Axonal Transport in Rats after Galactose Feeding

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Summary. Axonal transport was studied in galactose-fed rats. Precursors for proteins and glycoproteins were injected into the fifth lumbar spinal ganglion and retrograde axonal transport as well as slow axonal transport were estimated at different time intervals during the following 4 weeks. Galactose-feeding was found to produce a progressive reduction of the retrograde axonal transport of glycoproteins in intact nerve (2.9 ± 0.2 (arbitrary units) after five days of galactose-feeding as compared to 1.9 ± 0.7 after 28 days, $2p = 0.025$). The slow axonal transport velocity of structural proteins was reduced from 0.97 ± 0.09 mm/day to 0.84 ± 0.04 mm/day, $2p = 0.0030$. The present findings as well as structural and electrophysiological abnormalities of the nerves of galactose-fed rats are similar to the changes found in diabetic rats. The similarities point to a decisive role for glucose or its metabolites in the development of diabetic neuropathy.

Key words: Galactose-feeding, axonal transport, diabetic neuropathies, nerve crush.

In peripheral nerve of rats galactose-feeding, as well as induction of diabetes by streptozotocin, produces structural, functional and biochemical abnormalities [3, 10, 13, 15]. In both models the conduction velocity is slowed presumably due to a reduction in myelinated nerve fibre calibre [6, 7, 10, 13, 15]. Furthermore, accumulation of sugar alcohols and water occurs, associated with an endoneurial oedema [3, 13, 15, 19].

The reduced nerve fibre calibre in streptozotocin diabetes is due to axon abnormalities [6, 7, 10]. Therefore, we have recently examined the intra-

axonal transport of proteins and glycoproteins in this model. We found a very early decrease in the retrograde flux in intact and crushed diabetic nerves [11, 18] as well as a decreased velocity for structural proteins carried in the anterograde direction [12].

The purpose of the present experiment was to examine whether axonal transport changes similar to those in the diabetic rat can also be produced by galactose-feeding. If this is the case the galactose model points to the significance of sugar molecules rather than to hormonal factors for the development of peripheral nerve abnormalities in diabetes.

Materials and Methods

Weight matched (Table 1) male Wistar rats, 24 weeks old, were divided into galactose-fed and control groups. The diets used for galactose and control feeding were similar to those used by others in peripheral nerve studies [3, 13–15, 19]. The galactose diet consisted of two parts of D-galactose and three parts of the standard diet. Controls were fed a vitamin feed mixture (65% carbohydrate, 30% protein and 5% fat). All animals had free access to water and food during the experiment, until the time of sacrifice.

Retrograde axonal transport was estimated in intact and crushed nerves after 5 and 28 d on the diet. Slow axonal transport velocity was estimated after 29 d of galactose-feeding. In 5 control and 4 galactose-fed rats of the latter group wet weight, dry weight and water content of a 15 mm segment of the left sciatic nerve was measured as previously described [8, 13].

Experimental Procedures

Retrograde axonal transport was estimated with L-(³⁵S) methionine (Amersham, 700–1100 Ci mmol⁻¹) as precursor for protein and L-(6-³H) fucose (Amersham, 26 Ci mmol⁻¹) as precursor for glycoproteins. In the slow axonal transport experiment (L-2,3,4,5-³H) proline (Amersham, 117 Ci mmol⁻¹) was used as precursor for protein. The precursors were concentrated by freeze-drying and dissolved in a buffered salt solution to give a final concentration of 5 μ Ci μ l⁻¹ ³⁵S-methionine and 10 μ Ci μ l⁻¹ ³H-fucose or of 10 μ Ci μ l⁻¹ ³H-proline.

Table 1. Body weight, nerve wet weight and nerve water content of control and galactose-fed rats during the various experimental periods. Values are mean $\pm$ SD (n)

Experimental period	Group	Body weight at the start of the experiment (g)	Body weight at the time for precursor injection (g)	Body weight at the end of the experiment (g)	Nerve wet weight (mg cm ⁻¹)	Nerve water content (mg cm ⁻¹)
5 days diet (retrograde transport)	Control	411 $\pm$ 34 (8)	429 $\pm$ 39 (8)	—	—	—
	Galactose	408 $\pm$ 20 (9)	419 $\pm$ 28 (9)	—	—	—
28 days diet (retrograde transport)	Control	406 $\pm$ 41 (9)	446 $\pm$ 39 (9)	—	—	—
	Galactose	379 $\pm$ 47 (6)	346 $\pm$ 44 (6)	—	—	—
29 days diet (slow transport)	Control	413 $\pm$ 16 (8)	421 $\pm$ 19 (8)	448 $\pm$ 23 (8)	8.7 $\pm$ 1.0 (5)	6.0 $\pm$ 0.9 (5)
	Galactose	402 $\pm$ 24 (8)	409 $\pm$ 26 (8)	403 $\pm$ 24 (8)	11.8 $\pm$ 0.5 (4)	9.1 $\pm$ 0.4 (4)

