmacology, Kyoto Prefectural University of Medicine, Kyoto, Japan.

Unless the expression of aldose reductase (AR) was upregulated, sorbitol was not accumulated under high glucose conditions in Schwann cells isolated from adult rat sciatic nerves (1). In the peripheral nerve, however, Schwann cells of both myelinated and un-myelinated fibers are ensheathed by the basement membrane. To evaluate the effect of basement membrane formation on sorbitol accumulation, we examined the sorbitol levels in Schwann cells pre-cultured for 12 days in the medium containing 50 μ g/ml ascorbic acid and 20nM progesterone that elicit basement membrane synthesis on the plate coated with extracellular matrix. In the cells cultured in ascorbic acid containing medium, type IV collagen, one of the basement membrane components, was detected. When these cells were incubated with 50mM glucose for 6 days, a significant level of sorbitol accumulation was observed without any change in the activity of AR and SDH. Fidarestat, an inhibitor of AR, decreased the sorbitol levels in a dose-dependent manner. Increased fructose levels in these cells were simultaneously suppressed by the addition of fidarestat. Our findings therefore suggest that accumulation of sorbitol in Schwann cells takes place under diabetic conditions, only when cells are ensheathed by the basement membrane.

(1) Suzuki, T., et al. (1999). J. Neurosci. Res., 57, 495-503.

IMPROVEMENT OF PERIPHERAL NERVE STRUCTURE BY 15-MONTH ALDOSE REDUCTASE INHIBITION WITH FIDARESTAT IN STZ-INDUCED DIABETIC RATS

Kato N¹, Mizuno K¹, Makino M¹, Suzuki T¹ and Yagihashi S.² ¹R&D Department, Sanwa Kagaku Kenkyusho Co., Ltd., Mie, Japan; ²Department of Pathology, Hirosaki University School of Medicine, Hirosaki, Japan.

There has been no report documenting the effects of long-term aldose reductase (AR) inhibition one year or more on peripheral neuropathy of streptozotocin-induced diabetic rats (STZ-rats). Fidarestat (SNK-860) is a potent AR inhibitor that possesses the beneficial effects on the nerve dysfunction in early stage of diabetic rats (1). In the present study, we investigated the effects of 15 months of treatment with fidarestat on functional, morphological and metabolic abnormalities in the peripheral nerve of STZ-rats. Fidarestat treatment inhibited the slowing of F-wave conduction velocity and motor nerve conduction velocity by about 70% and normalized the slowing of sensory nerve conduction velocity. Morphometric analysis of myelinated fibers demonstrated that the frequencies of abnormal fibers such as paranodal demyelination, Wallerian degeneration and axonal sequestration were reduced to the extent of normal levels by fidarestat. Axonal atrophy, reduction of myelin sheath thickness and ratio of large fibers were also inhibited. The effects of fidarestat on functional and morphological changes were accompanied by correction of the increased sorbitol, fructose and decreased *myo*-inositol in nerve. It was thus demonstrated that long-term inhibition of increased polyol pathway flux by fidarestat was beneficial for the development of functional and structural neuropathy in STZ-rats.

(1) Mizuno, K., et al. (1999). J. Diab. Comp., 13,141-150.

HYPERALGESIA MEDIATED BY SPINAL PROSTAGLANDIN E₂ AND SIGNALLING THROUGH THE p38 PATHWAY IN DIABETIC RATS

Calcutt NA, Freshwater J, Campana WM.* Departments of Pathology and *Anesthesiology, University of California San Diego, La Jolla CA 92093-0612, USA.

Diabetic rats exhibit hyperalgesia following paw formalin injection, despite reduced spinal release of the excitatory neurotransmitters substance P and glutamate. In normal rats, spinal sensitization to sensory input involves local release of prostaglandin E₂ (PGE₂). We used microdialysis to measure spinal PGE₂ release following paw formalin injection in conscious control and STZ-diabetic rats. In control rats, there was an increase in spinal PGE₂ levels to $260 \pm 14\%$ of basal ($p < 0.01$) in the first 5 minutes after paw formalin injection and thereafter levels were not different from basal. Diabetic rats showed a similar increase ($254 \pm 25\%$) but also exhibited a second period of release ($266 \pm 20\%$; $p < 0.01$ vs. basal) 15-20 minutes after the stimulus. Systemic delivery of the NSAID indomethacin (10 mg/kg IP) or intrathecal delivery of an EP₁ receptor antagonist (30 μ g) prior to paw formalin injection both significantly reduced the number of paw flinches in diabetic rats 10-40 minutes after injection (17 ± 9 and 20 ± 8 , respectively) compared to vehicle treated diabetics (42 ± 8). Formalin-evoked flinching was

also significantly reduced by spinal delivery of the p38 stress-activated protein kinase inhibitor SB203580 (1–30 μ g IT) in both control and diabetic rats, with higher concentrations required for efficacy in diabetic rats. Hyperalgesia following paw formalin injection in diabetic rats is accompanied by increased spinal PGE₂ release and activation of the EP₁ receptor while signaling through the p38 pathway also participates in maintaining formalin-evoked flinching.

