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Neurogenic cutaneous vasodilatation and plasma extravasation in diabetic rats: effect of insulin and nerve growth factor

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1 Neurogenic vasoactive responses in rat skin were investigated following 8 weeks of streptozotocin-induced diabetes to determine the effect of diabetes and of treatment with insulin and nerve growth factor (NGF) treatment.

2 Diabetic rats were divided into three groups: untreated; insulin (4 IU day^{-1} by s.c. implant weeks 4–8) treated; Nerve Growth Factor, NGF, (0.2 mg kg^{-1} three times weekly, weeks 4–8) treated. A fourth group served as a non-diabetic control.

3 Electrical stimulation of the saphenous nerve (10 V, 2 Hz, 1 ms for 30 s) increased blood flow in the ipsilateral paw skin, as measured by laser Doppler flowmetry. The peak increase was similar between groups, but the time taken for flow to return to a steady baseline was significantly ($P<0.01$) reduced in untreated diabetic rats, when compared with non-diabetic controls, but not significantly reduced in the insulin- or NGF-treated diabetic groups.

4 A second stimulation of the saphenous nerve (10 V, 2 Hz, 1 ms for 5 min) produced plasma extravasation, measured by the extravascular accumulation of ^{125}l-albumin, in the skin. Plasma extravasation was significantly attenuated ($P<0.001$) in the untreated diabetic group, but not the insulin-treated group, compared to non-diabetic controls. Plasma extravasation was present, though reduced, in the NGF-treated group.

5 Plasma extravasation induced by intradermal injections of substance P with and without CGRP was similar in all groups indicating no decrease in vascular responsiveness to exogenously applied neuropeptides. The results suggest that release of neuropeptides is diminished in diabetes and that treatment with either insulin or NGF can restore neurogenic microvascular vasoactive responses towards normal.

Keywords: Diabetic neuropathy; rat skin microvasculature; neurogenic inflammation substance P; CGRP; insulin; nerve growth factor, NGF

Introduction

Peripheral neuropathy is a major complication associated with diabetes mellitus. Sensory neurone (mainly C and Aδ fibers) dysfunction occurs in patients who often present with impaired sensory responsiveness and accompanying cutaneous abnormalities (Dyck, 1992; Levy *et al*., 1992). Loss of nociception and axon-reflex vasodilatation is thought to lead to many of the complications seen in these patients, such as foot ulceration. The mechanisms underlying diabetic neuropathy are complex, but microvascular responsiveness appears to be compromised with reduced flare responses to stimulation in skin reported (Aronin *et al*., 1987). Sensory nerves not only transmit sensory information, but are also able to act upon the local environment by releasing biologically active species. Neuropeptides including the tachykinins (substance P and neurokinin A), as well as calcitonin gene-related peptide (CGRP), are well documented as present in the peripheral endings of sensory nerves and to be released from the peripheral terminals of these nerves to act in a potent manner to mediate vasoactive effects at the microvascular level. These may be acute, involved in maintaining homeostasis, or alternatively they may influence ongoing inflammatory or wound healing processes, depending on circumstance (Holzer, 1992; Brain, 1996 for reviews).

The electrical or chemical stimulation of sensory nerves in animal models has been an essential component in the study of sensory nerve function. For example the stimulation of the sensory saphenous nerve in the anaesthetized normal rat leads to increased blood flow and oedema formation in the innervated area of hind paw skin which can be analysed by quantitative methods (Escott & Brain, 1993). Evidence indicates that CGRP acting via $CGRP_1$ receptors, is the major mediator of the vasodilator component as the response can be largely inhibited by using the CGRP antagonist, $CGRP_{8-37}$, (Escott & Brain, 1993; Escott *et al*., 1995). By comparison substance P, acting via tachykinin NK_1 receptors, is the major mediator of increased microvascular permeability, which leads to the observed oedema formation (Edmonds-Alt *et al*., 1993). Indeed, neurogenic oedema formation can be inhibited using a range of selective NK_1 antagonists (e.g. SR140333, (Edmonds-Alt *et al*., 1993)).

An insulin-sensitive depletion of substance P and CGRP has been shown to occur in sensory nerves in rats made diabetic with streptozotocin (Diemel *et al*., 1992) and this depletion has been suggested to be involved in some of the microvascular problems associated with diabetes. Growth factors (especially nerve growth factor, NGF, a member of the neurotrophin family) exerts essential trophic effects with respect to survival and integrity of sensory nerves (Lindsay *et al*., 1994). A reduction in availability of NGF in peripheral tissues such as skin and in the uptake and transport of NGF in nerves, occurs in diabetic neuropathy (Hellweg *et al*., 1994). NGF increases levels of both substance P and CGRP *in vitro* (Lindsay & Harmar, 1989) and *in vivo* in diabetic rats (Apfel *et al*., 1994; Diemel *et al*., 1994). Additionally, agents which increase NGF production *in vivo* can increase substance P and CGRP levels in sciatic nerve of diabetic rats (Garrett *et al*.,

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1997). Indeed NGF administration has been shown to normalize the response to thermal and mechanical sensation in diabetic rats (Apfel *et al*., 1994). Thus it is hypothesized that reduced endogenous NGF contributes to the neurological dysfunction that relates to an altered microvascular function in diabetic neuropathies and that the administration of NGF may be a useful therapeutic approach.

