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Ultrastructural changes in the hypothalamic supraoptic nucleus of the streptozotocin-induced diabetic rat

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ABSTRACT

Ultrastructural and morphometric studies were undertaken on the hypothalamic supraoptic nucleus of streptozotocin-induced diabetic rats over a period of 1 y. At 3 d, a few dendrites showing electron-dense cytoplasm and dilated rER were dispersed in the neuropil among seemingly normal neuronal somata. At 1–6 months, the somata contained numerous vacuoles of various sizes which probably originated from fragmented and dilated rER. Numerous unidentifiable vacuolated and electron-dense neuronal profiles were also seen in the neuropil. At 9–12 months, the number of degenerating electron-dense axon terminals and dendrites was markedly increased in diabetic rats. Glial cells containing electron-dense debris in their cytoplasm were involved in phagocytosis. At all time intervals studied, the mean cross-sectional cell area and mean cross-sectional nuclear area of supraoptic nuclei neurons of diabetic rats were significantly increased in comparison with age-matched controls injected with normal saline. The causative factors for these changes are not clear. However, it is suggested that the osmotic stress caused by chronic dehydration in the diabetic animals may be partly or wholly responsible for these ultrastructural changes.

Key words: Diabetes mellitus; neurodegeneration; osmotic stress.

INTRODUCTION

In recent years, there have been numerous investigations into changes in the central nervous system (CNS) in experimentally induced diabetes. DeJong (1950) was the first to suggest that the CNS was involved in severe diabetes mellitus. Later Reske-Nielsen & Lundbaek (1964) and Olsson et al. (1968) gave detailed descriptions of CNS lesions in diabetes, especially the perivascular changes. Luse et al. (1970) demonstrated that the cerebral cortex in early onset diabetic Chinese hamsters showed neuronal changes as evidenced by enlarged mitochondria in dendrites, dense fibrillar axoplasm, focal myelin degradation and residual space after loss of degenerated neurite processes. Recently neuroaxonal and dendritic dystrophic changes have been described in the autonomic nervous system of BB rats (Yagihashi & Sima, 1986). Ultrastructural changes in the gracile nucleus and nucleus ventralis posterolateralis have been studied in alloxan and streptozotocin-induced diabetic rats as well as in spontaneously diabetic BB rats (Tay & Wong, 1991*a*,*b*, 1992). In these studies, degeneration of neuronal profiles was characterised by electron-dense cytoplasm, swollen mitochondria and dilated endoplasmic reticulum (ER) in the dendrites as well as electron-dense axoplasm, vacuolation, swollen mitochondria and clumping of synaptic vesicles in the axon terminals. Any toxic effect of streptozotocin on the PNS (Jakobsen, 1979; Schmidt et al. 1983), CNS (Tay & Wong, 1991*b*) and sympathetic ganglia (Monckton & Pehowich, 1982) has been excluded.

The morphology of various hypothalamic nuclei has also been studied in chemically induced diabetic animals. Swollen neuronal processes, accumulation of glycogen, hypotrophy of tanycytes, axonal changes, dilated and fragmented ER, increased number of microtubules and loss of organelles have been reported in the median eminence and arcuate nucleus of male rats 1 y after streptozotocin treatment (Bestetti & Rossi, 1980; Rossi & Bestetti, 1981). Bestetti et al. (1987) described reduced cytoplasmic and nuclear areas in the neurons of the preoptic-