HYPERALGESIA IN DIABETIC RATS IS ALLEVIATED BY A PROSAPOSIN-DERIVED PEPTIDE

Freshwater J and Calcutt NA. Department of Pathology, University of California San Diego, La Jolla CA 92093-0612, USA.

The saposin C-derived peptide TX14(A) prevents onset of functional and structural disorders in the peripheral nerve of diabetic rats. We have now investigated the ability of TX14(A) to alleviate behavioral indices of abnormal pain perception in adult female rats 4–6 weeks after onset of STZ-induced diabetes. Untreated diabetic rats exhibited tactile allodynia (response threshold = 3 ± 1 g) compared to age-matched controls (10 ± 1 g). A single ip injection of TX14(A) transiently alleviated tactile allodynia, with an effect that was maximal 6 hours (11 ± 1 g) after injection and diminished within 48 hours. Maximal efficacy was seen with a 1 mg/kg dose while no effects were noted in control rats. Control rats exhibited a transient thermal hyperalgesia ($77 \pm 5\%$ of baseline paw withdrawal latency) 15 minutes after intrathecal delivery of substance P (30 nmol) that resolved within 30 minutes. Untreated diabetic rats exhibited substance P evoked thermal hyperalgesia of similar magnitude ($82 \pm 5\%$ at 15 minutes) but of greater duration ($83 \pm 4\%$ at 1 hour). Intrathecal delivery of TX14(A) 30 minutes before intrathecal substance P was without effect on the transient thermal hyperalgesia in control rats ($74 \pm 9\%$ at 15 minutes). In diabetic rats, the prolonged thermal hyperalgesia was abolished by prior intrathecal delivery of TX14(A), although the transient thermal hyperalgesia ($72 \pm 8\%$ at 15 minutes) remained. These studies show that TX14(A) can rapidly alleviate diabetes-induced allodynia and hyperalgesia for up to 48 hours.

HIGH DOSE THERAPY WITH ALPHA-LIPOIC ACID IMPROVES CUTANEOUS VASOMOTOR RESPONSE IN PATIENTS WITH DIABETIC NEUROPATHY

Tretjakovs P, Jurka A, Pirags V. Latvian Institute of Experimental and Clinical Medicine; Dept. of Endocrinology, P. Stradins University Hospital, Riga, Latvia.

The aim of our study was to evaluate the effect of alpha-lipoic acid on the postocclusive hyperaemic response in type 2 diabetes (NIDDM) patients with diabetic peripheral neuropathy. Subjects: 36 NIDDM patients with diabetic peripheral neuropathy were divided in two groups according to the dosage of alpha-lipoic acid: 18 patients received 600 mg (D1) and 18 patients received 1800 mg (D2) alpha-lipoic acid orally for 4 weeks. 20 healthy subjects were selected as controls (C). Patient groups were matched for age, sex, body

mass index and HbA_{1c}. Subjects with peripheral vascular disease, hypertension, microalbuminuria and dyslipidemia were excluded. Methods: Before and after therapy we recorded changes in the cutaneous blood flow induced by 3 min arterial occlusion (cuff above the knee) on the pulp of the big toe using laser Doppler (LD) fluxmetry (*PeriFlux* 4001, Perimed). Basal LD flux (b-LDF; PU), the percent increase of b-LDF (Δ LDF; %) and the time to peak LD flux (t-LDF; s) were calculated (*PeriSoft* program). Results: Before therapy b-LDF on the pulp was significantly higher (mean \pm SD; Mann-Whitney U test) in diabetic patients (D1: 116.1 ± 47.4 , D2: 99.8 ± 55.6 vs C: 58.5 ± 22.0 PU, $p < 0.01$), LDF was decreased in diabetic patients, and t-LDF was significantly shorter in diabetic patients (D1: 22.8 ± 8.6 , D2: 24.5 ± 7.1 vs C: 39.8 ± 9.7 s, $p < 0.05$) in comparison to controls. Oral therapy for 4 weeks with a high dose (1800 mg daily) of alpha-lipoic acid restored Δ LDF ($111.8 \pm 44.9\%$ vs $72.7 \pm 11.0\%$ before therapy, $p < 0.05$). Conclusion: Our results suggest that high dose of alpha-lipoic acid improves responsiveness of cutaneous microvessels to arterial occlusion test in diabetic patients with peripheral neuropathy.

