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ATP-sensitive K^+ channel effects on nerve function, Na^+, K^+ ATPase, and glutathione in diabetic rats

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Abstract

Some vasodilators correct nerve conduction velocity and endoneurial blood flow deficits in diabetic rats. It is not known whether vasa nervorum has ATP-sensitive K^+ (K_{ATP}) channels that mediate vasodilation, or whether K_{ATP} channels could modulate peripheral nerve function. Therefore, we examined the effects of 2 weeks treatment with the K_{ATP} channel openers, celikalim and WAY135201 (R-4-[3, 4-dioxo-2-(1, 2, 2-trimethyl-propylamino)-cyclobut-1-1-enylamino]-3-methoxy-benzonitrile), on sciatic nerve blood flow, conduction velocity, Na^+–K^+ ATPase activity and glutathione content after 6 weeks of untreated streptozotocin-diabetes in rats. Blood flow and motor conduction velocity, 47.6% and 20.3% reduced by diabetes, respectively, were completely restored by both celikalim and WAY135201 treatments. Diabetes diminished sciatic Na^+–K^+ ATPase activity by 47.6% and this was 80–90% corrected by the K_{ATP} channel openers. Sciatic nerve glutathione content, 30.3% reduced by diabetes, was unaffected by celikalim or WAY135201. Thus, K_{ATP} channel openers had marked beneficial effects on nerve perfusion and function in experimental diabetic neuropathy, and may be suitable for further study in clinical trials.

Keywords: Nerve conduction; Blood flow; K^+ channel opener; Na^+, K^+ ATPase; Oxidative stress; Glutathione; Neuropathy; Diabetic rat

1. Introduction

Impaired perfusion makes a major contribution to the etiology of peripheral nerve dysfunction in patients with diabetes mellitus and in experimental models (Tuck et al., 1984; Cameron and Cotter, 1994; Tesfaye et al., 1994). Other putative factors considered to be important include elevated oxidative stress and impaired neural-free radical protection mechanisms (Nagamatsu et al., 1995) and reduced nerve Na^+, K^+ ATPase activity (Greene et al., 1992). The nerve perfusion deficit in diabetic rats can be prevented or corrected by some vasodilator treatments. The result is an improvement in conduction velocity and attenuation of the development of resistance to ischemic conduction failure (Cameron and Cotter, 1994). However, despite normal conduction velocity for treatment with the α_1-adrenoceptor antagonist, prazosin, the Na^+, K^+ ATPase activity defect was not prevented (Cameron et al., 1991).

Nerve content of the reduced form of glutathione (GSH) is diminished by diabetes (Nagamatsu et al., 1995). GSH levels are restored by treatment with aldose reductase inhibitors and α-lipoic acid (Nagamatsu et al., 1995; Hohman et al., 1997), both of which improve nerve blood flow (Cameron et al., 1994a, 1998). It is not known whether restoration of GSH by these drugs depends directly on their metabolic effects or on their action to improved nerve blood flow. Thus, ischemia/reperfusion in diabetic nerve would produce free radicals that could diminish GSH content and, theoretically, restoration of blood flow might also restore GSH.

ATP-sensitive K^+ (K_{ATP}) channels are found in several cell types including pancreatic β cells, cardiac myocytes, vascular and other smooth muscle, and neurons (Tosaki et al., 1995; Yokoshiki et al., 1998). K_{ATP} channel openers cause membrane hyperpolarization, leading to vascular

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smooth muscle vasorelaxation sufficient to increase blood flow in several circulations including heart and brain (Tosaki et al., 1995; Zimmerman et al., 1997). Diabetes affects vascular K_{ATP} channel systems, for example, aorta and cerebral vessels from diabetic rats showed impaired relaxation to K_{ATP} channel openers (Kamata et al., 1989; Cameron and Cotter, 1992; Zimmerman et al., 1997). It is not known whether K_{ATP} channels are present in vasa nervorum or whether they could influence nerve blood flow. In the context of neuropathy, this matter is of particular interest because the suphonylurea drugs used in type II diabetes to improve insulin release close K_{ATP} channels could conceivably have adverse effects mediated by K_{ATP} channels on vasa nervorum, axons or Schwann cells.

