Anterograde Fast Component of Axonal Transport During Insulin-Induced Hypoglycemia in Nondiabetic and Diabetic Rats

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SUMMARY

To elucidate the pathogenesis of the peripheral neuropathy associated with hypoglycemia the anterograde fast component (aFC) of axonal transport was studied in nondiabetic rats during acute and prolonged insulin-induced hypoglycemia and in streptozocin-diabetic (STZ-D) rats with acute hypoglycemia. [^{35}S]methionine and [^{3}H]fucose were injected into the dorsal root ganglion (L5) to label protein and glycoprotein, respectively. During the 4 h of transport, thigh temperature was maintained constant. Acute severe hypoglycemia (1.5 ± 0.2 mM) was associated with a 36% decrease in the amount of aFC (2.3 ± 0.7% in the test group vs. 3.6 ± 0.8% in the controls), whereas transport velocity was unaffected. Prolonged hypoglycemia, obtained by pretreatment with insulin for 3 days, prevented the decrease in amount of aFC. In STZ-D rats, acute severe hypoglycemia (1.5 ± 0.6 mM) produced a similar but less-pronounced decrease of aFC. We conclude that hypoglycemia is associated with alterations in axonal transport that could play a role in development of neuropathy. Prolonged hypoglycemia protects axonal transport against the effects of glucopenia, and an untreated diabetic state maintained for several days has a partially protective effect against episodes of hypoglycemia. *Diabetes* 36:853–58, 1987

Low levels of glucose can be inadequate for normal function of neural tissues and may threaten structural integrity. We have recently shown that insulin-induced hypoglycemia can produce a peripheral neuropathy with acute breakdown of the axon in rats (1), and similar findings have been made in insulin-treated diabetic rats (2,3). The peripheral nervous system under normal conditions depends on glucose as the major substrate for energy production, and its utilization is not influenced by physiologic insulin concentrations (4). Because the fast axonal transport system is ATP dependent (5), reduction of the delivery of integral proteins carried by the transport might be the functional mechanism involved in axonal degeneration in hypoglycemia.

There are two earlier studies on axonal transport during hypoglycemia. Mendell et al. (6) reported a decreased velocity of the anterograde fast component (aFC) in diabetic rats accidentally rendered hypoglycemic by insulin treatment. In contrast, Tomlinson and James (7) found that a decrease in fast axonal transport seen during hypoglycemia was normalized if hypothermia was avoided.

Our study was designed to investigate the effect of hypoglycemia on the aFC during strict control of body temperature in nondiabetic and diabetic rats. In addition, severe and moderate hypoglycemic conditions were compared during acute lowering of blood glucose levels and after a period of sustained hypoglycemia.

MATERIALS AND METHODS

Male Wistar rats (inbred in the animal house of the Institute of Pathology, Municipal Hospital of Aarhus, for >20 yr) aged 15–18 wk and weighing 305–450 g were subjected to hypoglycemia by insulin treatment.

Acute hypoglycemia. At −18 h (1600 h), acute hypoglycemia was induced by injection of 10–12 IU s.c. of a very long acting insulin [heat-treated Ultralente bovine insulin (Novo, Copenhagen, Denmark), pH 5.5, duration of effect from 6 to >24 h, maximum effect at 12–18 h]. At −2 h, food was removed from the animals, and at 0 h, proteins of aFC were labeled. Blood was taken from a tail vein at −18, −10, −2, 0, 2, and 4 h after labeling of the transported material, and blood glucose was measured by a glucose oxidase method (Glox, Kahi Diagnostica, Stockholm, Sweden). Animals whose blood glucose was <2.0 mM (<36 mg/dl) at 0

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and 2 h were designated *acute severe hypoglycemic*. Rats that did not fulfill this criterion were designated *acute moderate hypoglycemic*.

