MOTOR AND SENSORY CONDUCTION IN PERIPHERAL NERVES OF UNANAESTHETIZED STREPTOZOTOCIN DIABETIC AND NORMAL RATS DURING ISCHEMIA*

J. DAVID, G. P. WAGLE AND R. S. GREWAL**

CIBA-GEIGY Research Centre, Goregaon, Bombay – 400 063

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Summary : Motor nerve conduction velocities (MNCVs) of ventral peripheral tail nerves of unanaesthetized streptozotocin diabetic rats were examined in comparison to age matched normal rats under normal and ischemic conditions. A miniature blood pressure cuff was applied to the base of the tail and was adjusted to provide complete vascular occlusion for 30 min. MNCVs were recorded during ischemia and in the post-ischemic period. MNCVs were markedly reduced during ischemia in normal rats but were unchanged in diabetic rats. Conversely, the sensation of heat induced pain was retained to an equal extent in normal and diabetic rats during ischemic states. The abnormal resistance to ischemia of MNCV of peripheral nerves is an early and sensitive index of nerve dysfunction and precedes slowing of MNCV in diabetic rats. The results suggest that initial peripheral nerve abnormalities in diabetic rats may be related to biochemical changes rather than axonopathy and may have heuristic significance for clinical diabetic neuropathy.

Key words : streptozotocin diabetes, nerve conduction velocity, motor nerve, sensory nerve, sensory threshold, thermal pain, ischemia

INTRODUCTION

Abnormal preservation of nerve function during ischemic states has been shown to occur in diabetic subjects (5, 9). Steiness (9) was the first to show that virtually all patients with diabetes mellitus had better retention of vibratory sensation during ischemia than did normal subjects. Gregersen (5) demonstrated that the average fall in motor nerve conduction velocity (MNCV) during ischemia, was significantly larger in normal individuals

* Contribution No. 660 from the CIBA-GEIGY Research Cente, Bombay.

** Present address ; CIBA-GEIGY Ag., Klybeckstrasse, Basel, Switzerland.

than in diabetics. In contrast, he found that heat-induced pain remained unchanged during ischemia in both diabetic subjects and normal volunteers.

In vitro aspects of isolated sciatic nerve function under hypoxic conditions have been examined in normal and diabetic rats (8). These studies confirmed that the peripheral nerves of alloxan diabetic rats were more resistant to inactivation by hypoxia than nerves from healthy rats. In order to determine if *in vitro* studies on hypoxic nerves (reported by Seneviratne and Peiris) (8) would apply to *in vivo* studies, we investigated motor and sensory aspects of peripheral tail nerve function during a state of arrested blood flow, in unanaesthetized and age matched streptozotocin diabetic and normal rats (3). We have previously demonstrated that the earliest, detectable significant slowing of tail nerve MNCV occurred six weeks after induction of streptozotocin diabetes (4). In this study we used rats one week after streptozotocin treatment with blood glucose levels above 300 *mg/dl*, but showing no significant difference in tail nerve MNCV when compared to age matched controls. A further objective of this study was to determine if abnormal resistance to ischemia preceded slowing of MNCV in diabetic rats.

MATERIALS AND METHODS

Nine week old CF female rats, 180 to 200 g, were divided into normal and streptozotocin (50 *mg/kg*, iv) groups. One week post-streptozotocin rats with a nonfasting blood glucose concentration in excess of 300 *mg/dl* and age matched normal rats were employed. Blood samples were obtained from the right orbital sinus and blood glucose levels were determined with the Autoanalyzer using the Hoffman method. Estimations were done on each animal just prior to determination of MNCV. *In vivo* serial MNCV measurements were done on the ventral peripheral tail nerves of conscious diabetic and normal rats, according to a procedure described in detail previously (4).

Blood pressure measurements and arrest of blood flow were done on the rat tail using a plethysmographic method (1). The occlusion pressure of 300 mm Hg, which was well above the systolic pressure, was maintained by means of a miniature blood pressure cuff applied to the root of the tail at a self retaining pressure device for a 30 min period. The equipment employed for arresting the blood flow and for recording the MNCV is shown in detail in Fig. 1. The same netural loci were stimulated throughout the experiment. Amplitude of action potentials and MNCV's were measured before, during and after ische-

mia. During occlusion, recordings were taken at 5, 15 and 30 min. Post-ischemic MNCV's were recorded at 10, 15 and 30 min, after release of cuff pressure.