PRE-PERCEPTUAL PAIN SENSORY RESPONSES (N1 COMPONENT) IN TYPE 1 DIABETES MELLITUS (DM): A LASER EVOKED POTENTIALS (LEPs) STUDY

Rossi P¹, Serrao M¹, Gabriele A², Morano A², Di Mario U², Pozzessere G¹. ¹Istituto di Clinica delle Malattie Nervose e Mentali, Il Clinica Medica, ²Università "La Sapienza," Rome, Italy.

Background. LEPs may be a useful and sensitive diagnostic tool for assessing the function of small-myelinated fibers (A δ) in diabetic neuropathy (DN). This statement is based on recent studies measuring the N2-P2 vertex potentials. This component is strongly influenced by attention processes and may reflect cognitive processing of nociceptive inputs. It represents a drawback of LEP studies. Recently, it has been demonstrated that a smaller N1 component is not influenced by attention shifts and is thought to be more specific for the sensory-discriminative aspects of pain. Aim of the study. To evaluate the integrity of A δ -fibers in type 1 DM by measuring the N1 component of LEPs. Subjects. 12 healthy volunteers and 20 diabetics divided in two groups: Group 1 included 11 patients staged as N0; group 2 included 9 patients staged as N1 or N2. Methods. N1 component following hand (LEPH) and foot (LEPF) stimulation was measured using a simple fronto-temporal derivation. Results. A clear N1 component was recorded in 12/12 of controls, 9/11 of patients in group 1 and 6/9 of patients in group 2. No significant difference was found for LEPH. LEPF was significantly prolonged in group 2 patients (controls = 202 ± 10.1 ms; group 1 = 208 ± 12.3 ms; group 2 = 240.4 ± 14.3 ms, $p < 0.05$). 2 patients in group 1, and 4 patients in group 2 presented abnormal LEPF values. Discussion. N1 component following foot stimulation is abnormally prolonged in type 1 diabetic patients with clinical or electrophysiological evidence of DN. A subclinical hypoalgesia was found in asymptomatic patients as well. These data suggest that LEP abnormalities are secondary to a small fiber dysfunction.

EXPRESSION OF O-ACETYL SIALIC ACID ON CEREBRAL MICROCIRCULATION IN A GLYCINE OR TAURINE TREATED DIABETIC RAT MODEL

Noe A, Pedro Z, Ricardo L*, Edgar Z*, Alejandra B, Guillermo C. Department of Pharmacology, *Department of Biochemistry; Instituto Nacional de Enfermedades Respiratorias, Calz. de Tlalpan 4502, Col. Seccion XVI, Mexico, D.F.

Expression of sialic acid is altered in Diabetes mellitus. This modification has also been involved with both vascular and neurologic diseases, and with the increase of non enzymatic glycosylation of proteins. In our opinion, the lectins were very useful with specificity for sialic acids in order to determine the level of sialic acid expression on cerebral microcirculation in a diabetic Wistar rat model with streptozotocin. In this model, the glycine (1%) and taurine (0.5%) aminoacids were placed in drinking-water by six months. At the end of this time, the animals were sacrificed, their brains surgically removed and frozen in liquid nitrogen, and the specimens cut in serial sections. Immediately, the sections were incubated with different biotin-labelled lectins specific to sialic acid using peroxidase-labelled avidin as second ligand and H₂O₂ chromogen. The results showed greater O-acetyl sialic acid expression in cerebral capillaries of untreated diabetic rats than in glycine-, taurine-treated diabetic rats or than in control animals. The minor sialic acid expression may be an indicator of degenerative diseases such as Alzheimer's or the vascular disease of diabetic patients and probably is related to cellular protective properties of the glycine and taurine aminoacids. These first protective characteristics that have been observed in both ischemia with cellular ATP depletion models, suggest the utilization of aminoacids glycine or taurine in diabetic patient in order to avoid the development of microinfarcts.

