The effect of non-enzymatic glycation of serum proteins on their permeation into peripheral nerve in normal and streptozotocin-diabetic rats

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Summary. The permeation of native and non-enzymatically glycated albumin and immunoglobulin G into the endoneurium of the sciatic nerve of rats was examined in acute experiments. Low amounts of native albumin entered both in control and streptozotocin-diabetic animals with no significant difference between them. The entry of glycated albumin was significantly greater both in control and diabetic rats, especially in the former. Permeation of native and glycated immu-

noglobulin G was not detectable over the time course of the experiment. It is concluded that glycation of albumin enhances its permeation into the nerve. This may be relevant to the increased amounts of endoneurial albumin that are detectable in human diabetic neuropathy.

Key words: Streptozotocin diabetes, blood-nerve barrier, non-enzymatic glycation, serum proteins.

The main functional alteration of the vasculature associated with diabetes is abnormal leakage of proteins from the circulation. This has been clearly documented in the kidney and retina of diabetic rats [1], as well as in new vessels formed in granulation tissue [2], although there have been conflicting reports concerning the permeability of blood vessels in peripheral nerve [3–6]. Peripheral nerve possesses a blood-nerve barrier which has been related to the presence of tight junctions between the endothelial cells of endoneurial vessels.

In general, studies in experimental diabetes [4, 5] have failed to show any increased permeability of the blood-nerve barrier to large molecules but have detected an increase for smaller molecules [6]. In human diabetic neuropathy, on the other hand, an increased content of plasma macromolecules, including albumin and immunoglobulin has been found in the endoneurium [7, 8] which has been interpreted as indicating an increased permeability of the blood-nerve barrier. Morphological studies in human diabetic neuropathy have shown reduplication of the basal lamina around endoneurial capillaries and hyperplasia of the endothelial cells [9–13], but it is not clear whether these changes would be expected to increase permeability. Fenestration of endoneurial capillaries has been described [14] but only as a very rare occurrence.

We have tried to resolve these conflicting findings in terms of non-enzymatic glycation of circulating proteins secondary to the hyperglycaemia associated with diabetes. Albumin, the major species of circulating pro-

tein, has been reported to permeate more easily through the microvasculature when glycated [15]. We have, therefore, measured the permeation into the sciatic nerve of both ¹²⁵I-albumin and ³H-glycosyl albumin in control and streptozotocin-diabetic rats to determine whether diabetes causes increased permeability, and the permeation of ¹²⁵I-immunoglobulin G (¹²⁵I-IgG) and ³H-glycosyl IgG to examine the effect, if any, of the size of the permeating species.

Materials and methods

Animal maintenance

Sprague-Dawley rats weighing about 300 g were injected through the tail vein with streptozotocin (STZ) dissolved in 0.9% saline to a dose of 65 mg/kg body weight. Control rats were injected with an equivalent amount of 0.9% saline. All rats were checked for glycosuria approximately 2 days after the injection. Rats were provided with standard food and water ad libitum for 8 weeks from the date of injection.

Preparation of labelled compounds

Rat albumin (essentially fatty acid-free) and rat immunoglobulin (Sigma Chemical Company Ltd., UK) were iodinated by the iodogen method [16] using [¹²⁵I] sodium iodide (Amersham International Ltd. UK). Glycated rat albumin and rat IgG were prepared ac-

cording to the method of Day et al. [17] using D-[³H]glucose (Amersham International). The glycated compounds were separated from the unreacted glucose on a Sephadex G25 column.

Injection of tracers

Rats were anaesthetized with halothane and oxygen and their inferior vena cava cannulated. The tracer was injected in a volume of approximately 1.0 ml after the removal of a similar volume of blood. At 20 min after the injection, both sciatic nerves were removed, cleaned of all adhering tissue and their wet weight determined. They were then prepared for radioactive counting. A sample of blood was also removed for counting at this stage. The tissue to blood ratio (TBR) of the isotope was calculated according to the formula:

$$\text{TBR} = \frac{\text{DPM/mg wet weight sciatic nerve}}{\text{DPM/100 } \mu\text{l blood}}$$

Assessment of vascular space

⁵¹Cr-labelled erythrocytes prepared by the method of Gray and Sterling [18] were injected as described above to determine whether there was a difference between the vascular space available in control and STZ-diabetic animals.

Statistical analysis

The results were compared using both the parametric Student's *t*-test and the non-parametric Mann-Whitney-Wilcoxon test. Both gave identical levels of significance.

Results

The blood glucose concentration in the rats injected with STZ was 37.15 ± 6.2 mmol/l (mean $\pm$ SEM). In the control animals it was 11.85 ± 3.8 mmol/l.

The TBR values for the native and glycated albumin and IgG are given in Table 1. Those for native albumin did not differ between the control and diabetic animals. The TBR values for glycated albumin, on the other hand, were significantly greater than those for native albumin, both in the diabetic and control animals ($p < 0.01$ for both). The

values were also significantly higher in the control rats as compared with the diabetic rats.