The aim of this investigation was to examine the effects of the K_{ATP} channel openers, WAY135201 and celikalim on sciatic conduction velocity, endoneurial blood flow, Na^+, K^+ ATPase activity and GSH content in diabetic rats.

2. Materials and methods

2.1. Experimental groups and diabetes induction

Parallel studies were performed in which diabetes duration was 8 weeks and drug treatment was given over the final 2 weeks. Diabetes was induced in mature 19-week-old male Sprague–Dawley rats, obtained either from Charles River (Kingston, NY, USA) or from Aberdeen University breeding colony, by intraperitonial (i.p.) injection of streptozotocin (Sigma, St Louis, MO, USA or ZENECA, Macclesfield, UK) freshly dissolved in sterile saline. The rats were injected with approximately 0.4–0.5 ml, depending on body weight, of a 42.5 mg ml^{-1} solution to give a streptozotocin dose of 42–43 mg kg^{-1}. One study, performed in Princeton, examined sciatic endoneurial Na^+, K^+ ATPase activity and GSH content. The other study, carried out in Aberdeen, was concerned with nerve function and blood flow.

Experimental groups consisted of nondiabetic and diabetic controls, diabetic and nondiabetic rats treated with WAY135201, R-4-[3, 4-dioxo-2-(1, 2, 2-trimethyl-propylamino)-cyclobut-1-1-enylamino]-3-methoxy-benzonitrile (Wyeth-Ayerst, Princeton, NJ), daily by gavage as a suspension in 0.5% methylcellulose at a dose of 3.0 mg kg^{-1}, and diabetic rats treated with celikalim (Wyeth-Ayerst; 0.25 mg kg^{-1}, by gavage, dissolved in distilled water).

2.2. Sciatic nerve Na^+, K^+ ATPase activity and glutathione levels

At the end of the treatment period, animals were killed by thiobutabarbital overdose. The right and left sciatic nerves were then rapidly removed from their vertebral exit to the common peroneal bifurcation, cleaned of adherent muscle and connective tissue and placed on ice for measurement of Na^+, K^+ ATPase activity or frozen on dry ice for subsequent quantification of glutathione levels. Preliminary experiments showed that the biochemical parameters measured were unaffected by the anaesthetic conditions (halothane, thiobutabarbital or CO_2) under which the nerve samples were taken (Hohman, unpublished observations).

Nerve Na^+, K^+ ATPase activity was assayed spectrophotometrically (340 mM) by monitoring at the disappearance of NADH in an enzymatic reaction that coupled Na^+, K^+ ATPase, pyruvate kinase and lactate dehydrogenase activities using a membrane-enriched sciatic nerve fraction as previously described (Cameron et al., 1999). In brief, sciatic nerves were desheathed, immersed in 20 mM Tris–HCl, pH 7.5, containing 150 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 1 mM EDTA and 0.5 M sucrose, and homogenized. A discontinuous three-step sucrose gradient was prepared with the homogenate sandwiched between 1.2 and 0.25 M sucrose layers. After centrifugation ($140{,}000 \times g$ for 60 min at 4°C), the opaque layer between the 1.2 and 0.5 M sucrose layers was collected, resuspended, recentrifuged, and the membrane enriched fraction was resuspended in 0.25 M sucrose buffer. Aliquots, 30 μl of the membrane-enriched fraction, were then added to 970 μl of 3 mM ATP (Tris salt), 1 mM phosphoenolpyruvate, 0.3 mM NADH, 18 U lactate dehydrogenase, and 18 U pyruvate kinase, prepared in 50 mM imidazole buffer, pH 7.3, with 100 mM NaCl, 10 mM KCl, and 5 mM $MgCl_2$. This reaction mixture was stabilized at 37°C for 16 min and NADH disappearance was monitored for 15 min without and with 750 μM ouabain. Ouabain-sensitive Na^+, K^+ ATPase activity was expressed as the amount of ATP converted to ADP, normalized to the protein content of the membrane-enriched fraction.