It has been previously shown that a reduced neurogenic oedema formation is observed in the skin of streptozotocin-induced diabetic rats (Gamse & Janscö, 1985). The aim of this study was to determine the effect of exogenously administered NGF, when compared with insulin treatment, on responses observed in the cutaneous microvasculature after administration of substance P and CGRP and on neurogenically-mediated vasodilatation and oedema formation after stimulation of the saphenous nerve in the rat.

Methods

Male Wistar rats (Charles River, U.K. weighing approx. 300 g at the start of the experiment were used. Rats were allowed free access to food and water throughout the study and were housed in groups with similarly treated animals. All experiments were carried out according to the Animals (Scientific Procedures) Act 1986.

Induction of experimental diabetes and treatments

Rats were divided into four groups, for each of the two experiments. All groups of animals were fasted overnight and three groups then made diabetic by a single intraperitoneal (i.p.) injection of streptozotocin (50 mg kg^{-1}, Sigma, U.K.). The fourth group served as a non-diabetic control. A blood sample was taken 3 days later from the tail vein and blood glucose measured using a glucose oxidase strip-operated reflectance meter (Reflolux II, BCL, Boehringer Mannheim). Any streptozotocin-treated animals with a blood glucose level below 15 mM were excluded from the study. Animals were maintained for 4 weeks, with body weights recorded weekly.

After 4 weeks, one group of diabetic animals received continuous-delivery (4 IU day^{-1}) subcutaneous, s.c., insulin implants (Linplant; Møllegaard, Ejby, Denmark) placed subcutaneously at the back of the neck under halothane anaesthesia (Stevens *et al*., 1994). Another group received human recombinant NGF treatment (0.2 mg kg^{-1} s.c. at the back of the neck, three times per week, Genentech, San Francisco, U.S.A.). The third group of diabetic animals remained untreated. The animals were then maintained for a further 4 weeks before blood flow and oedema experiments. Blood glucose levels were measured immediately at the start of these experiments.

Blood flow changes and oedema formation mediated by saphenous nerve stimulation (Experiment 1)

Rats were anaesthetized by i.p. injection of sodium pentobarbitone (Sagatal 60 mg kg^{-1}, May & Baker, UK). The tail vein was cannulated to allow the maintenance of anaesthesia with sodium pentobarbitone (15 mg ml^{-1} i.v., 0.1–0.4 ml, as required). Both hind limbs were shaved and depilated and both saphenous nerves dissected clear of surrounding tissue. The nerves were then ligated centrally and immersed in mineral oil for the duration of the experiment. Rats were placed on an automatic heating pad to maintain the body temperature at 36–38°C. The method was adapted from Escott & Brain (1993) to allow assessment of both blood flow change and oedema formation in the same animal using two separate stimulation periods.

A laser Doppler blood flow probe (Moor Instruments, U.K.) was placed over the area of skin innervated by the saphenous nerve (Escott & Brain, 1993). Rats were left at least 30 min after dissection until a steady basal blood flow was seen in both paws. One saphenous nerve was then placed on a bipolar platinum electrode and stimulated (10 V, 1 ms, 2 Hz for 30 s). The other paw acted as a sham control. The blood flow response in both paws was recorded on the laser Doppler flow meter (see Figure 2).

After 30 min, when the blood flow had returned to a steady level, the animals received ^{125}I-human serum albumin (1 μCi, Amersham International, U.K.) and Evans Blue dye (0.2–0.5 ml of 2.5% w/v in saline) i.v. via the tail vein in order to visualize and quantify plasma extravasation in paw skin (Brain & Williams, 1985; Escott & Brain, 1993). The same saphenous nerve was again lifted on to the platinum electrodes and stimulated (10 V, 1 ms, 2 Hz for 5 min).

Immediately after stimulation, a blood sample was taken by cardiac puncture and the animals killed by anaesthetic overdose and cervical dislocation. The skin of the innervated areas of both hind paws was removed and weighed. The radioactivity of plasma and skin samples was then measured in a gamma counter. The plasma extravasated was calculated and expressed as the volume of plasma per 100 mg tissue for stimulated and sham legs.

Cutaneous skin oedema formation induced by i.d. mediators (Experiment 2)

Plasma protein extravasation in rat skin, induced by the intradermal (i.d.) injection of test agents, was measured by the extravascular accumulation of i.v. ^{125}I-human serum albumin (Brain & Williams, 1985). Rats were anaesthetized and cannulated as before. The dorsal skin was shaved and marked out for injection of test substances according to a balanced, randomized plan with two sites per test agent. Test agents used were substance P (Sigma, U.K.), GR73632 (a gift from Dr D. Beattie, GlaxoWellcome, U.K.), CGRP (Peninsula Laboratories, U.K.). The rats received ^{125}I-albumin (1 μCi) and Evans Blue dye (0.2–0.5 ml of 2.5% w/v in saline, Sigma, U.K.) i.v. via the tail vein. Test agents were made up in modified Tyrode's balanced salt solution containing (mM) NaCl 136.89, KCl 2.68, NaH_2PO_4 0.42, $NaHCO_3$ 11.9, $MgCl_2$ 1.05 and glucose 5.5 and injected i.d. (0.1 ml $site^{-1}$). After a 30 min accumulation period, a blood sample was taken by cardiac puncture and the rats killed by cervical dislocation. The dorsal skin was then removed and injection sites punched out (16 mm diameter) and counted in a gamma counter (Wallac 1282, Wallac, U.K.). The plasma protein extravasation at each site was expressed as volume of plasma extravasated, calculated from the counts in 0.1 ml plasma.