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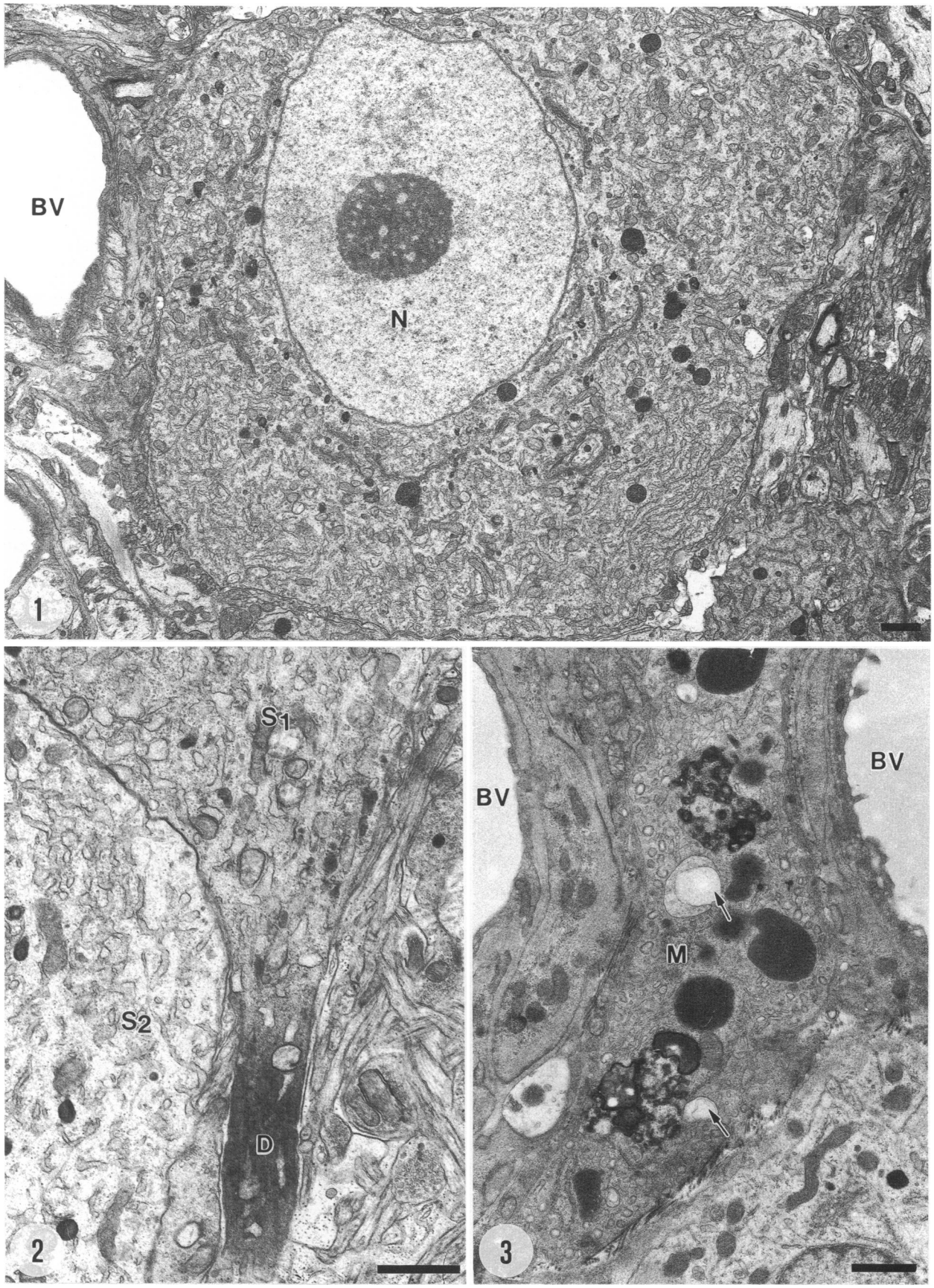


Fig. 1. Magnocellular neuron in the supraoptic nucleus. Note the pale spherical nucleus (N) with prominent centrally placed nucleolus. Electron-dense lysosomes, mitochondria, a well developed Golgi complex, rough endoplasmic reticulum and neurosecretory granules are distributed throughout the cytoplasm. BV, blood vessel. Control rat, 1 y after saline injection. Bar, 1 μm.

suprachiasmatic nuclei of streptozotocin-induced diabetic male rats. Lincoln et al. (1989) described the ultrastructural changes in the paraventricular and supraoptic nuclei (PVN and SON) as well as in the neurohypophysis of male rats 8 wk after streptozotocin-induced diabetes. However, their report did not show any morphological changes in rats after 3–12 months of diabetes. Therefore the present study was undertaken to ascertain the qualitative and quantitative structural changes in the hypothalamic SON of streptozotocin-induced diabetic rats at survival periods ranging from 3 d to 12 months of diabetes.