Tissue levels of reduced and oxidized glutathione were quantified with an enzymatic recycling assay as previously described (Cameron et al., 1999). In brief, frozen nerves were thawed in 200 ml of 5% sulphosalic acid, desheathed, weighed, minced and sonicated. The homogenate was centrifuged for 10 min at ($16{,}000 \times g$) and the pellet was discarded. The supernatant was diluted to 0.15% by sodium phosphate buffer (pH 7.5). Samples were maintained at 4°C during processing.

Standards were prepared from stock solutions of GSH and oxidized glutathione (GSSG). Aliquots of the diluted supernatant and standards were transferred to individual wells in a 96-well plate. The enzyme reaction was initiated with the addition of 100 ml of a reaction mixture to yield a final concentration of 0.2 mM NADPH, 0.15 mM DTNB, (5,5′-dithiobis [2-nitrobenzoic acid]) and 1 U of glutathione reductase in 100 mM sodium phosphate buffer with 1 mM EDTA. The reaction was monitored at 405 nm. To quantify the tissue GSSG, GSH was removed with 2-vinylpyridine (0.35 M). To optimize the activity of glutathione

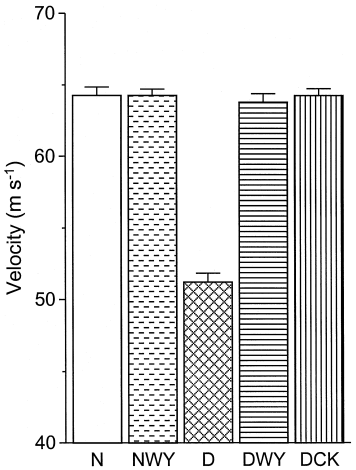

Fig. 1. Sciatic motor conduction velocity in nerve fibres to tibialis anterior muscle. N, nondiabetic control group, $n = 10$; NWY, nondiabetic rats treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 9$; D, 8-week diabetic rats, $n = 10$; DWY, 8-week diabetic rats treated for the last 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 9$; DCK, 8-week diabetic rats treated for the last 2 weeks with 0.25 mg kg^{-1} day^{-1} celikalim, $n = 10$. Error bars are SEM.

reductase in the recycling assay, sample pH was adjusted to 7.0 with triethanolamine.

2.3. Sciatic nerve motor conduction velocity and endoneurial blood flow

At the end of the treatment period, rats were anaesthetised with thiobutabarbital (Zeneca; 50–100 mg kg^{-1}), by i.p. injection. The trachea was cannulated for artificial ventilation. A carotid cannula was used to monitor mean systemic blood pressure. Motor conduction velocity was assessed as previously described (Cameron et al., 1996) between sciatic notch and knee in the nerve branch to tibialis anterior muscle, which is representative of the whole sciatic nerve in terms of susceptibility to diabetes and treatment effects. Rectal and nerve temperatures were monitored and regulated at 36.5–37.5°C.