Statistical analysis

Statistical analysis was determined on the original data with tests as described below. The effect of different systemic treatments on weights (Figure 1a) and on oedema responses to exogenous peptides (Figure 5) was calculated using two way analysis of variance, (ANOVA), followed with a one tailed *t*-test on population means. A paired *t*-test was used to determine effect of insulin treatment on glucose levels (Figure 1b) and on the comparison of plasma extravasation in stimulated compared with sham paws (Figure 4). One way ANOVA followed by Dunnett's *t*-test was used for all other analyses.

Results

Effects of diabetes on body weight and plasma glucose levels

The control (non-diabetic) animals grew steadily over the 8 week experimental period, as shown in Figure 1. The three groups of diabetic animals all failed to grow significantly during the first 4 weeks of the experiment. Insulin treatment (4 IU day^{-1}, s.c., from slow-release implant) during weeks 4–8 caused a significant increase in body weight, to a level approaching that seen in the control animals. Treatment with NGF during weeks 4–8 had no effect on body weight. Blood glucose levels were significantly elevated in all diabetic groups compared with non-diabetic controls, with the insulin-treated diabetic group showing a significantly reduced level compared with uncontrolled diabetics. Glucose levels in NGF-treated diabetics were not significantly different to those seen in untreated diabetics. Similar differences between groups were seen for rats in Experiments 1 and 2 and Figure 1 contains data from all rats used in the study.

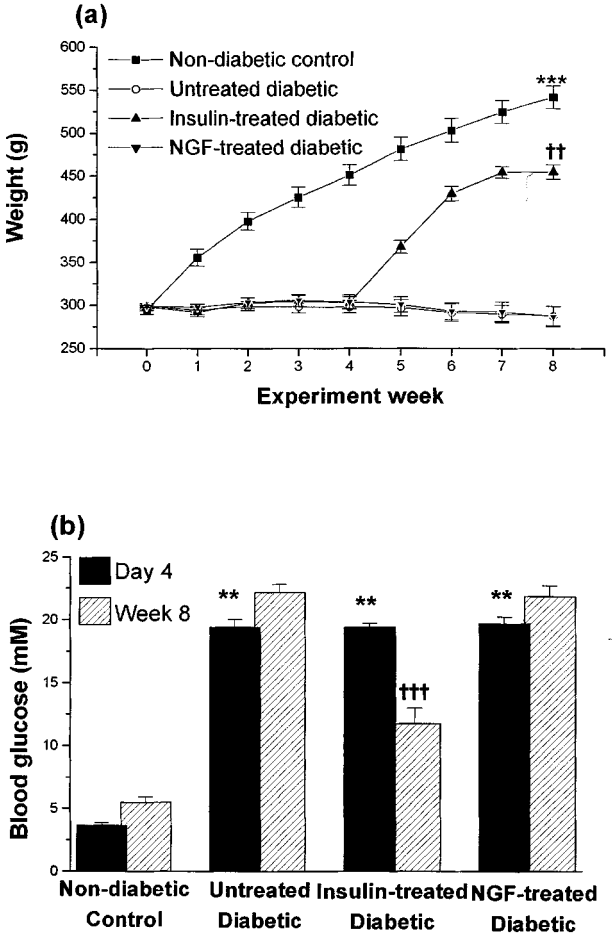

Figure 1 Effect of treatment on (a) weight and (b) plasma glucose levels. (a) shows the effect of treatment on weight of: untreated non-diabetic control rats, untreated diabetic rats, insulin-treated diabetic rats, and NGF-treated rats. The rats were weighed weekly and all results expressed as mean ± s.e.mean, $n = 16–20$. Final weights for the non-diabetic control group are significantly greater (***$P < 0.001$) than those of the untreated diabetic and NGF-treated diabetic groups. Final weights of insulin-treated diabetic rats are significantly different (††$P < 0.01$) from untreated diabetic and NGF-treated diabetic rats and significantly different to their own weights at the start of the week 4–8 insulin treatment, as determined by two way ANOVA, with *t*-test on population means. (b) shows the effect of treatment on glucose levels at day 4 after treatment and then on the day of experiment (at 8 weeks after treatment). Glucose levels were significantly (**$P < 0.01$) raised in all diabetic groups at 4 days (one way ANOVA with Dunnetts). By comparison glucose levels in the insulin-treated diabetic group was significantly lowered (†††$P < 0.001$) at 8 weeks compared with 4 days (paired *t*-test).

Blood flow changes and oedema formation mediated by saphenous nerve stimulation (Experiment 1)

Stimulation of the saphenous nerve (10 V, 2 Hz, 1 ms) for 30 s produced an increase in blood flow in the paw skin (Figure 2), as previously shown (Gamse & Saria, 1987; Escott & Brain 1993). The peak increase seen following stimulation did not differ significantly between the four groups, as shown in Figure 3a; further, the time to reach a peak response was not significantly different between these groups. However, the response was attenuated with respect to duration, as can be seen in Figure 2 and also by calculation of the calculation of area under the curve (flux versus time) as shown in Figure 3b. The results show that the response was reduced in untreated diabetic, but not in control or treated rats for 0–5, 0–10 and 0–15 min after stimulation. This indicates that the overall neurogenic vasodilator response is attenuated in diabetic rats, and that both insulin- and NGF-treatment can prevent this.