MATERIALS AND METHODS

Animals

Forty-eight male Wistar rats (200–250 g) were used for the present study. Rats were divided into 6 groups, each group consisting of 4 streptozotocin-injected rats and 4 controls injected with normal saline. The rats were housed in clean cages in a temperature-controlled room with a 14 h light and 10 h dark schedule and fed with rat chow and water ad libitum.

Induction of diabetes

Diabetes was induced under aseptic conditions. Under light ether anaesthesia, each rat was given a single i.v. injection of either streptozotocin (60 mg kg^{-1} body weight, freshly dissolved in 0.01 M citrate buffer, pH 4.5) or normal saline (0.9% sodium chloride) via the right external jugular vein, using a 1 ml syringe. Blood samples were collected by cutting the tip of the tail and blood glucose was measured using a blood glucometer (Ames Glucometer II) before induction, 24 h after induction and immediately before killing. Rats were considered diabetic when blood glucose concentrations exceeded 22 mmol l^{-1}.

Perfusion and electron microscopy techniques

Groups of 4 experimental and 4 control rats were perfused at intervals of 3 d and 1, 3, 6, 9 and 12 months postinduction. After the relevant survival period, each rat was anaesthetised with 1–1.5 ml of chloral hydrate (70 mg ml^{-1}). Before perfusion, tracheostomy was performed and artificial ventilation maintained with a Harvard rodent ventilator (model 683). 5 min before perfusion, 1000 units of heparin and 1 ml sodium nitrite per kg body weight were given by intracardiac injection. Each animal was then perfused through the left ventricle with 100 ml of Ringer's solution (pH 7.4) followed by 500 ml of fresh fixative (2% formaldehyde + 3% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4). The brains were removed immediately and immersed in the same fixative for 4 h at 4 °C. The brains were transferred into ice-cold 0.1 M cacodylate buffer (pH 7.4) containing 5% sucrose and stored at 4 °C overnight.

The following day, coronal sections (80 μm) were cut through the hypothalamus on an Oxford vibratome and collected in 0.1 M cacodylate buffer (pH 7.4). The sections were postfixed in 1% osmium tetroxide for 1 h, dehydrated in a graded ethanol series, flat-embedded in Araldite mixture and polymerised at 60 °C for 24 h. Areas containing the SON were trimmed out using a binocular microscope from the embedded-sections and mounted on Araldite filled capsules for ultrathin sectioning. The ultrathin sections were cut with a Reichert OMU4 ultramicrotome, doubly stained with uranyl acetate and lead citrate and examined in a Philips 400T electron microscope.

Morphometric techniques

Semithin sections (1 μm) from some of the tissue blocks containing SON were prepared using a Reichert OMU4 ultramicrotome. The sections were stained with 1% methylene blue. They were then viewed and analysed under an inverted microscope (Olympus IMT-2) which was linked to a Jandel video analysis system (JAVA software, version 1.20). 200 nucleated neurons containing nucleoli both in the control and diabetic rats of each group (3 d, 1, 3, 6, 9 and 12 months) were chosen randomly. The somata and nuclei were outlined and cross-sectional areas of the cells and nuclei were measured. Mean values were taken in all the measurements.