INSTABILITY OF CEREBRAL BLOOD FLOW IN DIABETIC PATIENTS WITH CARDIOVASCULAR AUTONOMIC NEUROPATHY AND ORTHOSTATIC HYPOTENSION

Mankovsky BN¹, Piolot R², Mankovsky O³, Ziegler D.² Institute of Endocrinology,¹ Kiev, Ukraine; German Diabetes Research Institute, Düsseldorf, Germany²; National Medical University, Kiev, Ukraine.³

Cardiovascular autonomic neuropathy (CAN) is a risk factor for stroke in people with diabetes mellitus, and orthostatic hypotension (OH) is a feature of CAN. Therefore, the purpose of this study was to evaluate the mean cerebral blood flow velocity (MFV) in response to standing up in patients with diabetes and OH. We studied 27 patients with diabetes mellitus, among whom 8 had CAN and OH (OH+, age: 46.4 ± 13.5 years, diabetes duration: 25.0 ± 11.0 years; mean ± SD), 7 had CAN but without orthostatic hypotension (OH-, age: 47.3 ± 12.7, diabetes duration: 26.4 ± 12.1 years), 12 had no evidence of CAN (CAN-, age: 44.1 ± 13.8, diabetes duration: 17.1 ± 10.2 years), and 14 control subjects (age: 42.6 ± 9.1 years). The MFV in the right middle cerebral artery was recorded using transcranial Doppler sonography in the supine position after 15 minutes of rest and immediately after standing up. The change from the supine position to standing resulted in a pronounced drop in mean blood

pressure in the OH+ group (−14.0 ± 20.9 mmHg), while there were no significant changes in blood pressure in the other groups. MFV in the supine position was not different between the diabetic (either with or without CAN or OH) and control groups. However, the relative percentage changes in MFV immediately after standing up were −23.3 ± 17.2% in OH+, +0.6 ± 14.6% in OH-, −2.3 ± 15.1% in CAN-, and −10.5 ± 11.1% in the control group (p < 0.05 for OH+ vs. the other groups). Patients with diabetes mellitus complicated by CAN and OH may show instability in cerebral blood flow regulation on standing. Whether this finding is associated with a higher susceptibility to stroke in these patients remains to be established in prospective studies.

LANGERHANS ISLET IMPLANTS IN DIABETIC PATIENTS. PRELIMINARY RESULTS

Alcázar-Montenegro H, *Alvarado-Vásquez N, Alcázar-Leyva S. Hans Selye Scientific Research Institute, A.C; *Department of Pharmacology, INER. Mexico.

Glycolysis, the Krebs cycle, oxidative phosphorylation and the pentose cycle are blocked in diabetes mellitus (DM). This results in alterations in the production of ATP and an increase in free radicals which damage protein lipids and DNAmt. In 1981, Cerami et al. stated that an excess of glucose is the cause of the classical complications in diabetics. The transplanting of pancreatic beta cells was proposed as a method of control for diabetes mellitus. This procedure in animals and humans has given rise to encouraging results, but only in patients receiving immunosuppressive therapy for renal transplants. On an experimental basis, we have implanted pancreatic beta islets and have obtained a reduction in glucose levels. In the present paper we evaluate the clinical and laboratory response of diabetic patients who, prior treatment with a modified form of RNAi (RNAi HAM) to induce the synthesis of IFN, received parenteral implants of Langerhans islets extracted from sheep. Immediately after, patients were given parenteral injections of thiamine pyrophosphate (X-2). Three days after the implant blood glucose levels had reduced. Clinical improvement was recorded through the disappearance of asthenia, adynamia and depression. The administration of hypoglycemic agents or insulin was reduced or suspended according to laboratory results. We conclude that the implanting of pancreatic islets is a good option for the treatment of DM, on condition that RNAi HAM is used to avoid rejection from immunological tolerance.

THE FREQUENCY OF ARTERIAL HYPERTENSION VERSUS ORTHOSTATIC HYPOTENSION IN DIABETIC PATIENTS

Ionescu-Tîrgoviste C, Tinu AM, Mircea E, Guja C, Vasilescu R, Cristescu J, Bratu D. Institute of Diabetes, Nutrition and Metabolic Diseases "N.C. Paulescu," Bucharest Romania.