Stimulation of the saphenous nerve of non-diabetic control animals (10 V, 2 Hz, 1 ms for 5 min) produced a significant plasma extravasation in the paw skin, as shown in Figure 4.

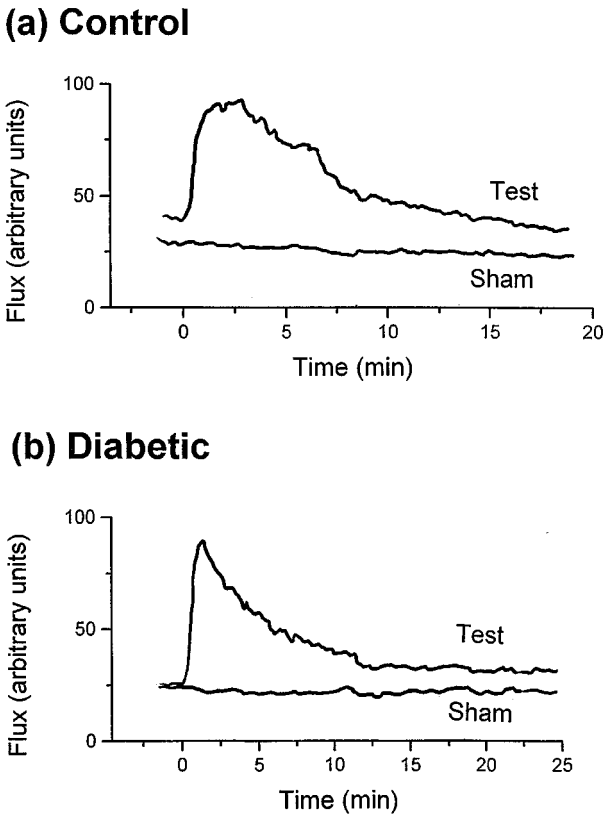

Figure 2 Effect of diabetes on neurogenic vasodilatation. Typical blood flow traces, as assessed by a laser Doppler flow meter, from a non-diabetic control (a) and a diabetic rat (b). Blood flux (arbitrary units) is shown on the *y*-axis, with time (min) after nerve stimulation (10 V, 2 Hz, 1 ms for 30 s) on the *x*-axis. The increased blood flow observed after stimulation is shown, together with the blood flow in the contralateral paw.

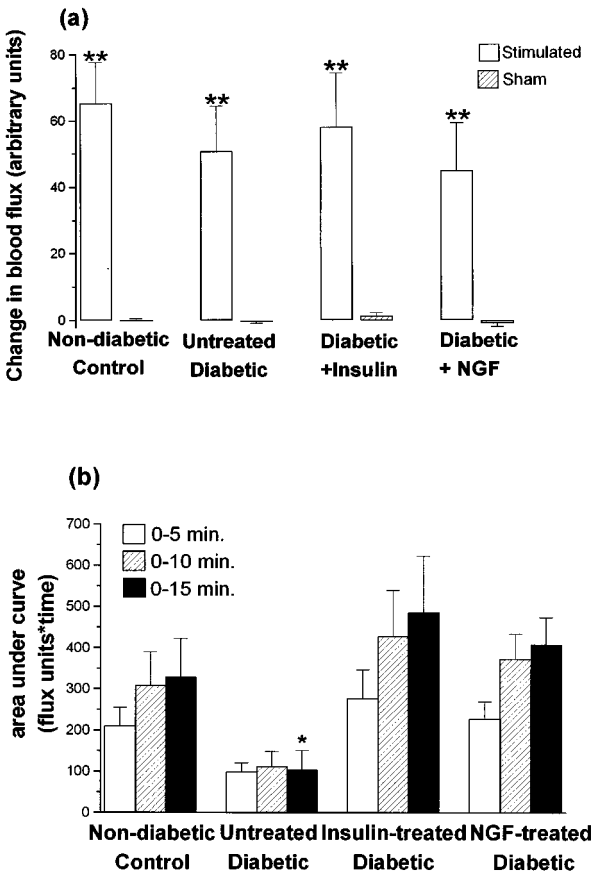

Figure 3 Effect of diabetes on neurogenic vasodilatation: modulatory effect of insulin and NGF. (a) shows the peak increase in blood flow (from basal) for the four treatment groups in ipsilateral paw skin after stimulation of the sensory saphenous nerve. The results for the contralateral paw, prepared as sham, are shown in the second column. All results are expressed as the mean ± s.e.mean flux, $n=6-10$. There was no significant difference in either the peak increase in blood flow or the basal blood flow between groups. Basal blood flow for the ipsilateral paws was as follows: untreated non-diabetic control rats, 41.3 ± 7.3, $n=8$; untreated diabetic rats, 38.7 ± 7.9, $n=10$; insulin-treated diabetic rats, 42.8 ± 6.7, $n=8$ and NGF-treated rats, 35.1 ± 8.6, $n=6$. (b) gives an indication of the duration of the response. Results are calculated as the area under the curve for flux units with time for 0–5 min after stimulation, 0–10 min after stimulation and 0–15 min after stimulation. All results are mean ± s.e.mean, $n=6-10$. Results differing significantly from control groups are shown as $^{*}P<0.05$, as assessed by ANOVA followed by Dunnett's post-test.