Table 2. Axonal transport of proteins and glycoproteins is estimated as the accumulation of activity at ligatures in 2 control groups and 2 galactose-fed groups. PA: accumulation proximal to a ligature estimating the anterograde axonal flux of material; DA: accumulation distal to a ligature estimating the retrograde axonal flux of material; CA: accumulation at the crush

	Group	Days on diet	n	Intact nerve			Crushed nerve	
				PA (pre-existing activity)	DA (pre-existing activity)	PA (pre-existing activity)	DA (pre-existing activity)	CA (pre-existing activity)
Protein transport (³⁵ S-methionine)	Control	5	8	2.0 $\pm$ 0.3	1.3 $\pm$ 0.3	3.0 $\pm$ 0.5	4.0 $\pm$ 0.2	23.0 $\pm$ 4.7
	Galactose	5	9	2.1 $\pm$ 0.2	1.4 $\pm$ 0.2	3.2 $\pm$ 0.6	3.6 $\pm$ 0.4	22.2 $\pm$ 4.2
	Control	28	9	2.1 $\pm$ 0.3	1.2 $\pm$ 0.2	3.2 $\pm$ 0.6	3.5 $\pm$ 0.3	24.4 $\pm$ 3.7
	Galactose	28	6	2.0 $\pm$ 0.4	0.9 $\pm$ 0.3	3.7 $\pm$ 0.9	2.8 $\pm$ 0.9	26.6 $\pm$ 2.7
2P	C5-G5			NS	NS	NS	0.034	NS
	C28-G28			NS	0.030	NS	0.064 (NS)	NS
	C5-C28			NS	NS	NS	0.0038	NS
	G5-G28			NS	0.0026	NS	0.031	0.041
Glycoprotein transport (³ H-fucose)	Control	5	8	3.2 $\pm$ 0.7	2.6 $\pm$ 0.4	5.1 $\pm$ 0.9	3.6 $\pm$ 0.5	24.7 $\pm$ 5.7
	Galactose	5	9	3.7 $\pm$ 0.3	2.9 $\pm$ 0.2	5.4 $\pm$ 0.7	3.0 $\pm$ 0.4	25.0 $\pm$ 3.1
	Control	28	9	3.3 $\pm$ 0.3	2.3 $\pm$ 0.5	5.4 $\pm$ 0.8	2.8 $\pm$ 0.3	24.2 $\pm$ 4.0
	Galactose	28	6	2.9 $\pm$ 0.4	1.9 $\pm$ 0.7	5.5 $\pm$ 0.5	1.9 $\pm$ 1.0	26.6 $\pm$ 1.6
2P	C5-G25			NS	NS	NS	0.017	NS
	C28-G28			NS	NS	NS	0.098 (NS)	NS
	C5-C28			NS	NS	NS	0.00098	NS
	G5-G28			0.00054	0.025	NS	0.010	NS

Fig. 1. The figure illustrates how the different ligatures are placed on the right and left sciatic nerve. The nerve segments designated by the symbols and the formulae for estimating anterograde (PA) and retrograde axonal transport (DA) by the accumulations at the two collection ligatures are shown

Anaesthesia was induced with sodium pentobarbitone (50 mg kg⁻¹) and body temperature was maintained by placing the rats on an electrically heated pad [16]. The precursor solution were injected through a micropipette into the 5th dorsal root ganglion as previously described [12, 16].

For examination of retrograde axonal transport right and left ganglion were injected 21 and 9 h before sacrifice, respectively. The left sciatic nerve was crushed and the right was left intact. The crush was produced by a ligature tightened against a glass rod at a constant site distally in the thigh 10 min after precursor injection. Two hours before sacrifice two ligatures separated by a 6 mm interval were placed on the right sciatic nerve in midhigh level and on the left side 15 mm proximal to the crush. The time intervals were chosen in accordance with the changes found for the retrograde axonal transport in streptozotocin diabetic rats [11, 18]. For

examination of the slow axonal transport the right dorsal root ganglion was injected 4 d after the start of galactose-feeding.