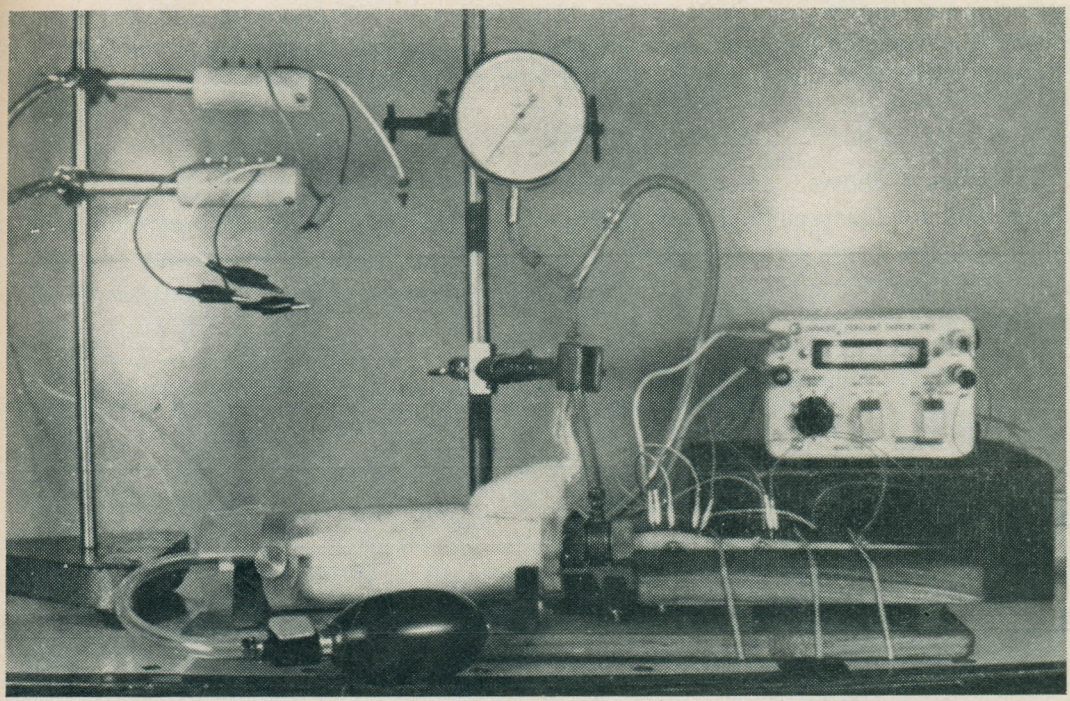

Fig. 1 : Unanaesthetized rat was placed supine in plexiglass holder with apertures for both legs. The ventral surface of the tail faced upwards and the tail was fastened to the board with rubber bands. The plethysmographic device (width=35 mm) was placed on the root of the tail and pressure was increased to 300 mm Hg (registered on the gauge) and maintained with the self retaining bulb. Stainless steel electrodes inserted into the ventral nerve are from left to right : 1,2 stimulating electrodes: 3, ground; 4,5 and 6,7 electrodes for recording of first and second action potentials. On the extreme left are the cathode followers (connection to electrodes not shown) and extreme right the constant current unit (Grass CCUIA) for recording stimulating current in milliamperes.

The tail-flick method (2) was used for testing the nociceptive response to a heat-induced stimulus. The response time, defined as the interval between the onset of the stimulus and the tail flick was measured electronically (to the nearest 0.1 sec). The radiant heat intensity was set at a level giving a mean control reaction time of 3 to 4 sec in saline treated controls. Rats were restrained in plexiglass tubes from which their tails extended (Fig. 1). Baseline latencies to tail-flick withdrawal from the radiant heat source were averages of the first three pretreatment trials (trials were done at intervals of 20 min) in unoccluded normal (n=210) and diabetic rats(n=146).