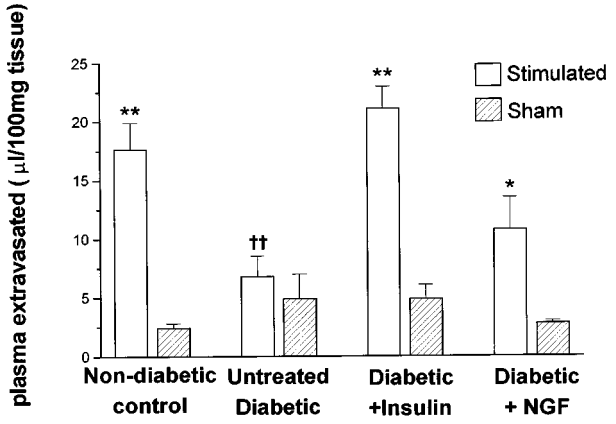

Figure 4 Effect of diabetes on neurogenic plasma extravasation. Plasma extravasation measured in paw skin after stimulation of the sensory saphenous nerve. Plasma extravasation into paw skin of the stimulated leg and the contralateral, prepared as sham, paw skin is shown. Results are mean ± s.e.mean, $n=8-10$. Extravasation in the stimulated paw differing significantly from that in sham paw are shown as $^{*}P<0.05$, $^{**}P<0.01$, as assessed by students t-test. Plasma extravasation in stimulated paw differing significantly from that in non-diabetic control are shown as $\dagger\dagger P<0.01$, as assessed by ANOVA with Dunnett's post test.

This plasma extravasation was significantly reduced in the untreated diabetic animals, such that no significant difference was seen in plasma extravasation between stimulated and sham paws. Insulin-treated diabetic rats responded to saphenous nerve stimulation with an extravasation that was not significantly different to that observed in the non-diabetic controls. NGF-treated diabetic animals showed a significant plasma extravasation in the stimulated paw compared to the sham. However, this plasma extravasation was partially, but significantly, reduced compared to non-diabetic controls.

Cutaneous skin oedema formation induced by i.d. mediators (Experiment 2)

The effect of i.d. substance P (30 pmol site^{-1}), either injected alone or with CGRP (20 pmol site^{-1}) is shown in Figure 5. This submaximal dose of substance P induced significant plasma extravasation in all groups. CGRP is known to increase cutaneous blood flow at this dose and, as a consequence, to act in a linear manner to potentiate inflammatory oedema induced by mediators of increased microvascular permeability (Siney & Brain 1996). It can be seen that plasma extravasation induced by substance P was increased in all groups when CGRP was co-injected, showing potentiation of the substance P-induced extravasation. Injection of CGRP alone induced no significant plasma extravasation. These results are in keeping with those previously reported (Brain & Williams 1985) in studies carried out in normal rats. Similarly, i.d. injection of the NK_1 agonist GR73632 (30 pmol site^{-1}) produced a significant plasma extravasation, as seen in Figure 5b. This was significantly increased when GR73632 (30 pmol site^{-1}) was co-injected with CGRP (20 pmol site^{-1}).

Discussion

This study showed a clear loss of neurogenic vasoactive responses in diabetic rats. Treatment with insulin prevented these deficits and treatment with NGF returned the neurogenic responses towards normal levels. The results suggest that insulin is more effective in reversing the neurogenic oedema response in this study but that both agents appear equally effective in restoring the neurogenic vasodilator response. This extends previous findings, which indicate that insulin has beneficial effects on neurogenic oedema responses in the diabetic rat, and importantly, reveal an ability of NGF to restore sensory nerve-mediated microvascular function in diabetes. The streptozotocin-treated rats exhibited characteristic features of Type 1 (insulin-dependent diabetes) in that they failed to gain weight, and suffered from hyperglycaemia. Treatment with NGF did not markedly affect these parameters, whereas insulin significantly attenuated both symptoms. The dose of NGF (0.2 mg kg^{-1}, s.c., three times weekly from 4 to 8 weeks) was chosen from previous studies where it has been shown to restore levels of substance P and CGRP in

the sciatic nerve without the hyperalgesic side effects which are seen with higher doses (Fernyhough *et al.*, 1995).