Aim: The aim of this study was to assess the relationship between supine high blood pressure and orthostatic hypotension both in Type 1 (T1DM) and Type 2 (T2DM) diabetic patients. **Patients and Methods:** Our study included 321 T2DM patients (153 M/168 F; mean age 62.3 (14.2 yr; duration of dis-

ease 12.1 (7.6 yr) and 116 T1DM patients (65 M/51 F; mean age 39.7 (9.2 yr); duration of diabetes 11.9 (8.1 yr). Patients with orthostatic hypotension were divided into 3 groups: A – without symptoms; B – mild/moderate symptoms (short and tolerable dizziness when standing); C – severe symptoms (persistent and disabling dizziness or even fainting in upright position). Results: *Arterial hypertension* was registered in 67.6% of T2DM patients (217 from 321 cases) and in 50.0% of T1DM patients (58 from 116 cases). *Orthostatic hypotension* (defined as a decrease in systolic blood pressure (30 mm Hg)) was encountered in 64.5% in T2DM patients (207 out of 321 cases) and in 60.3% of T1DM patients (70 out of 116 cases). From 207 T2DM patients with orthostatic hypotension, 105 were in Group A (50.7%), 89 in Group B (42.99%) and 13 in Group C (6.28%), while from 70 T1DM patients with orthostatic hypotension 14 were in Group A (20.0%), 51 in Group B (72.8%) and 5 in Group C (7.14%). An association of supine arterial hypertension with orthostatic hypotension was registered in 96 (29.9%) T2DM patients (68 of them receiving antihypertensive treatment) and in 25 (21.5%) T1DM patients (19 of which were on antihypertensive treatment). From the 18 patients with severe orthostatic hypotension (13 T2DM and 5 T1DM), supine arterial hypertension was registered in 5 cases (3 T2DM and 2 T1DM). In 4 of these 5 cases, patients were receiving antihypertensive treatment. Discontinuation of this treatment led to a decrease in the intensity of clinical signs of orthostatic hypotension in 4 out of 5 cases. An improvement of clinical symptoms of orthostatic hypotension was recorded in about 1/3 of hypertensive patients after discontinuation or just lowering of the dose of antihypertensive drugs (26 out of 87 cases). Conclusion: An association between hypertension and orthostatic hypotension is frequent both in T1DM and in T2DM, rising in difficulties for treatment. The treatment of hypertension in diabetic patients should take into account the possible orthostatic hypotension induced by some of the antihypertensive drugs.

ATROPHY OF FOOT MUSCLES IN TYPE 1 DIABETIC PATIENTS IN RELATION TO PRESENCE AND SEVERITY OF NEUROPATHY

Andersen H, Gjerstad MD, Jakobsen J. Department of Neurology, Aarhus University Hospital, Aarhus, Denmark.

Diabetic patients with neuropathy may develop motor dysfunction. In a previous study we found atrophy of the ankle extensors and flexors in neuropathic patients being more pronounced distally. This indicates that evaluation of the morphology of the foot muscles may be more sensitive for the detection of muscular atrophy in neuropathic patients. In this study we included 23 type 1 diabetic patients with a diabetes duration of 29 years (16–38) (median, range). For comparison, 23 age-, sex-, weight-, and height-matched control subjects were included. Using magnetic resonance imaging, the muscular morphology of the non-dominant foot was evaluated in all subjects. Applying stereological techniques on consecutive cross-sectional magnetic resonance images, the total volume of the foot muscles was determined. In addition, the diabetic patients were assessed clinically with a neuropathy symptom score and a neurological impairment score. Patients were also evaluated with motor nerve con-

duction studies and quantitative sensory examination. As compared with the healthy controls, the volume of the foot muscles was reduced in the diabetic patients being 117 cm³ (23–209) (median, range) and 166 cm³ (94–263) ($p < 0.005$), respectively. In the 8 non-neuropathic patients, the muscle volume was 106% (70–130) of the matched controls, whereas the 15 neuropathic patients had a muscle volume of only 49% (11–95) of the controls ($p < 0.001$). Furthermore, there was a close inverse relationship between the neurological impairment score and the total muscle volume ($r = -0.81$, $p < 1 \cdot 10^{-5}$). In conclusion, the volume of the foot muscles is reduced in long-term type 1 diabetic patients with neuropathy. The muscular atrophy is closely related to the severity of neuropathy. We suggest assessment of the volume of the foot muscles to be a sensitive method for the detection of motor dysfunction in diabetic neuropathy.

ARTERIAL RIGIDITY AND CARDIOVASCULAR SYMPATHETIC TONE IN HYPERTENSIVE OBESE AND TYPE 2 DIABETIC PATIENTS

Valensi P, Dabire H, Brahimi M, Platon P, Hadj-Brahim F, Attali JR. Department of Diabetology, Jean Verdier Hospital, Bondy. INSERM U337, Paris 6 University, France.