One week post-streptozotocin diabetic rats and age matched normal rats were selected for application of the blood pressure cuff to the base of the tail. Tail flick latencies (in seconds) were measured before and during occlusion at 5, 15 and 30 min. Post-occlusion latencies were recorded at 10, 15 and 30 min. To prevent tissue damage to the tail, the heat source was automatically cut off at 10 sec. Room temperature was maintained at 24°C for measurements of MNCV and tail flick latency.

RESULTS

Table I shows comparative blood pressure (BP) and blood glucose values for normal and streptozotocin diabetic rats, 10 to 57 weeks of age. The BP and blood glucose values of diabetic rats were significantly higher than controls at all age groups. An occlusion pressure of 300 mm Hg was employed which was well above the systolic BP of 10 week old diabetic rats.

TABLE I : Sequential values for blood pressure and blood glucose in normal and streptozotocin diabetic rats (streptozotocin, 50 *mg/kg* iv was given when rats were 9 weeks old).

Age in weeks	*Blood pressure (mm Hg)*			*Blood glucose (mg/dl)*		
	Normal (*n*=6)	*Diabetic* (*n*=5)	2*P*	*Normal* (n=10)	*Diabetic* (*n*=5)	2*P*
10	79±7.2	100±9.8	<0.2	108±2.4	381±1.4	<0.001
13	80±8.4	110±7.7	<0.05	107±2.1	385±14	<0.001
17	80±7.2	120±1.9	<0.001	102±2	425±34	<0.001
37	91±4.7	133±7.7	<0.005	106±2	378±17	<0.001
57	93±8.2	130±6.7	<0.01	93±2	401±13	<0.001

Values are mean ± SEM

Effect of ischemia on heat-induced pain response (tail-flick response).:

The mean latency to tail-flick in unoccluded, normal rats (n=210) was 4.09±0.27 sec and in unoccluded diabetic rats (n=146) was 4.20 ± 0.24 sec ($\bar{x}$ of 3 responses at 20 min intervals). Thus, the sensory threshold to a thermal stimulus was not significantly different in normal and diabetic rats under conditions of normal blood flow.

Table II-A shows the effect of ischemia on the heat induced pain response (as measured by the tail flick latency time) in age matched normal and diabetic rats. In normal rats, tail flick latencies were significantly reduced from a pre-occlusion value of 4.1±0.07 to 3.6±0.19 sec ($P<0.05$) and 3.7±0.05 sec ($P<0.005$) at 5 and 15 min following occlusion. No significant differences occurred thereafter. In contrast, in diabetic rats

TABLE II : Effect of ischemia on heat-induced pain response in 10 week old normal and diabetic* rats (tail flick latency in sec).

(A)

	Pre-occlusion	*During occlusion (min)*			*Post-occlusion (min)*		
		5	15	30	10	15	30
Normal rats (n=4)	4.1 ±0.07	3.6 ±0.19	3.7 ±0.05	4.2 ±0.1	4.1 ±0.32	4.2 ±0.34	4.0 ±0.1
*P		<0.05	<0.005	NS	NS	NS	NS
Diabetic rats (n=10)	4.1 ±0.12	5.1 ±0.48	4.4 ±0.21	4.4 ±0.31	4.2 ±0.27	4.2 ±0.23	4.2 ±0.39
*P		0.05	NS	NS	NS	NS	NS

(B)

Normal vs diabetic rats

P (significances are column wise)	NS	<0.02	<0.01	NS	NS	NS	NS

Values are $\bar{X}$ ±SEM. Significances are two tailed (Student's t test).
NS=Not significant.

*One week post streptozotocin.
*Significances are with respect to pre-occlusion values.

tail flick latency was elevated from a pre-occlusion value of 4.1±0.12 to 5.1±0.48 sec ($P<0.05$) at 5 min following occlusion, with no subsequent significant changes. During initial ischemic periods, normal rats experienced a lowering of sensory thresholds (hyperalgesia) whereas diabetic rats had a transient elevation of sensory threshold for 5 min, but essentially, retained the nociceptive response throughout ischemia.

When tail flick latencies were compared in normal and diabetic rats (Table II-B) the diabetic rats had significantly elevated latencies at 5 and 15 min following occlusion.