For the quantitation of abnormal neuronal profiles (axons, dendrites and somata) both in the control and diabetic rats at 3 d and 1, 3, 6, 9 and 12 months after streptozotocin treatment, the following procedure was followed. Ultrathin sections from tissue blocks containing SON were prepared for electron microscopy

Fig. 2. Electron-dense dendrite (D) projects from the soma (S1) of a magnocellular neuron. Note the dilated rER in the cytoplasm. S2, soma of an adjacent neuron. 3 d postdiabetes. Bar, 1 μm.

Fig. 3. Perivascular macrophage (M) containing degenerating electron-dense debris and vacuoles (arrows) in its cytoplasm. BV, blood vessels. 3 d postdiabetes. Bar, 1 μm.

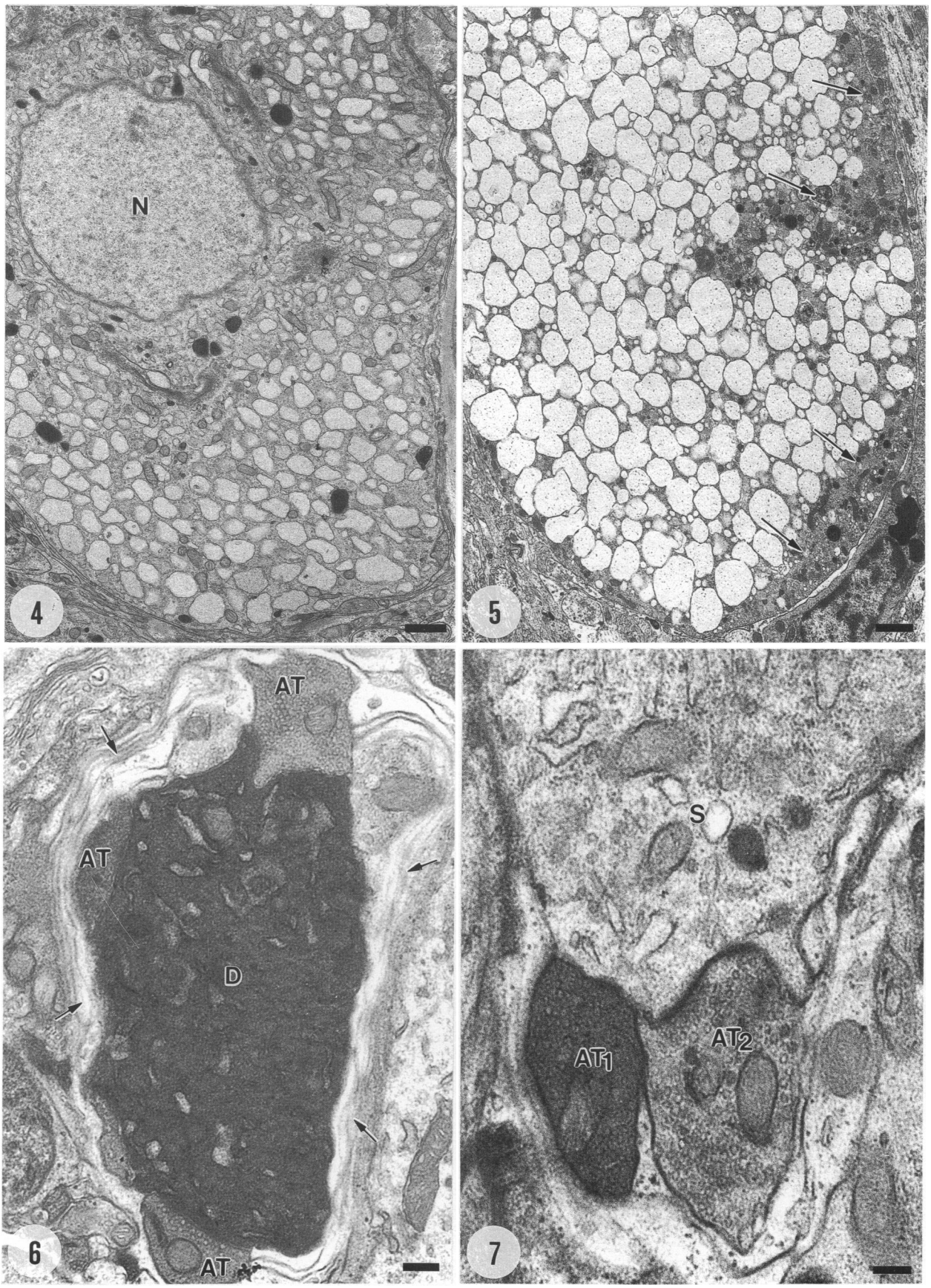


Fig. 4. Neuronal soma containing numerous vacuoles probably formed from fragmented and dilated rER. Note the intact nucleus (N), well preserved perinuclear Golgi saccules, lysosomes, mitochondria and neurosecretory granules in its cytoplasm. 3 months postdiabetes. Bar, 1 μm.

and then viewed under a Philips 400T electron microscope at ×5000 magnification. Abnormal neuronal profiles from a total of 10 random samples of the SON from each animal were counted and then analysed together. The frequency of abnormal axons and dendrites were calculated and expressed as per 1000 axons and dendrites respectively. The frequency of abnormal somata was expressed as per 100 nucleated somata. 4 rats from controls and diabetics at each time interval were studied.

The data obtained were expressed as means (derived by averaging the mean values of 4 animals) ± standard deviation, and then subjected to statistical analysis using Student's t test.