An increase of arterial rigidity and sympathetic activity has been suggested to contribute to essential hypertension. We have shown that vagal control of heart rate (HR) variations during standardized tests is similarly impaired in normotensive obese and type 2 diabetic patients. The aim was to compare cardiovascular vagosympathetic balance and the link between pulse pressure, an index of arterial rigidity, and sympathetic activity in normotensive and hypertensive obese and type 2 diabetic patients. Groups 1 and 2 consisted of 70 normotensive and 32 hypertensive obese patients, groups 3 and 4 of 18 normotensive and 14 hypertensive diabetic patients respectively. HR and blood pressure (BP) variations were studied with a plethysmographic system and spectral analysis (Finapres). During a 5 min-period at a controlled breathing rate, in the 4 groups, the high frequency peak of HR variations (vagal control) was significantly lower than in controls (19 healthy subjects), and the mid/high frequency peak ratio of HR variations was significantly increased. During a standing test, the mid-frequency peak of systolic BP variations (sympathetic activity) did not differ significantly in obese or diabetic patients, either normotensive or hypertensive, and in controls. This peak correlated significantly with pulse pressure in groups 2 and 4 and in the control group but not in groups 1 and 3. In conclusion, 1) spectral analysis confirms that in obese and diabetic patients vagal control of HR variations is similarly reduced and suggests that sympathetic activity is relatively increased; 2) in hypertensive patients sympathetic tone is not higher than in normotensive ones, but may contribute to arterial rigidity.

ABNORMALITIES OF THE FOOT LOADING PATTERN ARE PRESENT IN PATIENTS WITH MILD NEUROPATHY

Caselli A, Giacomozzi C*, Macellari V*, Latini V, Menzinger G, Uccioli L. University of Rome "Tor Vergata," *I.S.S., Rome.

Specific alterations of the foot loading pattern have been described in patients with severe diabetic neuropathy, however, some of these might be already present in the earliest stages of peripheral neuropathy. Aim: The aim of our study was to describe the loading pattern in diabetic patients without signs or symptoms of peripheral neuropathy (D), and with mild (DM) and moderate-severe (DS) neuropathy, classified according to the Neuropathy Disability Score (NDS), respect to controls (C). Materials and methods: We studied 20 D, 30 DM, 32 DS, age and duration of disease matched, respect to 36 C patients using a piezo-dynamometric platform that is able to detect the three components of the Ground Reaction Force (GRF) under selected foot sub-areas, namely heel, metatarsal and hallux.

(N %b.w.)	C (36)	D (20)	DM (30)	DS (32)
Fz	93.81 ± 8.4	94.87 ± 9.6	88.89 ± 7.9	87.31 ± 8.4*§
Heel Fx	15.3 ± 3.6	15.46 ± 3.7	14.35 ± 3.1	12.54 ± 3.5*§
Fy	3.37 ± 1.8	3.54 ± 1.9	3.42 ± 2.0	3.57 ± 1.7
Fz	89.99 ± 10.3	95.92 ± 7.6	92.65 ± 5.8	94.05 ± 9.0
Metal Fx	13.37 ± 2.5	13.89 ± 4.1	12.02 ± 2.1	12.63 ± 3.2
Fy	3.93 ± 2.1	4.45 ± 2.1	4.25 ± 2.0	4.37 ± 2.1
Fz	21.93 ± 9.2	18.07 ± 7.9	16.48 ± 7.7*	14.01 ± 6.2*
Hallux Fx	5.3 ± 2.3	4.67 ± 2.5	3.21 ± 1.4*§	3.06 ± 2*§
Fy	0.84 ± 0.7	0.47 ± 0.6	0.31 ± 0.43*	0.17 ± 0.3*

* < 0.05 vs C; § p < 0.05 vs D

Results: DM and DS showed a significant reduction of the vertical (Fz), the anteroposterior (Fx) and the mediolateral (Fy) components of GRF under the hallux respect to C and D. At the metatarsal sub-area the peak value of Fz was not significantly different among the four groups of patients, however DS showed an increase of the loading time in this area, expressed by a significantly higher Fz integral (C 2956 ± 430.5, D 3025 ± 521.9, DM 3332 ± 440.3, DS 3525 ± 695.3*§ N.msec). At the heel, only DS showed a reduction of the load. No significant differences were observed between C and D. Conclusions: Diabetic patients with mild neuropathy show specific changes in the loading pattern, mainly at the hallux area. Further changes are recorded at the heel and metatarsal only in patients with moderate-severe neuropathy and might justify the increased risk of foot ulceration in this group of patients.