Effect of ischemia on MNCV and amplitude of the action potential in ventral tail nerves :

Fig. 2 shows sequential MNCV and stimulus threshold values in unanaesthetized, non-fasted normal and diabetic rats with normal blood flow to the tail. In the diabetic

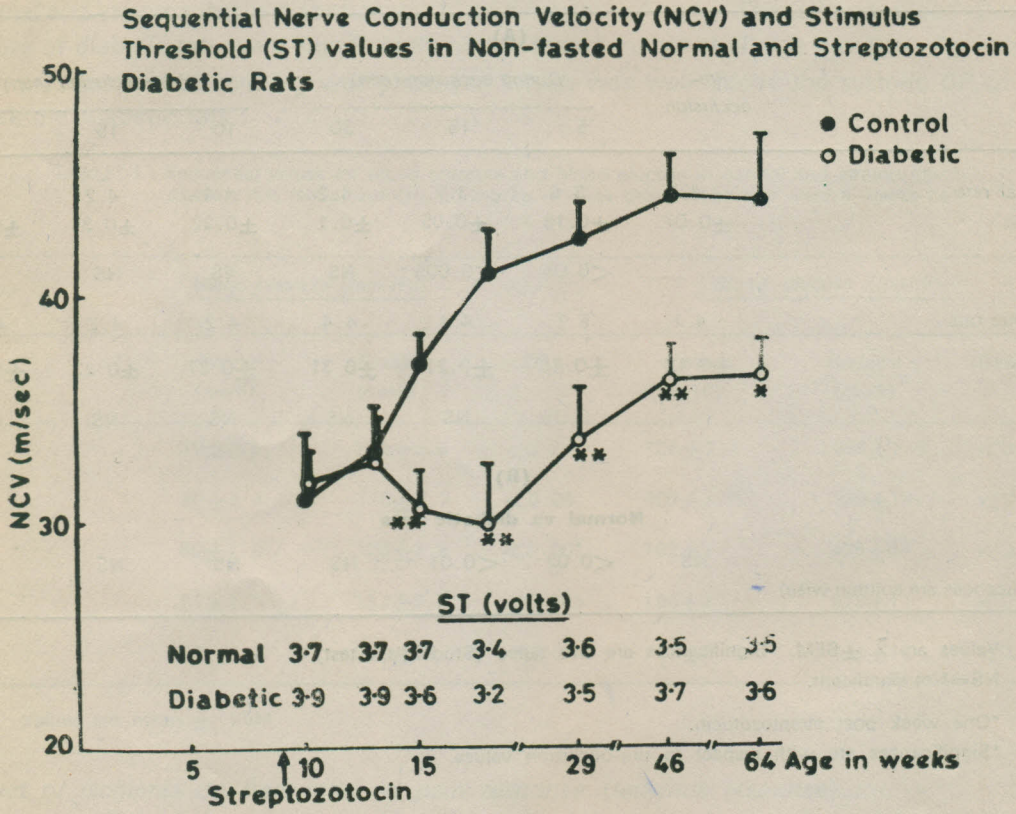

Fig. 2 : Sequential NCV in non-fasted normal and streptozotocin diabetic rats. Streptozotocin 50 *mg/kg* iv, was given at 9 weeks of age to the diabetic rats (at arrow). In these experiments, five stainless steel stimulating electrodes (2 mm interelectrode distance) were placed 30 mm caudal to the anal aperture, and inserted into the ventral nerve at the base of the tail. Two pairs of recording electrodes were also inserted 20 and 60 mm distal to the stimulating electrodes. Control rats (n=12), diabetic rats (n=8). Values are $\bar{x} \pm$SEM. Significances are with respect to controls of the same age. $^{*}P<0.05$, $^{**}P<0.01$. ST = Stimulus threshold in volts.

group, streptozotocin, 50 *mg/kg* iv, was given at 9 weeks of age (see arrow). A steady increase in MNCV up to 46 weeks was observed in normal healthy controls. On the other hand, diabetic rats showed a significantly reduced MNCV with respect to normal controls from 15 to 64 weeks.