Sciatic endoneurial blood flow was measured by microelectrode polarography and hydrogen clearance as previously described (Cameron et al., 1996, 1998). Briefly, core temperature of the rat was monitored and regulated at 37–38°C, using a rectal probe and radiant heat. The skin around the sciatic nerve incision was used to form a pool that was filled with mineral oil maintained at 35–37°C by radiant heat during blood flow measurements. Rats were given neuromuscular blockade using D-tubocurarine (Sigma; 2 mg kg^{-1} via the carotid cannula) and were artificially ventilated. The level of anaesthesia was monitored by observing any reaction of blood pressure to manipulation, and supplementary anaesthetic was given as necessary. A glass-insulated platinum microelectrode was inserted into the middle portion of the sciatic nerve, above its trifurcation, and polarised at 0.25 V with respect to a subcutaneous reference electrode. 10% H_2 was added to the inspired gas, the proportions of O_2 and N_2 being adjusted to 20% and 70%, respectively. When the H_2 current, recorded by the electrode, had stabilised, indicating equilibrium with arterial blood, the H_2 supply was shut off and N_2 delivery was increased appropriately. The H_2 clearance curve was recorded until baseline, the latter being defined as no systematic decline in electrode current over 5 min. This procedure was then repeated at another nerve site. After the experiment, clearance curves were digitised and mono-exponential or bi-exponential curves were fitted to the data by computer using non-linear regression software that employed the Marquardt algorithm and the least squares method for optimising goodness-of-fit (Prism, Graphpad, San Diego, CA, USA). The slow exponent was taken to reflect nutritive (capillary) flow (Day et al., 1989). Sciatic endoneurial flow parameters were calculated as previously described (Cameron et al., 1996).

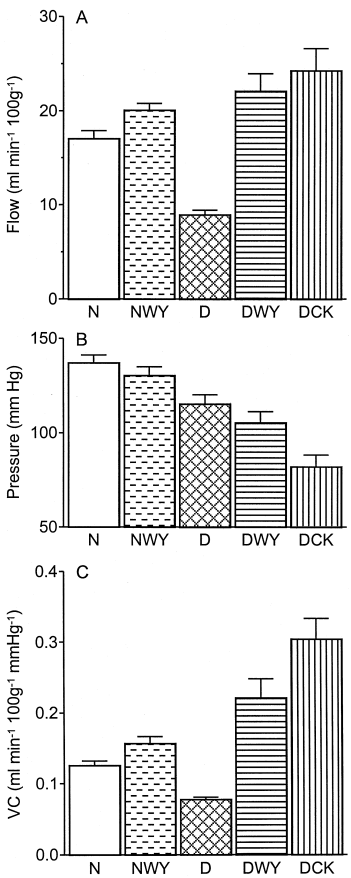

Fig. 2. Sciatic nutritive endoneurial blood flow (A), mean systemic blood pressure (B) and vascular conductance (C). N, nondiabetic control group, $n = 10$; NWY, nondiabetic rats treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 8$; D, 8-week diabetic rats, $n = 10$; DWY, 8-week diabetic rats treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 9$; DCK, 8-week diabetic rats treated for 2 weeks with 0.25 mg kg^{-1} day^{-1} celikalim, $n = 9$. Error bars are SEM.

2.4. Statistical analysis

Data are presented as group means ± SEM. They were given Bartlett's test for homogeneity of variances, followed by log transformation where appropriate before being subjected to one-way analysis of variance. When the overall significance ($P < 0.05$) was attained, between-group differences were established by post hoc analysis using the Student–Newman–Keuls test which corrected for multiple comparisons.

3. Results

The diabetic rats had a reduced body weight (379 ± 8 g) compared to the nondiabetic control group (519 ± 7 g; $P < 0.001$). There were no significant effects of WAY135201 (404 ± 8 g) or celikalim (370 ± 13 g) treatments on the body weight of diabetic rats or of WAY135201 on nondiabetic (487 ± 10 g) rats. Plasma glucose values were 30.8 ± 0.7 mM in untreated diabetic rats and they were not significantly different with WAY135201 (32.0 ± 0.6 mM) or celikalim (32.4 ± 0.6 mM) treatment. In comparison, plasma glucose levels for untreated and WAY135201-treated nondiabetic rats were 9.2 ± 0.4 and 9.0 ± 0.5 mM, respectively.