EFFECT OF RENAL DENERVATION ON URINARY TGF-β1, ALBUMINURIA AND RENAL CONTENT OF GLUT1 IN STZ-INDUCED DIABETIC RATS

Schmid H, Schaan B, Bertolluci M, Lacchini S, Machado U, Irigoyen MC. Porto Alegre, Brazil.

In long-term Diabetes Mellitus (DM) the progression of nephropathy has been related to the occurrence of autonomic neuropathy and hypertension. The STZ-diabetic rat was previously described by us as an animal model that develops albuminuria, as well as increased expression of cortical renal GLUT1 and of Transforming Growth Factor 1

(TGF1) and increased urinary excretion of TGF1. The aim of this study was to evaluate the effect of renal denervation (RD) upon urinary TGF-β1 (UTGF-β1) and cortical renal GLUT1 protein levels, relating these parameters to blood pressure (BP) levels and metabolic control. Streptozotocin injected rats (group D; n=13) and control animals (group C; n=13) were compared for their urinary albumin (UA), UTGF-1, BP and for their renal cortical and medullar GLUT1 protein abundance 45 days after the beginning of hyperglycemia. The animals were submitted to surgical RD or sham procedure within 30 days of STZ injection, and evaluations were done 15 days after the surgery. The effects of RD were confirmed by intra-renal decrease of norepinephrine levels. Results showed that RD determined no changes in UA or UTGF-β1, in both D and C rats. Blood pressure levels were similar between D and C and did not change as an effect of denervation. These results are shown in the table.

	Arterial Pressure (mmHg)	TGF-β1 (ng/mg cre)	GLUT1 (AU)
C (n=8)	115 8	10.6 3	3506 193
C-RD(n=5)	113 8	13.4 4	6905 288 #
D (n=6)	103 12	75.8 26 *	5204 516 #
D-RD(n=7)	105 14	111.8 24 *	4144 246 §

Mean ± SE- ANOVA; * p < 0.001 vs C and C-RD; # p < 0.05 vs C. cre = creatinine; AU = Arbitrary Units

In conclusion, in the STZ-rat with albuminuria complicating diabetes, RD produces a normalization of previously elevated cortical GLUT1 protein and no effect upon UTGF-β1 and UA, suggesting that these animals (with no hypertension) are protected from the deleterious effect of kidney denervation.

HEDGEHOG PROTEINS PLAY A ROLE IN PERIPHERAL NERVE REGENERATION

Engber TM¹, Allendoerfer KL², Berg A², Drake E², Mahanthappa N², Mozell R¹, Pepicelli C², Ranciato R², Reilly JO², Sah D¹, Wang S¹, Galdes A.¹ ¹Biogen Inc. and ²Ontogeny Inc., Cambridge, MA, USA.

Hedgehog proteins are embryonic induction factors that play a role in the development of the central and peripheral nervous systems. The Hedgehog proteins and their signaling pathway continue to be expressed in the adult nervous system, where their role is not known. In adult peripheral nerve, Sonic Hedgehog (Shh) is expressed in a subset of motor neurons, Desert Hedgehog (Dhh) is expressed in Schwann cells, and the Hedgehog receptor Patched (Ptc) and the Hedgehog-responsive transcription factor Gli are expressed predominantly in perineurial cells. We have used the mouse sciatic nerve crush model to assess whether the Hedgehog pathway plays a role in nerve regeneration. The sciatic nerve was crushed in adult, male CD-1 mice, and the expression of Dhh, Ptc-1, Ptc-2 and Gli-1 was examined 1, 3, 7 and 13 days later. The ability of exogenous and endogenous Hedgehog proteins to influence functional recovery after nerve crush was assessed by measuring toe spread

and ability to grip with the hindpaws. Little change in expression was seen 1 or 3 days after nerve crush. At 7 days and, to a greater extent, 13 days, there was a pronounced increase in expression of Dhh in Schwann cells and Ptc-1 in perineurial cells. Expression of Ptc-2 and Gli-1 increased not only in the perineurial layer, but also in the endoneurium. Treatment with a Shh-Ig fusion protein (1 mg/kg s.c. every other day) improved recovery of both toe spread and ability to grip. Conversely, treatment with an anti-Hedgehog neutralizing antibody (10 mg/kg s.c. every other day) slowed functional recovery, while an isotype control antibody had no effect. These findings suggest that endogenous Hedgehog proteins play a role in promoting regeneration following nerve injury and that administration of exogenous Hedgehog proteins can further accelerate recovery of injured peripheral nerves. Therefore, Hedgehog proteins may be useful in the treatment of nerve injury and peripheral neuropathies.