At the end of the experiment the rats were sacrificed under ether anaesthesia. The nerves were dissected out and axonal transport was stopped by freezing the nerve on a cooled copperblock (-70°C). The nerves were cut into 3 mm segments which were placed overnight in numbered vials with 1 ml icecold 10% TCA (trichloroacetic acid, w/w). On the following day the nerve segments were washed in an additional ml of 10% TCA for 1 h at room temperature. The TCA treated segments were dissolved in 150 μl Lumasolve (LUMAC) and counted for 10 min in 4 ml Lipoluma (LUMAC). The counts were corrected for quenching, cross-over and back-ground and expressed in dpm.

Calculations

Retrograde Axonal Transport after 5 d and 28 d on Galactose Diet

The axonal flux of retrograde transported material was estimated as the accumulation distal to the distal collection ligature. In addition, the experimental procedure allows estimation of the anterograde transport as the accumulation proximal to the proximal ligature. The formulae used for calculation of the accumulation were

$$\text{DA} = \frac{\frac{A_d}{S_d + S_i} - 1}{t} \times 3 \text{ and } \text{PA} = \frac{\frac{A_p}{S_p + S_i} - 1}{t} \times 3.$$

DA: distal accumulation, PA: proximal accumulation, A_d : distal collection segment (3 mm), A_p : proximal collection segment (3 mm), S_d : 6 mm segment distal to A_d , S_p : 6 mm segment proximal to A_p , S_i : 6 mm segment between the collection ligatures, and t : time interval in h between placing of the ligatures and stopping of axonal transport (Fig. 1). The accumulation at the crush was calculated using the formula $\text{CA} = \left(\frac{A_{cr}}{\frac{3}{2}S_d} - 1 \right) \times 9$ [18]. CA: accumulation at the crush, A_{cr} : 9 mm segment containing the crush and S_d : 6 mm segment proximal to A_{cr} . Assuming that the accumulations are caused only by axonal transport the proximal and the distal accumulation estimates the transport flux, the amount of material moving through a cross section of the nerve per unit time [11]. The crush accumulation measures the difference between the anterograde and retrograde transport. The unit for the flux is (pre-existing activity) $\text{mm}^{-1} \text{h}^{-1}$ and for the accumulation at the crush (pre-existing activity) mm^{-1} .

Slow Axonal Transport after 29 d on Galactose Diet

The distribution of activity in the nerve was measured after further 25 d when a bell-shaped wave of

activity, representing the slowly transported material, could be demarcated. The transport velocity was calculated partly as a mean velocity of all activity under the bell-shaped wave partly as the velocity of the wave peak [12]. The position of the wave was determined by the formula

$$\text{X wave} = \frac{A_1X_1 + A_2X_2 + \dots + A_nX_n}{A_1 + A_2 + \dots + A_n}$$

A_i : amount of activity in segment i ; X_i : distance between ganglion and segment i . For the mean velocity the formula was applied on all segments under the bell-shaped wave while the peak position was estimated by applying the formula on the 4 or 5 neighbouring segments with highest activity. Subsequently the velocity was estimated as the distance from the ganglion to the wave position divided by the time elapsed between precursor injection and stopping of axonal transport [12]. The velocity is given in mm d^{-1} .

Statistics

Differences were examined for statistical significance by Student's t test. Values are given in mean $\pm$ SD.

Results

Body weight for the different groups during the experimental period is given in Table 1. Whereas the controls rats showed a minor weight gain during the 4 week experimental period the galactose-fed rats failed to gain weight or lost weight.

Water Content of the Nerves

The nerve wet weight of the galactose-fed rats was increased by 40%, namely $11.8 \pm 0.04 \text{ mg cm}^{-1}$ compared to $8.7 \pm 0.11 \text{ mg cm}^{-1}$ in the control group ($2p = 0.00062$). The dry weight was $2.7 \pm 0.23 \text{ mg cm}^{-1}$ in the galactose-fed rats and $2.7 \pm 0.07 \text{ mg cm}^{-1}$ in the controls while the water content was 9.1 ± 0.04 and $6.0 \pm 0.16 \text{ mg cm}^{-1}$, respectively ($2p = 0.0048$).

Retrograde Axonal Transport after 5 d and 28 d of a Galactose-feeding

In Table 2 the values for the different accumulations in the intact and the crushed nerves are given. The distal accumulation for protein in intact nerves was decreased after 4 weeks by 25%. In crushed nerve the distal accumulation of protein and glycoprotein after 5 d of galactose-feeding was decreased by 10 and 17%, respectively.

For proximal accumulation no differences were seen between the galactose-fed rats and the control animals. There were also no significant differences between these two groups in the crush accumulation.