Fig. 3 shows the effect of arrested blood flow on MNCV in the ventral tail nerves of normal and diabetic rats, (one week post-streptozotocin). There were no significant differences in the MNCV of normal and diabetic rats before occlusion and at 5 and 15 min post-occlusion. 30 mins following occlusion, the MNCV of normal rats was highly

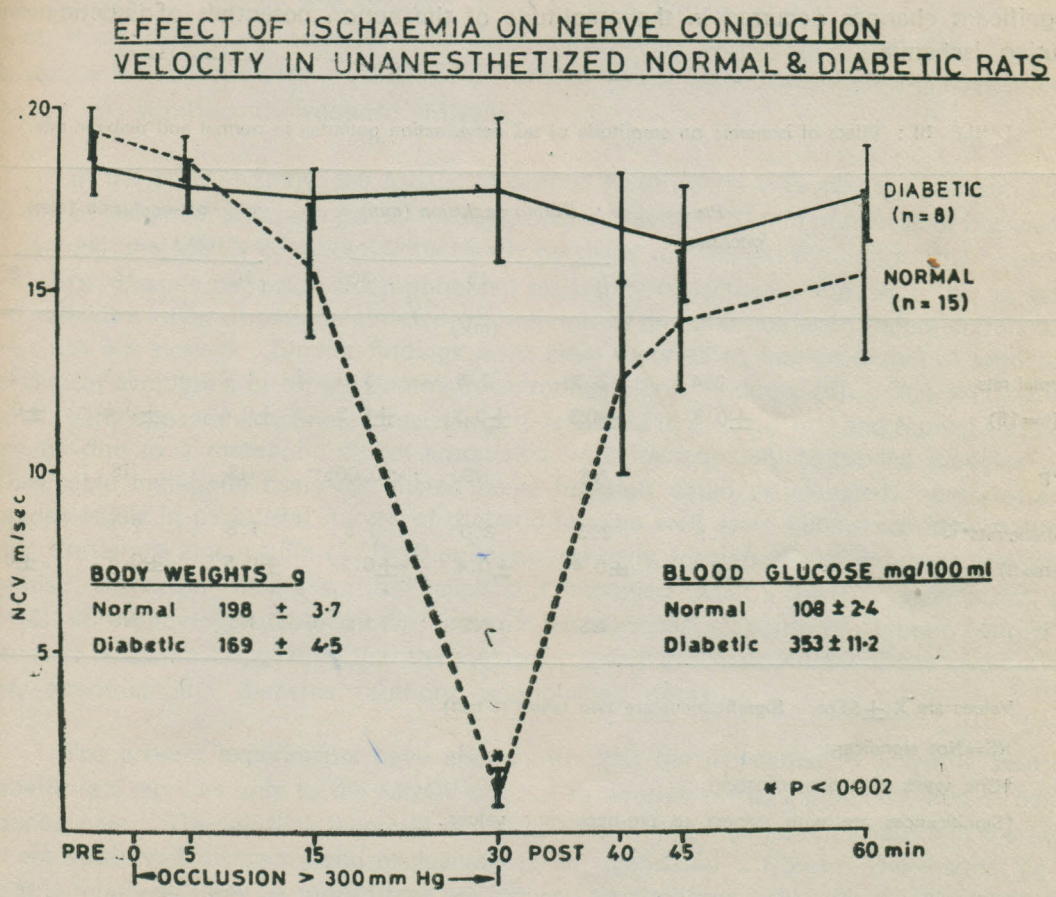

Fig. 3 : Effect of ischemia on MNCV in 10 week old unanaesthetized normal and diabetic rats (post-streptozotocin, 1 week). At post-occlusion 30 min, MNCV of normal rats is highly significantly decreased with respect to diabetic rats ($P<0.002$).

significantly decreased with respect to diabetic rats ($P<0.002$). 10 min after release of the occlusion, MNCV's of both normal and diabetic rats were not significantly different.

The MNCV of diabetic rats remained unaltered throughout the duration of ischemia, whereas the 60 min post-ischemic MNCV of normal rats, 15.4±2,26 m/sec, was less than the pre-occlusion value of 19.3±0.68 m/sec (not significant).

Table III shows that the amplitude of the action potential in normal rats was significantly decreased 30 min after ischemia, with respect to pre-occlusion values. No significant changes occurred in the amplitude of the action potentials of diabetic nerves during ischemia.