To study the effect of acute hypoglycemia in diabetes, 45 mg/kg i.v. streptozocin (STZ; Zanosar, Upjohn, Kalamazoo, MI) was administered. The rats were considered diabetic if blood glucose was >13.9 mM (>250 mg/dl) 24 h after STZ administration. Hypoglycemia was induced 10 days later by subcutaneous administration of crystalline insulin (Actrapid, Novo); 10 IU were given on the morning of the experiment. Every 2 h, 4 IU s.c. was administered, blood was drawn, and blood glucose levels were determined with a reflectance meter (Glucometer, Ames, Elkhart, IN) until they were ≤2 mM. This dosage was maintained during the axonal transport experiment to control blood glucose levels. Blood was drawn at 0, 2, and 4 h, and glucose was determined by the glucose oxidase method at a later time.

Prolonged hypoglycemia. To study whether adaptation to glucopenia occurs, a series of rats was pretreated for 3 days with the long-acting insulin preparation mentioned above: Four days before the axonal transport experiment, 4 IU s.c. was administered. On the following day, 8 IU was given, and the next day, 10 IU was given. The day before the experiment, 10–12 IU was administered exactly as in the experiment of acute hypoglycemia. During the pretreatment period, blood was drawn every 8 h, and blood glucose levels were determined by the glucose oxidase method. Rats with blood glucose <2.0 mM at 0 and 2 h were designated *prolonged severe hypoglycemics*, and rats with hypoglycemia above this level were designated *prolonged moderate hypoglycemics*. Untreated age- and weight-matched rats were used as controls.

Axonal transport experiment. During the transport experiment a needle thermistor was inserted into the musculature at midthigh level, and a drop in temperature was prevented by external heating.

The aFC was investigated as described earlier (8,9). Labeled methionine (L-[^{35}S]methionine, 37.4–55.0 TBq/mmol, Amersham, Arlington Heights, IL) was used as a marker for protein, and tritiated fucose (L-[5,6-^{3}H]fucose, 1.85–2.48 TBq/mmol, Amersham) was used to mark glycoprotein. The precursors were concentrated by freeze-drying and dissolved in a buffered salt solution to a final concentration of 185 and 370 kBq/μl, respectively. The right and left 5th lumbar dorsal root ganglia were exposed by a laminectomy performed during nembutal (25 mg/kg)/diazepam (5 mg/kg) anesthesia. This procedure was performed 24 h before the axonal transport experiment to allow the animal to recover before administration of the final insulin dose. At time 0 and 2 h the right and left dorsal root ganglia, respectively, were injected with 1 μl of the precursor solution during a short-lasting ether anesthesia. At 4 h the animals were killed by heart puncture during ether anesthesia, the ganglia and the sciatic nerves were removed, and axonal transport was stopped on a cold copper block (−40°C).

The nerves were cut into 3-mm segments and solubilized in 150 μl Lumasolve (Lumac, Basel, Switzerland). The activity was counted by liquid scintillation in 4 ml Lipoluma (Lumac) and expressed in disintegrations per minute. The distribution of activity displaying the transport wave (Fig. 1) was described by the following parameters: activity in the ganglion (4 h after injection), activity in aFC (4 h), the fraction of activity in aFC relative to the activity in the ganglion, the height of the plateau, the span of the wave front, the maximal transport velocity, and the lag time (8,9).

Statistics. Values are given as means ± SD. A model I ANOVA was used for determining differences among the three experimental groups (controls, acute severe hypoglycemics, and prolonged severe hypoglycemics) in nondiabetic rats. When the ANOVA showed that differences were present, the *F* test was used for comparisons between independent subsets of the groups (10). This analysis allowed control versus acute and prolonged severe hypoglycemic rats and acute severe hypoglycemic versus prolonged severe hypoglycemic rats to be compared. The study was not designed a priori for evaluation of different degrees of hy-