RESULTS

Streptozotocin-injected rats developed diabetes mellitus within 24 h after induction, with a blood glucose level exceeding 22 mmol l^{-1}. Most of the diabetic rats developed cataracts after 6 months. All animals showed hyperglycaemia (22 mmol l^{-1} and above) at the time of killing.

Qualitative analysis

Control animals. In semithin sections stained with methylene blue, the SON appeared as a compact mass of magnocellular neurons lying on each side of the optic chiasma. Under the electron microscope each magnocellular neuron had a round or oval nucleus with a prominent nucleolus (Fig. 1). The cytoplasm displayed a well developed Golgi apparatus, widely distributed cisternae of rough endoplasmic reticulum (rER), mitochondria, lysosomes, neurotubules and some neurosecretory granules. Axon terminals containing small spherical agranular vesicles made synaptic contacts with neuronal somata and dendrites as well as other axon terminals. Dendrites were characterised by the presence of microtubules, ER and mitochondria in their pale cytoplasm.

Experimental animals

3 d postdiabetes. At 3 d, a few neuronal profiles were affected. Some dendrites emerging from seemingly normal neuronal somata contained dilated rER in their electron-dense cytoplasm (Fig. 2). However, most of the other cytoplasmic organelles in the somata appeared normal. Perivascular macrophages containing vacuoles and electron-dense debris in their cytoplasm were also present in the neuropil (Fig. 3).