In our first series of experiments, electrical stimulation of the saphenous nerve was used to stimulate neuropeptide release and neurogenic inflammatory responses. We adapted an established method to allow analysis of two separate responses. One response is vasodilator and due to a peak of increased cutaneous blood flow which then falls back towards basal levels after 20 min with no oedema formation. The other response is principally a neurogenic oedema response, due to both increased blood flow and increased microvascular permeability although it is not possible to measure the blood flow changes due to limitations with respect to our laser Doppler flowmetry technique. We used the same rat to measure both of these responses. Initially the nerve was stimulated for 30 s, leading to the vasodilator response, then after the blood flow returned to levels close to baseline, the nerve was stimulated for 5 min, which led to the irreversible oedema response. Although the peak increases in blood flow after the first stimulation of the sensory nerve was similar in all rats, the duration of the vasodilator response was attenuated in diabetic rats. The reason for the lack of effect on the peak response in diabetes is unclear. There has been much discussion on the role of various vasoactive mediators in diabetes, one of which is nitric oxide, thus mediators such as nitric oxide may be playing an altered vasodilator role in the diabetic rat (Stephens *et al.*, 1995) and influencing these results. Interestingly, whilst sensory nerve-mediated vasodilatation is principally mediated by CGRP (Escott & Brain, 1993), we have evidence that the initial neurogenic vasodilatation may not be (Escott *et al.*, 1995) and this may be relevant to the present findings. The neurogenic oedema formation was significantly reduced in diabetic rats, in agreement with previous studies (Gamse & Jansćo, 1985). The reduction in plasma extravasation was prevented by insulin treatment and was partially prevented by NGF treatment. It is interesting to note that reduced levels of substance P but not of CGRP have been reported in the skin of patients displaying mild diabetic neuropathy (Anand, 1996). Levels of both peptides are reduced in more severely neuropathic patients, indicating that the levels of CGRP may be more tolerant of diabetes-induced NGF depletion. This may be due to the fact that substance P is only found in small-diameter fibers, thought to be primarily affected in diabetic neuropathy, while CGRP is found both colocalized with substance P and in larger fibers, which may be less affected.

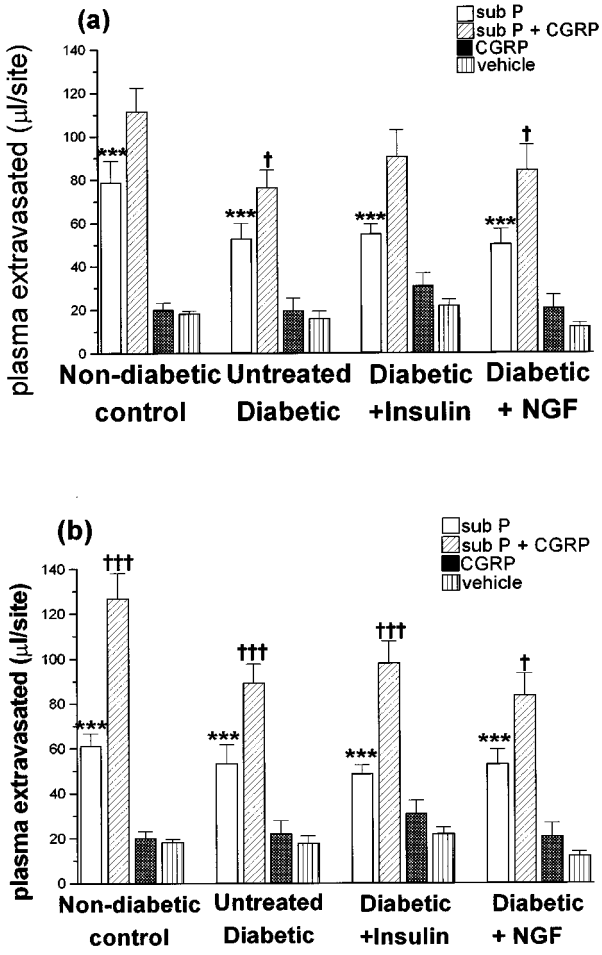

Figure 5 Effect of exogenous neuropeptides on plasma extravasation. (a) Shows the effect of plasma extravasation induced by intradermal substance P (30 pmol site^{-1}), alone and in the presence of CGRP (20 pmol site^{-1}) in the four experimental groups. The plasma extravasation induced by CGRP (20 pmol site^{-1}) and Tyrode (vehicle) is also shown. All results are plotted as mean$\pm$s.e.mean, $n=8-10$. Results differing significantly from Tyrode (vehicle)-injected sites are represented as $***P<0.005$. Results differing significantly from sites receiving substance P alone are represented as $\dagger P<0.05$. Significance assessed by two-way ANOVA followed by one-tailed *t*-test on population means. (b) Shows the plasma extravasation induced by intradermal NK_1 agonist GR73632 (30 pmol site^{-1}) in the absence and presence of CGRP (20 pmol site^{-1}), in the four experimental groups. The extravasion induced by CGRP (20 pmol site^{-1}), and Tyrode (vehicle), is also shown. All results are plotted as mean$\pm$s.e.mean, $n=8-10$. Results differing significantly from Tyrode (vehicle)-injected sites are represented as $***P<0.005$. Results differing significantly from sites receiving GR73632 alone are represented as $\dagger P<0.05$ and $\dagger\dagger\dagger P<0.005$. Significance assessed by two-way ANOVA followed by one-tailed *t*-test on population means.

In our second series of experiments, diabetic rats were found to respond to intradermal injection of substance P and CGRP, in a similar manner to normal rats. Submaximal doses of substance P, the NK_1 agonist GR73632 and CGRP were chosen. Substance P is a potent mediator of increased microvascular permeability which acts *via* NK_1 receptors and activation of mast cells (Brain & Williams, 1985), whilst GR73632 is a selective NK_1 agonist in rat skin (Siney & Brain, 1996). CGRP is a potent vasodilator, which does not by itself increase microvascular permeability. However, CGRP potentiates inflammatory oedema formation induced by mediators of increased microvascular permeability such as substance P as a consequence of its vasodilator activity (Brain & Williams, 1985). We have used this as an indirect assay of the vasodilator activity of CGRP. These results suggest that in the diabetic rat neurogenic responses are not markedly affected by a modulation of substance P or CGRP neuropeptide receptor-mediated mechanisms.