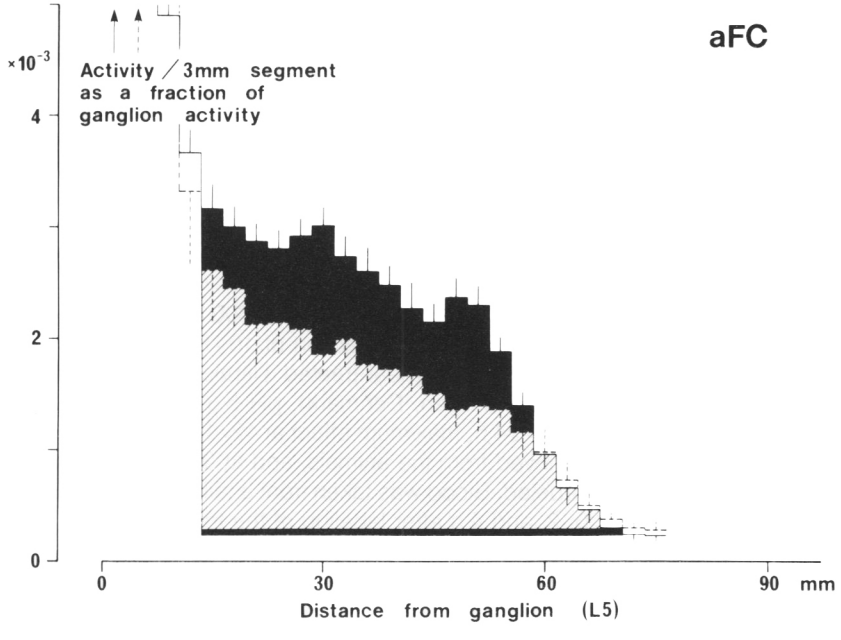

FIG. 1. Distribution of labeled-protein activity along sciatic nerve 4 h after injection of [^{35}S]methionine shown as fraction of activity in ganglion. Values ± SE for each 3-mm segment are given. *Solid lines* and *black area* indicate control values; *broken lines* and *open area* indicate nondiabetic group with acute severe hypoglycemia; *hatched area* indicates overlapping of areas. Amount of aFC is arbitrarily defined as amount of activity >12 mm distal to ganglion.

TABLE 1
Data on experimental and control animals grouped by hypoglycemic state

Hypoglycemic state	*n*	Age* (wk)	Weight* (g)	Weight change† (g)	Insulin‡ (IU)	Blood glucose§ (mM)	Temperature§‖ (°C)
Control						6.1 ± 0.7	
None	13	16.2 ± 0.5	371 ± 31				37.4 ± 0.4
Insulin treated							
Acute							
Severe	9	16.2 ± 0.4	351 ± 15		10.5 ± 0.8	1.5 ± 0.2	37.0 ± 0.3
Moderate	5	16.3 ± 0.6	374 ± 21		10.2 ± 0.4	2.4 ± 0.6	37.2 ± 0.2
Prolonged							
Severe	9	16.5 ± 0.3	369 ± 24	27 ± 11	10.4 ± 1.7	1.4 ± 0.4	37.5 ± 0.6
Moderate	6	16.5 ± 0.3	395 ± 25	17 ± 10	10.7 ± 2.5	2.3 ± 0.4	37.4 ± 0.8
Diabetes							
None	9	16.5 ± 0.9	376 ± 40	−40 ± 25		18.8 ± 1.5	37.1 ± 0.6
Acute severe	9	16.4 ± 0.9	392 ± 43	−54 ± 29	22.7 ± 8.4	1.5 ± 0.6	37.5 ± 0.6

Values are means ± SD.
*At start of study.
†During study.
‡Dosage on last day of study.
§During axonal transport experiment.
‖Intramuscular temperature of the thigh.

poglycemia. We found, however, the results regarding the moderate hypoglycemic rats to be of interest. This group was compared with the severe hypoglycemic rats by Student's *t* test, but note that the statistical evaluation of these differences is not part of the ANOVA and, consequently, not as valid. In diabetic rats, differences between hypoglycemic and hyperglycemic animals were compared by Student's *t* test. The limit of significance was 5%.