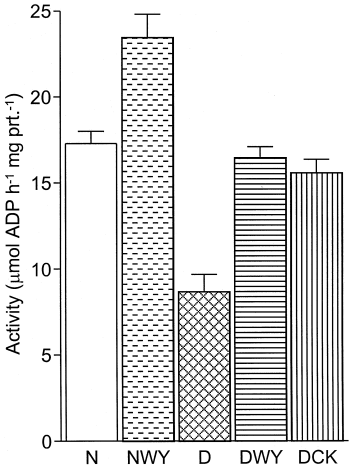

Fig. 4. Sciatic endoneurial ouabain-sensitive Na^+, K^+ ATPase activity. N, nondiabetic control group, $n = 21$; NWY, nondiabetic group treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 10$; D, 8-week diabetic group, $n = 13$; DWY, 8-week diabetic group treated for the last 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 20$; DCK, 8-week diabetic group treated for the last 2 weeks with 0.25 mg kg^{-1} day^{-1} celikalim, $n = 5$. Error bars are SEM.

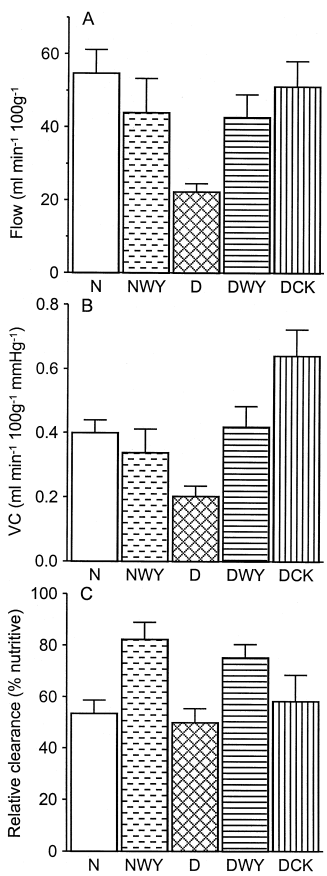

Fig. 3. Sciatic total endoneurial blood flow (A), vascular conductance (B) and relative nutritive clearance (C). N, nondiabetic control group, $n = 10$; NWY, nondiabetic rats treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 8$; D, 8-week diabetic rats, $n = 10$; DWY, 8-week diabetic rats treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 9$; DCK, 8-week diabetic rats treated for 2 weeks with 0.25 mg kg^{-1} day^{-1} celikalim, $n = 9$. Error bars are SEM.

Sciatic motor conduction velocity (Fig. 1) was 20.3 ± 1.0% reduced by 8 weeks of diabetes ($P < 0.001$). WAY135201 and celikalim treatment for 2 weeks corrected this deficit to the extent of 96.4 ± 4.6% ($P < 0.001$) and 99.9 ± 3.6% ($P < 0.001$), respectively, such that values were not significantly different from those of the nondiabetic control group. Treatment of nondiabetic rats with WAY135201 did not affect conduction velocity.

Nutritive (capillary) sciatic endoneurial blood flow (Fig. 2A) was 47.6 ± 2.9% reduced ($P < 0.001$) by diabetes. WAY135201 and celikalim more than corrected this deficit ($P < 0.001$), producing flow values that were supernormal by 29.7 ± 10.8% ($P < 0.05$) and 43.9 ± 13.9% ($P < 0.01$), respectively. Treatment of nondiabetic rats with WAY135201 caused a 17.7 ± 4.3% flow increase that did not reach statistical significance. Mean systemic blood pressure (Fig. 2B) was modestly reduced by diabetes (15.9 ± 3.6%; $P < 0.05$). There was a further marked reduction with celikalim treatment (29.0 ± 5.6%; $P < 0.001$) whereas WAY135201 caused a smaller decrease (8.7 ± 5.2%) that was not statistically significant. Systemic pressure in WAY135201-treated nondiabetic rats was also not significantly reduced (4.8 ± 3.4%). Endoneurial vascular conductance (Fig. 2C) values reflected these blood pressure changes. Thus, there was a 38.2 ± 2.7% reduction ($P < 0.001$) in conductance with diabetes, which was overcor-

rected by WAY135201 and celikalim treatment ($P < 0.001$) giving conductances that were $76.4 \pm 21.3\%$ ($P < 0.001$) and $142.5 \pm 23.3\%$ ($P < 0.001$) supernormal, respectively. WAY135201 treatment of nondiabetic rats increased conductance ($25.0 \pm 7.8\%$) into the top quartile of the nondiabetic range but this was not statistically significant.