TABLE III : Effect of ischemia on amplitude of tail nerve action potential in normal and diabetic rats.

	Pre-occlusion	*During occlusion (min)*			*Post-occlusion (min)*		
		5	15	30	10	15	30
			(*mV*)				
Normal rats	2.4	2.3	1.8	1.0	1.7	2.1	2.1
(n=15)	±0.3	±0.3	±0.3	±0.3	±0.4	±0.5	±0.5
*P		NS	NS	P<0.005**	NS	NS	NS
Diabetic rats†	2.5	2.3	2.0	1.5	1.8	2.1	2.4
(n=9)	±0.5	±0.4	±0.4	±0.3	±0.5	±0.6	±0.6
*P		NS	NS	NS	NS	NS	NS

Values are $\bar{X}$ ±SEM. Significances are two tailed (t test).

NS=Not significant.

†One week post streptozotocin.

*Significances are with respect to pre-occulusion values..

DISCUSSION

The results show that the MNCV and amplitude of action potential of tail nerves of diabetic rats show an abnormal resistance to ischemia, even before electrophysiologic evidence of significantly decreased MNCV could be demonstrated.

It is evident that the motor nerve conduction velocity (MNCV)of peripheral nerves of diabetic rats undergo changes during ischemia that are very different from nerves of normal rats. Conversely, no difference in heat-induced pain perception during ischemia was observed in normal and diabetic rats. From a functional and physiological viewpoint, these results are significant as they conclusively demonstrate that the abnormal resistance to ischemia seen in diabetic peripheral nerves precedes the electrophysiological change of significantly reduced MNCV.

In the present study, the MNCV of normal healthy rats was almost abolished after 30 min of ischemia, although partial recovery occurred within 10 min of release of occlusion. In contrast, the MNCV of diabetic rats clearly persisted unchanged throughout the ischemic and post-ischemic periods. Such abnormal resistance to ischemia appears to be an early and sensitive index of peripheral nerve dysfunction in diabetic rats even before conduction velocities are slowed. Similar findings have been reported in human diabetics who had no clinical symptoms or other abnormal electrophysiologic findings (6). One explanation for this early appearance of neural resistance to ischemia in diabetic rats and human subjects may be due to a metabolic defect associated with inadequately controlled diabetes. A conceivable metabolic basis for altered nerve function could be elevated sorbitol and fructose levels in peripheral nerves of diabetic rats, as well as in human autopsy material (10). Protamine zinc insulin (PZI) 10 units, s.c./rat daily, for two weeks inducedn ormoglycaemia, decreased neural sorbitol levels and improved MNCV (4,10). Administration of PZI, 48h following streptozotocin, prevents the abnormal response of diabetic peripheral nerves to ischemia, suggesting that the metabolic defect precedes structural changes during early streptozotocin diabetes (authors, unpublished data).

The present experiments have also shown that the resistance to ischemia seen in diabetic rats, applies only to the MNCV but not to another sensory modality, that of heat-induced pain. The tail flick response is a spinal reflex and thermal nociceptors in the rat's tail are subserved discreetly and exclusively by unmyelinated C fibres. The thermal threshold is relatively high, as these fibres are induced to discharge only with skin temperatures of 40°–45°C. Thermal nociceptors show little adaptation in contrast to low threshold

mechanical receptors. Heat-induced pain persisted during ischemia in both diabetic as well as normal rats. Unmyelinated fibres are most resistant to anoxia and hence preservation of function under ischemic conditions is possible. Jakobsen (7) has shown that the number of unmyelinated fibres, mean fibre size and calibre spectra in peripheral nerves of normal and diabetic rats are not significantly different, although the smooth endoplasmic reticulum was decreased by 40% in diabetic rats. These structural findings may account, in part, for the lack of differentiation in pain responses during ischemia in diabetic and normal rats. Gregersen (5) showed that heat-induced pain perception remained unchanged during ischemia in diabetics and normal people, similar to our findings in experimental animals. These findings on ischemia and nerve conduction in diabetic rats may have relevance in the early diagnosis and treatment of diabetic neuropathy.

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