RESULTS

Data on the experimental and control animals by hypoglycemic state are shown in Table 1. The dose of insulin given during the last 18 h before the transport experiment was very similar in the four nondiabetic groups. In comparison, diabetic animals needed approximately twice the dosage. The degree of hypoglycemia in the acute hypoglycemics compared with the pretreated groups was the same, and intramuscular temperature of the thigh was maintained constant in all groups. In Fig. 2 the blood glucose values obtained before the axonal transport experiment are given for all groups.

aFC in hypoglycemic nondiabetic rats. Data of amount and transport kinetics of [^{35}S]methionine-labeled proteins are given in Table 2. No significant differences are seen between the amounts of label incorporated by the ganglia and the amount of aFC. However, the fraction of activity in aFC in relation to ganglion activity and the plateau level of the transport curve were significantly different among the groups. Acute severe hypoglycemia was associated with a reduced amount of transport compared with that seen with prolonged severe hypoglycemia (Fig. 3). In the acutely hypoglycemic rats, transported amount was significantly less for severe hypoglycemics than for moderate hypoglycemics (Fig. 3). The kinetics of the transport, i.e., the span of the wave front, velocity, and lag time, were all unaffected by hypoglycemia. The same pattern of transport alterations was found for [^{3}H]fucose-labeled glucoproteins (Table 3).

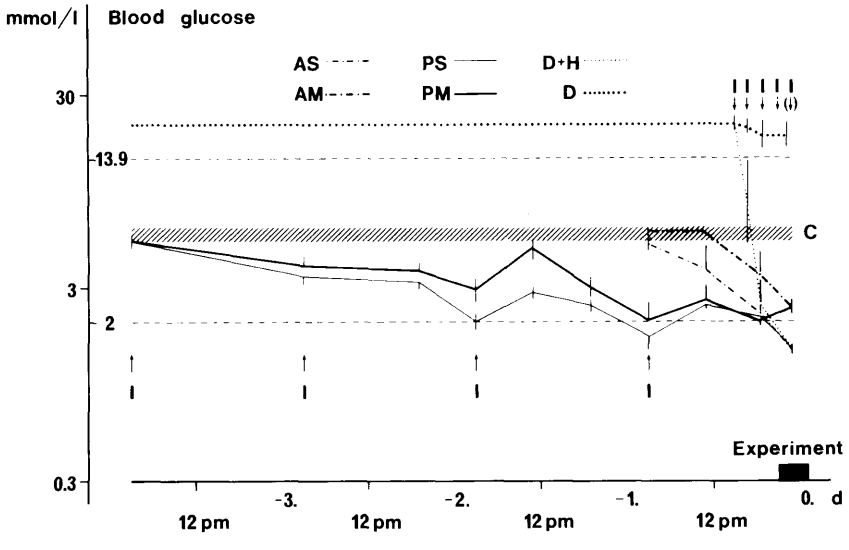

FIG. 2. Blood glucose levels before axonal transport experiment given for various groups. C, control; AS, acute severe hypoglycemia; AM, acute moderate hypoglycemia; PS, insulin-pretreated severe hypoglycemia; PM, insulin-pretreated moderate hypoglycemia; D, untreated diabetes; D + H, diabetes with acute severe hypoglycemia. Values are means ± SE. Note logarithmic scale.