The TBR values for IgG were uniformly low and did not differ between the diabetic and control animals. The penetration of IgG into nerve, therefore, appears to be negligible and there was no detectable influence of glycation, at least over the time course of the experiment.

The possibility that the changes in TBR could have been the result of volume changes in the vascular compartment rather than reflecting an increased permeability of glycated albumin was investigated by measuring the TBR for ⁵¹Cr-labelled erythrocytes, both in control and diabetic animals. The values were very small (0.12 ± 0.05 and 0.11 ± 0.03 for control and diabetic animals, respectively) and did not differ significantly from each other.

Discussion

Our results suggest that the increased permeability of albumin through blood vessels in diabetes is caused not simply by structural changes in the vascular endothelium but instead by an increase in the glycation of albumin. This is in keeping with the findings of Sampietro et al. [15] who examined microcirculation of the hamster cheekpad using fluorescent video microscopy and found that, unlike native albumin, glycated albumin leaked out of the microvascular beds. Further confirmation for this is provided by Williams et al. [19] who showed that glycated albumin is ingested *in vitro* by isolated microvessels whereas unmodified albumin is excluded. Furthermore, Kumagai et al. [20] have found that the modification of albumin by the covalent addition of primary amine groups, which alters its isoelectric point from about 4 to about 9, facilitates its entry from the circulation to the cerebrospinal fluid. In the non-enzymatic glycation of albumin it is the low pK lysine residue(s) that are thought to be the major site of glycation [17].

It is likely that the necessary condition for the transfer of substances across the blood-nerve barrier are specific to a particular molecular species and probably dependent on size, charge and carbohydrate content of that species. For example, Rechthand et al. [6] showed an increase in the permeability of the blood-nerve barrier of STZ-diabetic rats for a small non-electrolyte such as mannitol but not for a larger molecule such as microperoxidase.

In the present study we also found a low level of entry of albumin into rat sciatic nerve with no difference between diabetic and non-diabetic animals but enhanced transfer of non-enzymatically glycated albumin. This would accord with the studies on sural nerve biopsies from patients with diabetic neuropathies that found an increased endoneurial albumin content [7, 8]. It is possible that the radioimmunoassay used was unable to differentiate between native and glycated albumin; the latter would be present in elevated levels in diabetic patients. Poduslo et al. [8] suggested that there was no general extravasation of plasma proteins and that a mechanism "other than size indiscriminate extravasation of plasma proteins is involved". The lesser permeability to glycated albumin in our diabetic animals as compared with control

Table 1. Sciatic nerve tissue to blood ratios for non-glycosylated and glycosylated albumin and IgG in control and streptozotocin-diabetic rats (means $\pm$ SEM)

	Control rats	Diabetic rats	<i>p</i>
Native albumin	0.45 ± 0.07 (9)	0.32 ± 0.12 (8)	n. s.
<i>p</i>	< 0.01	< 0.01	
Glycosylated albumin	2.08 ± 0.34 (13)	0.87 ± 0.16 (12)	< 0.01
Native IgG	0.27 ± 0.05 (8)	0.19 ± 0.04 (8)	n. s.
<i>p</i>	n. s.	n. s.	
Glycosylated IgG	0.29 ± 0.06 (8)	0.29 ± 0.07 (8)	n. s.

Number of animals in parenthesis; n. s. = not significant

rats is unexplained. A possible explanation would be partial saturation of a transport mechanism.

In a comparative study on a number of tissues, Williamson et al. [21] found a small increase in ^{125}I -bovine serum albumin (BSA) permeation of the sciatic nerve in spontaneously diabetic female rats and a higher increase in rats with severe STZ diabetes but no significant increase in rats with mild STZ diabetes. This was shown to be an aldose reductase-linked phenomenon that was modulated by sex steroids. We were unable to show any increase in the permeation of the diabetic sciatic nerve with ^{125}I -albumin although our methods were similar. The difference could arise from their use of BSA as opposed to our use of rat albumin. We were also unable to show any permeation of IgG into the sciatic nerve over a 20 min period. Seitz et al. [22], however, have shown the presence of biotinylated IgG in the inner layers of normal mouse perineurium and in the endoneurium 4 days after the intraperitoneal injection of tracer. Poduslo et al. [8] found increased levels of IgG and IgM in diabetic patients and even higher values in those with diabetic neuropathy. It is possible that a different mechanism operates over an extended time period.

In conclusion, it would appear that there are many different factors affecting the permeation of molecules into nerve and that non-enzymatic glycation is of particular importance for albumin. Changes in the endoneurial environment will influence nerve fibre function. The consequences of increased amounts of protein in endoneurial fluid can at present only be a matter for speculation.

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