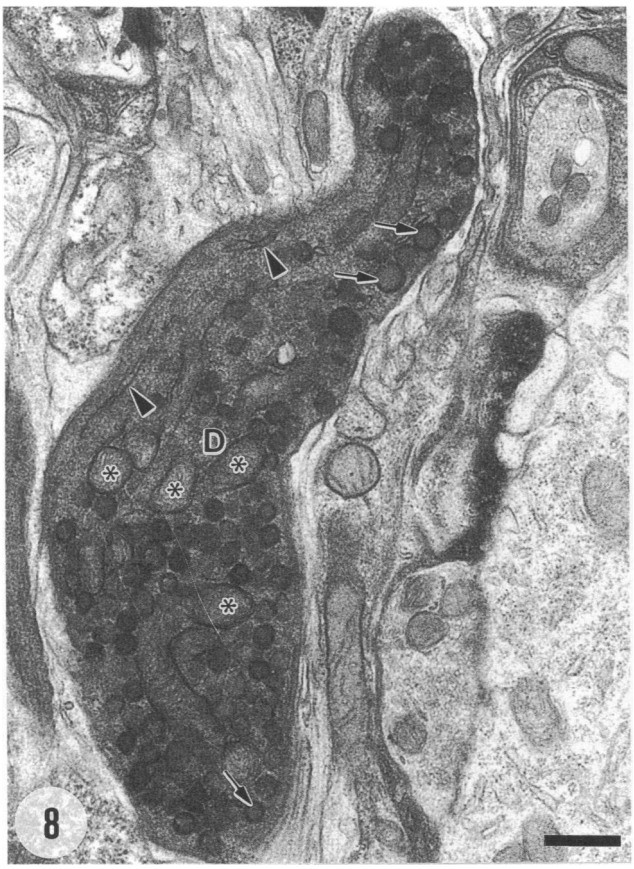
1–6 months postdiabetes. During these survival periods, the hypertrophied somata contained numerous electron-lucent vacuoles of various sizes which appeared to originate from fragmented and dilated rER (Figs 4, 5). The affected somata were characterised by the presence of a spherical nucleus, well preserved paranuclear Golgi saccules, lysosomes and mitochondria in their vacuolated cytoplasm (Fig. 4). In severely affected somata, the vacuoles occupied most of the cytoplasmic area with the cell organelles pushed to the periphery (Fig. 5). The large vacuoles appeared to consist of dilated rER cisternae that had anastomosed with each other. Numerous unidentifiable highly vacuolated and electron-dense profiles were also present in the neuropil. Macrophages with ingested material could also be seen in the neuropil and most of them were in the process of digesting engulfed materials.

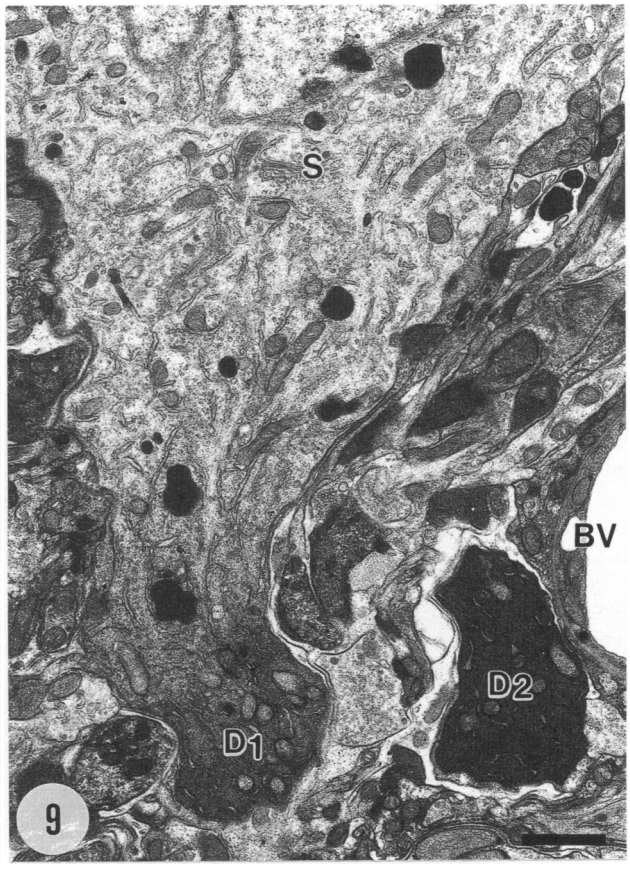
9–12 months postdiabetes. During these survival periods, a significant wave of degeneration was observed in the neuropil of the SON. The electron-dense axon terminals and dendrites were readily identified in the neuropil (Figs 6–9). Abnormal axon terminals were characterised by an electron-dense granular axoplasm containing clustered small spherical agranular vesicles and swollen mitochondria (Figs 6, 7). Some of these abnormal axon terminals were presynaptic to abnormal dendrites (Fig. 6) as well as seemingly normal somata (Fig. 7). Abnormal dendrites were characterised by an electron-dense granular cytoplasm containing swollen mitochondria and dilated ER (Figs 6, 8, 9) and were postsynaptic to normal as well as abnormal axon terminals (Fig. 6). Some abnormal neuronal profiles containing mitochondria, ER and swollen neurosecretory granules in their electron-dense granular cytoplasm appeared to be neurosecretory dendrites (Fig. 8). The structural nature of the dense material in the abnormal neuronal profiles is difficult to define even at higher magnifi-