Diabetes and K_{ATP} channel opener treatment affected the total (capillary plus large vessel and shunt) endoneurial blood flow (Fig. 3A). There was a $59.3 \pm 4.2\%$ reduction ($P < 0.001$) with diabetes which was returned to the nondiabetic range by WAY135201 ($P < 0.05$) and celikalim ($P < 0.01$) treatments. Conductance (Fig. 3B) was $49.1 \pm 8.0\%$ decreased ($P < 0.01$) by diabetes, and this was corrected by WAY135201 ($P < 0.01$) and celikalim ($P < 0.001$). WAY135201 treatment of nondiabetic rats did not significantly alter the total endoneurial flow or conductance. The percentage of H_2 clearance via nutritive perfusion (Fig. 3C), an index of the pattern of endoneurial blood flow and degree of arteriovenous shunting (Day et al., 1989; Cameron et al., 1996), varied between groups. It was not significantly altered by diabetes or celikalim, however, in WAY135201-treated diabetic and nondiabetic groups, the nutritive component was elevated by $50.9 \pm 10.2\%$ ($P < 0.05$) and $54.1 \pm 12.3\%$ ($P < 0.05$), respectively.

Sciatic nerve ouabain-sensitive Na^+, K^+ ATPase activity (Fig. 4) was $49.8 \pm 5.8\%$ reduced ($P < 0.001$) by 8 weeks of diabetes. This was largely corrected by WAY135201 ($90.4 \pm 7.7\%$; $P < 0.001$) and celikalim ($80.1 \pm 9.2\%$; $P < 0.001$) treatments. WAY135201-treated nondiabetic rats had Na^+, K^+ ATPase activity that was $35.6 \pm 7.9\%$ supernormal ($P < 0.01$).

The total glutathione and GSH contents (Fig. 5) of sciatic nerve were $30.3 \pm 3.1\%$ ($P < 0.001$) and $30.1 \pm 2.7\%$ ($P < 0.001$) reduced by diabetes, respectively. Neither measure was significantly altered by WAY135201 treatment of nondiabetic or diabetic rats or by celikalim treatment of diabetic rats.

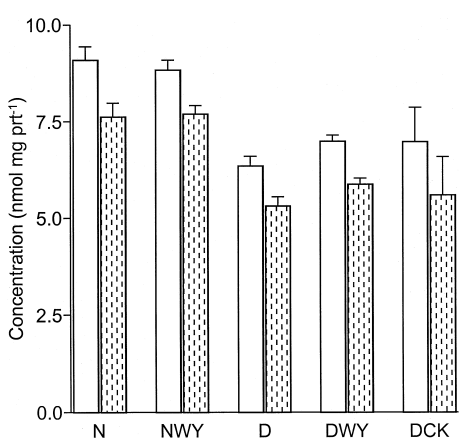

Fig. 5. Sciatic total glutathione (open bars) and GSH (stippled bars). N, nondiabetic control group, $n = 21$; NWY, nondiabetic rats treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 9$; D, 8-week diabetic rats, $n = 21$; DWY, 8-week diabetic rats treated for 2 weeks with 3 mg kg^{-1} day^{-1} WAY135201, $n = 21$; DCK, 8-week diabetic rats treated for 2 weeks with 0.25 mg kg^{-1} day^{-1} celikalim, $n = 5$. Error bars are SEM.