TABLE 2
Characteristics of aFC during hypoglycemia in nondiabetic rats after labeling of proteins with [^{35}S]methionine

Hypoglycemic state	n	Total activity ($\times 10^3$ dpm) Ganglion*	Total activity ($\times 10^3$ dpm) aFC*	aFC/ganglion† (%)	Plateau/ganglion† ($\times 10^{-3}$)	Span* (mm)	Transport velocity* (mm/h)	Lag time* (min)
Control								
None	11	318 ± 174	11.8 ± 7.4	3.6 ± 0.8	2.6 ± 0.6	14 ± 4	17.1 ± 1.4	10 ± 16
Insulin treated								
Acute								
Severe	8	265 ± 162	7.1 ± 5.8] $2P < .05$ (vs. Moderate)	2.5 ± 0.9] $2P < .01$ (vs. acute Moderate); [$2P < .001$ (vs. prolonged Severe)	2.0 ± 0.9] $2P < .05$ (vs. acute Moderate); [$2P < .01$ (vs. prolonged Severe)	17 ± 6	17.4 ± 1.1	22 ± 11
Moderate	5	413 ± 171	16.2 ± 7.3	3.9 ± 0.5	3.3 ± 0.7	15 ± 5	17.4 ± 1.1	15 ± 13
Prolonged								
Severe	9	411 ± 259	16.6 ± 11.3	3.9 ± 0.4	3.5 ± 0.7	15 ± 3	17.4 ± 2.5	14 ± 24
Moderate	6	625 ± 399	28.0 ± 17.0	4.4 ± 0.4	3.6 ± 0.5	15 ± 2	17.6 ± 1.5	16 ± 16

aFC, anterograde fast component; aFC/ganglion, fraction of activity in aFC relative to activity in ganglion; plateau/ganglion, height of plateau of wave as fraction of activity in ganglion; span, span of wave front; lag time, time between precursor injection and start of transport.
*Differences among groups not significant (NS).
†$2P < .001$ among groups.

aFC in hypoglycemic diabetic rats. In the diabetic animals the relative amount of [^{35}S]methionine-labeled protein was reduced (Table 4). The reduction was to only 40% of that observed in nondiabetic animals with a similar degree of hypoglycemia. No significant differences were found in aFC transport of ^{3}H-labeled glycoproteins. Transport kinetics also remained unchanged during severe hypoglycemia in diabetic rats. Although a direct statistical evaluation is not possible between the control group and the diabetic group without hypoglycemia, the similarity of the aFC parameters in the two groups corroborates our previous finding that aFC is unaffected by the hyperglycemia associated with the diabetic state (11).

DISCUSSION

Our study demonstrates that severe hypoglycemia (<2 mM) is associated with a decrease in the amount of protein-related activity carried by the aFC of the axonal transport system in peripheral nerves relative to the amount incorporated in the ganglion. Because the absolute amount of activity in aFC is also decreased, although not statistically significantly, we interpret the finding as a decrease in the amount of protein in aFC.

When a hypoglycemic episode was preceded by a moderate degree of sustained hypoglycemia for ~3 days, no decrease in amount of aFC occurred. This protection of the fast transport system is probably the result of either the utilization of substrates other than glucose for energy production or an improved uptake of glucose. The adaptation of neural metabolism to hypoglycemia is well known. Thus, human and rat brains use ketone bodies as the major fuel during starvation and insulin-induced hypoglycemia (12,13). Peripheral nerves can also utilize ketone bodies. In vitro studies of rabbit peripheral nerves have demonstrated that hydroxybutyrate in a glucose-free medium can maintain ATP levels and oxygen uptake at an almost normal level (4).