ALDOSE REDUCTASE-DEFICIENT MICE ARE PROTECTED FROM REDUCTION OF NERVE CONDUCTION ASSOCIATED WITH DIABETES

Ho ECM^{1,2}, Lam KSL², Chung SSM¹, Chung SK.¹ ¹Institute of Molecular Biology and Department of Medicine,²The University of Hong Kong, Pokfulam, Hong Kong, SAR, China.

Previously, we have shown that aldose reductase (AR) mRNA is strongly expressed by Schwann cells and not expressed by the endothelial cells in the sciatic nerve. Additional over-expression of AR in the Schwann cells lead to further reduction of motor nerve conduction velocity (MNCV) in diabetic and more prominently in galactosemic mice, suggesting AR's contribution to hyperglycemic and galactosemic lesions in the nerve. Here, we report the deletion of AR gene in mice to study its physiological functions and to understand its role in the pathogenesis of diabetic neuropathy. The homozygous AR-deficient mice showed no apparent developmental or reproductive abnormalities except they drink and urinate significantly more than their wildtype littermates due to a partially defective urinary concentrating ability. These AR knockout mice are then converted to become diabetic by streptozotocin (STZ) treatment to determine the effects of AR-deficiency on the MNCV. The diabetic mice, whether they are AR deficient or wildtype mice, drank and urinated similarly. Whereas the wildtype showed significant reduction in MNCV after 4 weeks of STZ treatment ($p <$

0.01), the AR-deficient mice did not show any significant reduction in MNCV. Interestingly, the AR-deficient mice have less body weight reduction after 4 weeks of diabetes compared to those of wildtype mice treated to STZ ($p < 0.05$) suggesting that AR deficiency has an overall beneficial effect on diabetic mice.

ONE YEAR FOLLOW-UP IN DIABETIC PATIENTS AFTER SURGICAL TREATMENT OF CARPAL TUNNEL SYNDROME

Reljanovic M, Barada A, Bilic R, Kovijanic J, Vuk Vrhovac Institute, Dugi dol 4a, Zagreb; Orthopedic Clinic, Zagreb, Croatia.

The aim of the study was to evaluate clinical and neurophysiological changes in diabetic patients who had the surgery of carpal tunnel syndrome (CTS). The group consisted of 61 diabetic patients with the clinical and neurophysiological signs of CTS, with mean age 50.3 (± 27.3) and diabetes duration 13.5 years (± 8.9). The response to treatment was evaluated using visual analogue scale (VAS) for pain and for paresthesias prior to surgery, as well as 3 and 12 months after. At the same time we compared the median motor distal latency, amplitude of motor nerve action potentials, sensory conduction velocity and amplitude of sensory nerve action potentials in distal segment of median nerve as well as vibration perception threshold. The improvement was found for pain (baseline VAS 4.5 cm vs. follow-up VAS 1.5 cm; Wilcoxon $p = 0.00365$) and for paresthesias (baseline VAS 5.4 cm vs. follow-up VAS 0.8 cm; Wilcoxon $p = 0.00000$) after 3 months. Subjective pain and paresthesias assessment remained at the improved level after 12 months of follow-up. Neurophysiological parameters measured were shown to improve after 3 and 12 months of follow-up period: median motor distal latency (baseline 5.9 ms/6 cm, 3 months 4.8 ms/6 cm, 12 months 4.1 ms/6 cm; ANOVA $p < 0.00000$), amplitude of median motor action potential (baseline 4.8 mV, 3 months 5.6 mV, 12 months 6.6 mV., ANOVA $p < 0.01040$), median sensory conduction velocity in distal segment (baseline 37.9 m/s, 3 months 43.0 m/s, 12 months 47.3 m/s, ANOVA $p < 0.00464$), vibration perception threshold (baseline 9.8 V, 3 months 7.6 V, 12 months 6.8 V; ANOVA $p < 0.00006$). Concerning our results, the clinical and neurophysiological parameters supported surgery as the effective treatment for CTS in diabetics.