4. Discussion

The K_{ATP} channel openers, celikalim and WAY135201, markedly improved sciatic neurovascular function in diabetic rats; motor conduction velocity was completely restored and perfusion was supernormal. Blood flow was also increased by WAY135201 in nondiabetic rats. This suggests that K_{ATP} channels are present on vasa nervorum. The data support the theory that vascular changes are basic factors in the aetiology of diabetic neuropathy. Celikalim and WAY135201 were particularly effective in diabetic rats, which may reflect their actions against a background of elevated smooth muscle tone. The latter could be due to the changes in the properties of the smooth muscle itself or in the local influences of vasoconstrictors and dilators. Diabetic deficits in the vasa nervorum nitric oxide and prostacyclin systems have been noted (Ward et al., 1989; Kihara and Low, 1995; Maxfield et al., 1997). This elevates reactivity to vasoconstrictors (Maxfield et al., 1997), further exacerbated by changes in the angiotensin II and endothelin-1 systems (Cameron and Cotter, 1996). In cerebral arteries, the nitric oxide defect causes smooth muscle depolarisation and increased tone (Zimmerman et al., 1997).

K_{ATP} channel-mediated vasodilation of the aorta is diminished by diabetes, despite an increase in the number of channels (Kamata et al., 1989; Cameron and Cotter, 1992; Glocker and Quast, 1997). This could reflect changes in other vasorelaxation systems such as nitric oxide. The link between membrane potential, intracellular Ca^{2+} and smooth muscle tone may also be altered by diabetes (Glocker and Quast, 1997). Nonetheless, vasa nervorum responded well to chronic K_{ATP} channel opener treatment in diabetic rats.

The physiological role of vasa nervorum K_{ATP} channels is unknown. Regulation of the channel opening by energy stores and ATP/ADP ratio clearly does little for the endoneurium in diabetes, which is sufficiently hypoxic to compromise nerve function (Tuck et al., 1984). Drawing parallels with other vascular beds, vasa nervorum K_{ATP} channels may participate in vasodilator responses to several agonists, such as adenosine, histamine and prostacyclin (Marshall et al., 1993; Champion and Kadowitz, 1997). The hyperpolarising effect of K_{ATP} channel openers will close voltage-gated Ca^{2+} channels. Thus, the data for celikalim and WAY135201 on neurovascular function in diabetic rats are consistent with the effects of dihydropyridine Ca^{2+} channel blockers (Cameron and Cotter, 1994; Kapelle et al., 1994).

The K_{ATP} channels in rat vascular smooth muscle may be closed by angiotensin II, a protein kinase C mediated action (Kubo et al., 1997). Such modulation of a K_{ATP}

channel system that normally contributes to vasa nervorum vasodilation may be important for the development of diabetic neuropathy. Thus, oxidative stress in diabetes elevates angiotensin II levels (Cameron et al., 1994b), which contributes to reduced nerve blood flow. Conduction velocity and perfusion are improved by angiotensin converting enzyme and angiotensin AT_1 receptor antagonists (Cameron and Cotter, 1994). Moreover, diabetes increases vascular protein kinase C activity, partly as a result of diacylglycerol synthesis from elevated glucose and partly stimulated by oxidative stress (Koya and King, 1998). Recent studies showed that protein kinase C inhibition improves nerve perfusion and function (Cameron et al., 1999; Jack et al., 1999). Thus, oxidative stress-related events in diabetes may contribute to vasa nervorum K_{ATP} channel closure and decreased vascular conductance, which can be countered by K_{ATP} channel openers.

The two K_{ATP} channel openers did not have identical cardiovascular effects. At the doses used, both drugs normalized nutritive and total endoneurial blood flow in diabetic rats. However, compared to celikalim, WAY135201 treatment altered the pattern of blood flow to favour greater hydrogen clearance by the nutritive component. It is possible that this subtle distinction resulted from an action biased towards a subtype of K_{ATP} channels involved in the dilation of arterioles controlling capillary perfusion, rather than those involved with arterio-venous shunt flow. WAY135201 treatment also caused less hypotension than celikalim, which could reflect specificity for a subset of K_{ATP} channels, although this may also be a dose effect. However, the measurements were made in anaesthetized rats, a state that exaggerates the hypotensive effects of peripheral vasodilators (Cotter and Cameron, 1998). Further experiments in conscious rats would be necessary to assess any differences in the systemic haemodynamic actions of WAY135201 and celikalim. In conscious man, K_{ATP} channel openers have modest hypotensive effects at doses that increase forearm blood flow (Fox et al., 1991).