The results further the hypothesis that reduced endogenous NGF levels underlie the neurological and microvascular complications in diabetes. This hypothesis is supported by studies in man (Anand, 1996) and in the rat (Fernyhough *et al.*, 1995) which show that the expression of NGF is reduced in skin together with the uptake into nerves, retrograde nerve transport and thus delivery of NGF to nerve ganglia (Hellweg *et al.*, 1994) which may be further influenced by a reduction in the expression of trkA receptors (Maeda *et al.*, 1996). An observed reduction of substance P, CGRP and respective mRNAs can be reversed by NGF treatment (Apfel *et al.*, 1994; Diemel *et al.*, 1994), and by treatments which increase NGF production (Garrett *et al.*, 1997) which is in keeping with the knowledge that these neuropeptides are produced from genes which are regulated by NGF (Lindsay & Harmar, 1989).

Therefore the findings of the present study are in keeping with the principle that administration of neurotrophic factors may be of value in neuropathies, especially as a reduction of these neuropeptides and of NGF has been detected in diabetic patients (Anand *et al*., 1994). It has been previously shown that NGF protects against behavioural changes in streptozotocin-induced diabetic rats (Apfel *et al*., 1994). The effect of NGF on the neurogenic inflammatory response has not been, to our knowledge, previously investigated; although a reduced neurogenic response in the diabetic rat was first reported by Garcia-Leme *et al*. (1974) after stimulation of the saphenous nerve and confirmed in a related study (Gamse & Jansćo, 1985). A reduction of neurogenic inflammation has also been recently shown in the gingivomucosal tissue in the rat (Györfi *et al*., 1996). The conclusion from the study of Gamse & Jancsó (1985) was that the loss of the neurogenic response was a consequence of microvascular changes, rather than neuropeptide depletion. This is not supported by the results of the present study. However Gamse & Janscó (1985) looked at responses 10 days after injection of streptozotocin, whilst in this study we investigated responses after 8 weeks. Thus, we could be detecting a more chronic stage of the process in this model. The consequence of these changes are difficult to analyse at this stage. The neuropeptides have potent vasoactive effects and could have a physiological role in the regulation of microvascular tone and permeability in tissues such as skin and in the inflammatory response (Brain, 1996). A diminished response in diabetic rats in a model of acute inflammation has been reported (Garrett *et al*., 1996); although in that study NGF levels increased in the joint after injury and it was suggested that an uncoupling of NGF with substance P may occur during the neuronal dysfunction in diabetes.

In summary, we suggest that the reduced neurogenic vasoactive responses seen in this study may be related to the neuronal dysfunction and microvascular complications observed in diabetes. The responses may be reversed by either insulin or NGF treatment.

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References

ANAND, P. (1986). Neurotrophins and peripheral neuropathy. *Phil. Trans. R. Soc. Lond. B.,* **351,** 449 – 454.

ANAND, P., WARNER, G., PARRETT, A., KOPELMAN, P., GHATEI, M.A., BLOOM, S.R., SINICROPI, D.V. & WILLIAMS CHESTNUT, R.E. (1994). Nerve growth factor related small fibre dysfunction in human diabetic polyneuropathy. *Ann. Neurol.,* **36,** 284a.

APFEL, S.C., AREZZO, J.C., BROWNLEE, M., FEDEROFF, H. & KESSLER, J.A. (1994). Nerve growth factor administration protects against experimental diabetic sensory neuropathy. *Brain Res.,* **634,** 7 – 12.

ARONIN, N., LEEMAN, S.E. & CLEMENTS, R.S., JR. (1987). Diminished flare response in neuropathic patients: comparison of effects of substance P, histamine and capsaicin. *Diabetes,* **36,** 1139 – 1143.

BRAIN, S.D. (1996). Sensory neuropeptides in the skin. In *Neurogenic Inflammation*. eds. P. Geppetti and P. Holzer, pp. 229 – 224. Boca Raton, Florida: CRC Press Inc.

BRAIN, S.D. & WILLIAMS, T.J. (1985). Inflammatory oedema induced by synergism between calcitonin gene-related peptide (CGRP) and mediators of increased vascular permeability. *Br. J. Pharmacol.,* **86,** 855 – 860.

DIEMEL, L.T., BREWSTER, W.J., FERNHOUGH, P. & TOMLINSON, D.R. (1994). Expression of neuropeptides in experimental diabetes; effects of treatment with nerve growth factor or brain-derived neurotrophic factor. *Mol. Brain Res.,* **21,** 171 – 175.

DIEMEL, L.T., STEVENS, E.J., WILLARS, G.B. & TOMLINSON, D.R. (1992). Depletion of substance P and calcitonin gene-related peptide in sciatic nerve of rats with experimental diabetes; effects of insulin and aldose reductase inhibition. *Neurosci. Lett.,* **137,** 253 – 256.

DYCK, P.J. (1992). New understanding and treatment of diabetic neuropathy. *N. Engl. J. Med.,* **326,** 1287 – 1288.