Distal accumulation was statistically decreased in crushed nerves from 25 to 28 weeks of age in control rats. A similar fall was seen in the galactose-fed rats. In contrast no changes were observed in the intact nerves of the control group while the galactose-fed group demonstrated a fall from 5 d to 28 d of feeding for both precursors.

Slow Axonal Transport Velocity

The mean transport velocity for component "a" in galactose-fed rats and controls was 0.84 ± 0.04 mm d⁻¹ and 0.97 ± 0.09 mm d⁻¹, respectively ($2p = 0.0030$). The velocity of the peak was also decreased in the galactose-fed group, 0.96 ± 0.09 mm d⁻¹ versus 1.04 ± 0.03 mm d⁻¹ in the control group ($2p = 0.037$).

Discussion

The present experiments have shown that galactose-feeding in rats produces a progressive decrease in retrograde axonal flux of protein and glycoprotein in intact and crushed peripheral nerves and a decreased transport velocity of slowly advanced structural proteins. In our previous experiments on the streptozotocin diabetic rat we found similar changes [11, 12, 16, 18], suggesting that the intra-axonal dynamics in these two models may be the same.

The axonal transport changes produced by galactose-feeding were less pronounced than in the streptozotocin model. After 4 weeks of galactose-feeding the retrograde flux of glycoprotein in intact and crushed nerve was reduced by 17 and 32% as compared to 28 and 60%, respectively, in diabetic rats [11, 18]. Furthermore the differences in the galactose model did not reach statistical significance at this time interval. For the transport velocity of slow component "a" the reduction was 13% in the present experiment as compared to 19% in the diabetic rats [12]. The explanation for these quantitative differences between the two models might be a less effective feeding in the galactose model. In fact the rats who had free access to the galactose diet lost weight because they did not eat as much as the rats offered the control diet.

Malmgren et al. [13] have measured the total cross sectional area of myelinated fibres in the sciatic nerves of rats of the same strain and age as used in the present experiment after 4 weeks on diet identical

to those used in the present study. They found an 11% reduction in the fibre area and a 9% slowing in conduction velocity in tail nerve. In accordance with this observation Sharma et al. [15] found a 10% reduction of the mean fibre 'diameter' after several months of galactose-feeding.

Among the material in slow component 'a', tubulin for the axonal microtubuli and various polypeptides for the neurofilaments have been demonstrated [4, 5]. The present finding of the decreased transport rate of this component, therefore, is a likely explanation for the reduced myelinated nerve fibre calibre in galactose-fed rats [2].

In the streptozotocin model we have reported earlier that 23–28 weeks old Wistar rats have a 9–14% reduction of the mean myelinated nerve fibre calibre and a 9% slowing of conduction velocity in tail nerve after 4 weeks duration of the diabetic state [6, 7, 10]. The abnormalities of fibre calibre, conduction velocity and axonal transport appear similar in the two models.

In contrast, endoneurial oedema is much more pronounced after galactose-feeding. Malmgren et al. [13] observed a 68% increase in nerve water content and a 104% expansion of the fascicular area between myelinated fibres. These figures correspond to an increase of 21 and 44% respectively in the streptozotocin model [8]. It was suggested by Malmgren et al. [9, 13] that the accumulation of sugar alcohols and water within intact diffusion barriers in the nerve might be responsible for the abnormalities of peripheral nerve structure and function in galactose-fed rats and in diabetes.

However, in the present galactose-feeding experiment the increase in water was more than twice that in the streptozotocin model despite a similar or even smaller change in axonal transport. We therefore suggest an alternative explanation for the peripheral nerve abnormalities in both models. In galactose-fed animals as well as in streptozotocin diabetic animals the decrease in retrograde transport could be the signal from the axon and its terminals to the protein synthesizing machinery in the nerve cell body [17] which results in the decreased transport rate of structural proteins subsequently leading to the reduction in nerve fibre calibre and conduction velocity. How the decrease in retrograde transport arises is unknown. One possibility could be the reduction in CDP-diglyceride: inositol phosphotransferase recently described by Clements [1]. This enzyme abnormality is related to the turn-over of myoinositol which might influence nerve conduction in streptozotocin diabetes in the rat.

Streptozotocin diabetes is associated with insulin deficiency and other hormonal abnormalities. Pre-

sumably none of these changes occur after galactose-feeding. The peripheral nerve abnormalities described in the present study have been produced by feeding rats an epimer of glucose. All of the results taken together therefore very strongly point to a decisive role of glucose or its metabolites in the pathogenesis of diabetic neuropathy.

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