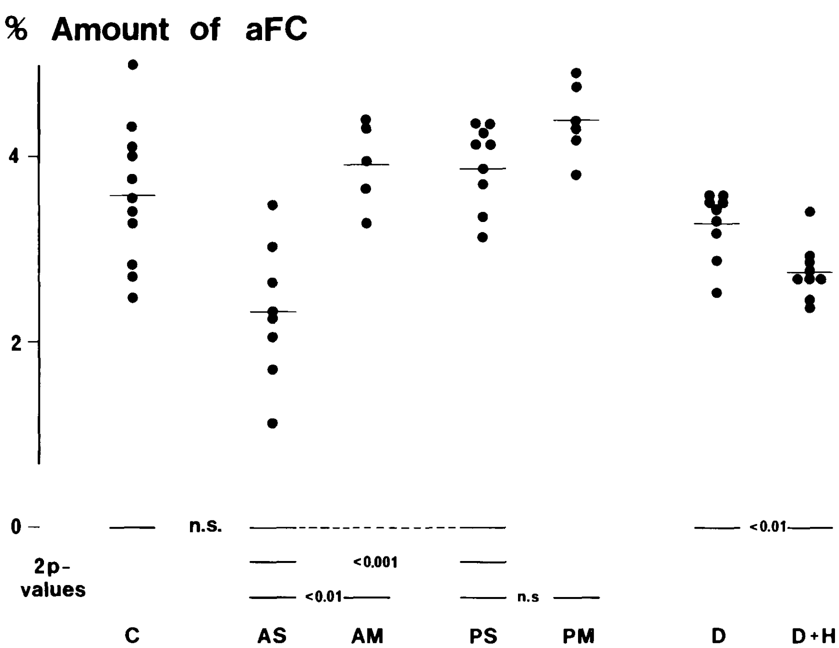

FIG. 3. Individual values of amount of [^{35}S]methionine-labeled aFC expressed as fraction of amount of activity in ganglion for all experimental groups. *Lines* adjacent to 2*P* values indicate subsets of groups tested against each other. C, control; AS, acute severe hypoglycemia; AM, acute moderate hypoglycemia; PS, insulin-pretreated severe hypoglycemia; PM, insulin-pretreated moderate hypoglycemia; D, untreated diabetes; D + H, diabetes with acute severe hypoglycemia.

TABLE 3
Characteristics of aFC during hypoglycemia in nondiabetic rats after labeling of glycoproteins with [^{3}H]fucose

Hypoglycemic state	*n*	Activity ($\times 10^3$ dpm) Ganglion*	Activity ($\times 10^3$ dpm) aFC*	aFC/ganglion† (%)	Plateau/ganglion‡ ($\times 10^{-3}$)	Span* (mm)	Transport velocity* (mm/h)	Lag time* (min)
Control								
None	10	142 ± 53	9.5 ± 4.3	6.6 ± 1.7	4.8 ± 1.3	14 ± 2	17.5 ± 1.3	17 ± 13
Insulin treated								
Acute								
Severe	6	153 ± 76	6.3 ± 2.4	4.4 ± 1.6	3.3 ± 1.0	15 ± 4	17.0 ± 1.2	16 ± 15
Moderate	4	253 ± 146	17.5 ± 11.3	6.6 ± 1.3	5.1 ± 1.4	12 ± 4	18.1 ± 0.5	25 ± 6
Prolonged								
Severe	6	109 ± 35	7.8 ± 3.7	6.9 ± 1.4	6.4 ± 2.0	17 ± 4	19.0 ± 2.7	36 ± 21
Moderate	4	165 ± 112	10.7 ± 3.9	6.2 ± 2.3	6.2 ± 2.3	12 ± 2	17.1 ± 0.5	22 ± 5

aFC, anterograde fast component; aFC/ganglion, fraction of activity in aFC relative to activity in ganglion; plateau/ganglion, height of plateau of wave as fraction of activity in ganglion; span, span of wave front; lag time, time between precursor injection and start of transport.
*Differences among groups not significant (NS).
†$2P < .05$ among groups.
‡$2P < .01$ among groups.

Therefore, adaptation of the energy-demanding process of axonal transport may be due to increased utilization of ketone bodies.

In our study, animals exposed to moderate hypoglycemia did not demonstrate impairment of axonal transport function in either the acute or pretreated condition. Because the dosage of insulin was similar in severe and moderate hypoglycemia, there is no indication that a direct insulin effect caused the impairment of axonal transport function.

The decrease in amount of aFC during acute severe hypoglycemia could stem from either a defect in all nerve fibers or an arrest of transport in a few fibers preceding structural breakdown. Impairment, however, is probably not the result of fiber degeneration, because ultrastructural segregation of the axon in transection experiments takes 24–48 h to develop, compared to the few hours of severe hypoglycemia used in our study. The aFC consists of membranous material that during transport and after arrival at the terminals is incorporated into the axolemma and nerve endings. It is evident that failure to supply these elements can lead to the axonal degeneration described previously (1,14).