EDMONDS-ALT, X., DOUTREMEPUICH, J.-D., HEAULME, M., NELIAT, G., SANTUCCI, V., STEINBERG, R., VILAIN, P., BICHON, D., DUCOUX, J.-P., PROIETTO, V., VAN BROECK, D., SOUBRIE, P., LE FUR, G. & BRELIERE, J.-C. (1993). *In vitro* and *in vivo* biological activities of SR140333, a novel potent non-peptide tachykinin NK_1 receptor antagonist. *Eur. J. Pharmacol.,* **250,** 403 – 413.

ESCOTT, K.J., BEATTIE, D.T., CONNOR, H.E. & BRAIN, S.D. (1995). Trigeminal ganglion stimulation increases facial skin blood flow in the rat: a major role for calcitonin gene-related peptide. *Brain Res.,* **669,** 93 – 99.

ESCOTT, K.J. & BRAIN, S.D. (1993). Effect of a calcitonin gene-related peptide antagonist (CGRP8-37) on skin vasodilatation and oedema induced by stimulation of the rat saphenous nerve. *Br. J. Pharmacol.,* **110,** 772 – 776.

FERNYHOUGH, P., DIEMEL, L.T., BREWSTER, W.J. & TOMLINSON, D.R. (1995). Altered neurotrophin mRNA in peripheral nerve and skeletal muscle of experimentally diabetic rats. *J. Neurochem.,* **64,** 1231 – 1237.

GAMSE, R. & JANCSÓ, G. (1985). Reduced neurogenic changes in inflammation in streptozotocin-diabetic rats due to microvascular changes but not to substance P depletion. *Eur. J. Pharmacol.,* **118,** 175 – 180.

GAMSE, R. & SARIA, A. (1987). Antidromic vasodilatation in the rat hindpaw measured by laser Doppler flowmetry: pharmacological modulation. *J. Auton. Nerv. Syst.,* **19,** 105 – 111.

GARCIA LEME, J., BÖHM, G.M., MIGLIORINI, R.H. & DE SOUZA, M.Z.A. (1974). Possible participation of insulin in the control of vascular permeability. *Eur. J. Pharmacol.,* **29,** 298 – 306.

GARRETT, N.E., KIDD, B.L., CRUWYS, S.C. & TOMLINSON, D.R. (1996). Effect of streptozotocin-diabetes on knee joint inflammation-induced changes in substance P and nerve growth factor in the rat. *Mol. Brain Res.,* **42,** 272 – 278.

GARRETT, N.E., MALCANGIO, M., DEWHURST, M. & TOMLINSON, D.R. (1997). Alpha-lipoic acid corrects neuropeptide deficits in diabetic rats via induction of trophic support. *Neurosci. Lett.,* **222,** 191 – 194.

GYÖRFI, A., FAZEKAS, A., FEHÉR, E., ENDER, F. & ROSIVALL, L. (1996). Effects of streptozotocin-induced diabetes on neurogenic inflammation of gingivomucosal tissue in the rat. *J. Periodont. Res.,* **31,** 249 – 255.

HELLWEG, R., RAIVICH, G., HARTUNG, H.-D., HOCK, C. & KREUTZBERG, G.W. (1984). Axonal transport of endogenous nerve growth factor (NGF) and NGF receptor in experimental diabetic neuropathy. *Exp. Neurol.,* **130,** 24 – 30.

HOLZER, P. (1992). Peptidergic sensory neurons in the control of vascular functions: mechanisms and significance in the cutaneous and splanchnic vascular beds. *Rev. Physiol. Biochem. Pharmacol.,* **121,** 49 – 146.

LEVY, D.M., TERENGHI, G., GU, X.-H., ABRAHAM, R.R., SPRINGALL, D.R. & POLAK, J.M. (1992). Immunohistochemical measurements of nerves and neuropeptides in diabetic skin: relationship to tests of neurological function. *Diabetologia,* **35,** 889 – 897.

LINDSAY, R.M. & HARMAR, A.J. (1989). Nerve growth factor regulates expression of neuropeptide genes in adult sensory neurons. *Nature,* **337,** 362 – 364.

LINDSAY, R.M., WIEGAND, S.J., ALTAR, C.A. & DISTEFANO, P.S. (1994). Neurotrophic factors: From molecule to man. *Trends in Neurosciences,* **17,** 182 – 190.

MAEDA, K., FERNHOUGH, P. & TOMLINSON, D.R. (1996). Regenerating sensory neurones of diabetic rats express reduced levels of mRNA for GAP-43, g-preprotachykinin and the nerve growth factor receptors, trkA and $p75^{ngfr}$. *Mol. Brain Res.,* **37,** 166–174.

SINEY, L. & BRAIN, S.D. (1996). Involvement of sensory neuropeptides in the development of plasma extravasation in rat dorsal skin following thermal injury. *Br. J. Pharmacol.,* **117,** 1065–1070.

STEPHENS, M.J., FELDMAN, E.L. & GREENE, D.A. (1995). The aetiology of diabetic neuropathy: the combined roles of metabolic and vascular defects. *Diab. Med.,* **12,** 566–579.

STEVENS, E.J., CARRINGTON, A.L. & TOMLINSON, D.R. (1994). Nerve ischaemia in diabetic rats: time-course of development, effect of insulin treatment plus comparison of streptozotocin and BB models. *Diabetologia,* **37,** 43–48.

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