Diabetes reduced nerve GSH and GSSG content to a similar extent. This suggests that impaired antioxidant protection by the glutathione system results from reduced synthesis rather than defective recycling of GSH from GSSG. The latter would decrease the GSH/GSSG ratio rather than diminish the total glutathione. While the glutathione deficit would potentially contribute to oxidative stress, it does not provide evidence for markedly elevated free radical activity in nerve endoneurium, which would tend to decrease the GSH/GSSG ratio. Furthermore, correction of nerve blood flow by K_{ATP} channel openers did not improve glutathione levels, indicating that their reduction was not directly caused by ischaemia or ischaemia–reperfusion effects. Aldose reductase inhibitors and α-lipoic acid restore nerve glutathione content (Nagamatsu et al., 1995; Hohman et al., 1997) and also corrected neurovascular dysfunction in diabetic rats. However, the results with K_{ATP} channel openers suggest that an action on nerve glutathione is not necessary for improved conduction velocity.

As an alternative (or addition) to the vascular hypothesis, reduced nerve Na^+, K^+ ATPase activity may cause conduction slowing in experimental diabetes (Greene et al., 1992). Celikalim and WAY135201 largely corrected the diabetic Na^+, K^+ ATPase deficit and WAY135201 also increased Na^+, K^+ ATPase activity in nondiabetic rats without effects on conduction velocity. This is not a general property of vasodilators; α_1-adrenoceptor antagonist treatment increased conduction velocity and blood flow without affecting Na^+, K^+ ATPase activity (Cameron et al., 1991; Cotter and Cameron, 1998). Neuronal/glial cell effects of K_{ATP} channel openers might explain Na^+, K^+ ATPase increases. Thus, if K_{ATP} channels were found on these cells, loss of intracellular K^+ or a reduced K^+/Na^+ ratio could cause compensatory upregulation of Na^+, K^+ ATPase pumps. A similar phenomenon is seen in galactosaemic rats; a model of elevated polyol pathway flux, oxidative stress and vascular protein kinase C activation (Mizisin and Calcutt, 1991; Love et al., 1996; Koya and King, 1998). Galactosaemia causes diabetes-like deficits in endothelium-dependent vasorelaxation, nerve perfusion and conduction velocity (McManis et al., 1986; Cameron and Cotter, 1993). As a result of galactose–Na^+ cotransport, endoneurial Na^+ content doubles (Mizisin et al., 1986). This is accompanied by elevated nerve Na^+, K^+ ATPase activity (Mizisin and Calcutt, 1991), perhaps in response to increased Na^+ leakage into cells. Together, these findings suggest that Na^+, K^+ ATPase activity is not closely linked to conduction velocity.

Reduced excitability and decreased conduction velocity may result from Na^+ channel inactivation due to chronic depolarisation in diabetic rat nerves (Brismar and Sima, 1981). Similar changes occur when nondiabetic rat nerves are exposed to anoxia (Brismar, 1981). If K_{ATP} channel openers cause axonal hyperpolarisation, then resting membrane potential and Na^+ channel status would be restored, allowing greater activation upon stimulation. This action would complement the beneficial vascular effects of K_{ATP} channel openers.

In conclusion, celikalim and WAY135201 treatment markedly improved sciatic nerve perfusion and function in diabetic rats. Thus, K_{ATP} channel openers may be candidates for further investigation in clinical trials for diabetic neuropathy. The data also caution that the use of K_{ATP} channel antagonists in glycemic control could potentially have adverse neurological effects, although no studies have shown that such drug therapy is a risk factor for neuropathy.

Acknowledgements

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