During preliminary experiments in our laboratory it was confirmed that hypoglycemia and hypothermia are associated events. Because fast axonal transport is highly temperature dependent, care was taken to maintain normothermia in all experimental groups. With maintenance of temperature throughout the study, transport velocity, lag time from precursor injection until start of transport, and span of the wave front remained unchanged in hypoglycemia. Mendell et al. (6) claimed a decreased velocity in insulin-treated diabetic rats. No special measures to prevent changes in temperature were described in their report. Therefore, the most likely explanation of their finding is inadvertent hypothermia (7).

In our study the same kind of impairment of the axonal transport system as observed in nondiabetic animals during acute severe hypoglycemia occurred in STZ-diabetic rats in which the fast axonal transport is otherwise normal (11). Although the same degree of hypoglycemia was obtained in nondiabetic and diabetic rats, the impairment of the aFC was less severe in the diabetic rats. This partial protection of the axonal transport system during acute hypoglycemia might be due to decreased energy demands of diabetic nerves (15) or adaptation to an increased exploitation of ketone bodies (12,13). Whatever the mechanism, it appears that untreated diabetes partially protects the peripheral nerves from the damage of a hypoglycemic episode.

Nevertheless, the start of insulin treatment can precipitate

TABLE 4
Characteristics of aFC during hypoglycemia in streptozocin-diabetic rats after labeling of proteins and glycoproteins with [^{35}S]methionine and [^{3}H]fucose, respectively

Hypoglycemic state	Precursor	*n*	Activity ($\times 10^3$ dpm) Ganglion	Activity ($\times 10^3$ dpm) aFC	aFC/ganglion (%)	Plateau/ganglion ($\times 10^{-3}$)	Span (mm)	Transport velocity (mm/h)	Lag time (min)
None		9	254 ± 75	8.4 ± 2.6	3.3 ± 0.4	2.6 ± 0.3	15 ± 4	16.4 ± 1.1	8 ± 16
Acute severe	[^{35}S]methionine	9	325 ± 160	8.7 ± 4.1	2.8 ± 0.3	2.4 ± 0.4	18 ± 6	16.0 ± 1.7	7 ± 24
None		9	248 ± 102	15.0 ± 7.4	6.0 ± 1.2	4.8 ± 0.7	14 ± 4	17.1 ± 1.0	18 ± 10
Acute severe	[^{3}H]fucose	9	231 ± 134	13.2 ± 6.5	6.0 ± 1.2	5.3 ± 2.1	16 ± 4	17.0 ± 1.4	20 ± 18

aFC, anterograde fast component; aFC/ganglion, fraction of activity in aFC relative to activity in ganglion; plateau/ganglion, height of plateau of wave as fraction of activity in ganglion; span, span of wave front; lag time, time between precursor injection and start of transport. Values are means ± SD.
*$2P < .01$; all others not significant.

the development of neuropathy (16). This type of neuropathy has been termed *insulin neuritis*, and its cause has remained a mystery. A further peculiar observation concerning this disease manifestation is the improvement that occurs after continuation of insulin therapy. We propose that the development of neuropathy during initial insulin treatment is due to impaired function of the fast axonal transport system during hypoglycemia. The subsequent improvement during maintained insulin treatment could be due to adaptation of energy utilization for the axonal transport system or could represent regeneration of peripheral nerve fibers after the initial hypoglycemic insult.

Furthermore, the loss of nerve cells and fibers in the peripheral nervous system of long-term diabetes is also unexplained. Episodes of hypoglycemia, which are common in diabetic patients (17), and fluctuating levels of blood glucose might impair the fast axonal transport, leading to breakdown of axons (14) and thus contributing to the development of diabetic neuropathy.

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