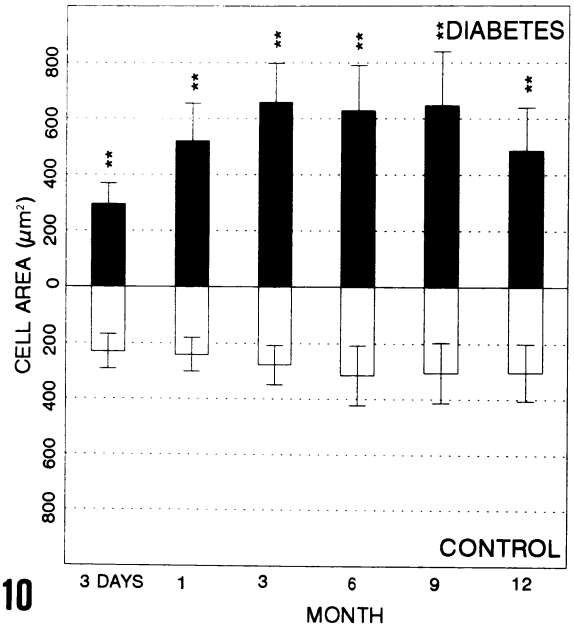
Fig. 5. Extremely vacuolated neuronal soma. The cytoplasmic organelles are pushed to the periphery of the soma (arrows). 3 months postdiabetes. Bar, 1 μm.

Fig. 6. An abnormal dendrite (D) is characterised by an electron-dense cytoplasm containing dilated ER. This dendrite is postsynaptic to several degenerating electron-dense axon terminals (AT) and is enveloped by glial processes (arrows). 1 y postdiabetes. Bar, 0.2 μm.

Fig. 7. An abnormal electron-dense axon terminal (AT1) and a seemingly normal axon terminal (AT2) are presynaptic to a neuronal soma (S). 1 y postdiabetes. Bar, 0.2 μm.







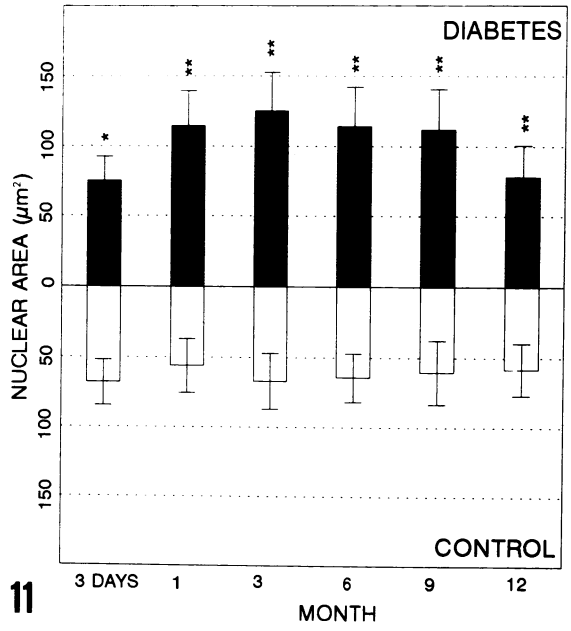


Fig. 8. An abnormal electron-dense neurosecretory profile (D) containing mitochondria (asterisk), ER (arrowheads) and numerous swollen neurosecretory granules (arrows) appears to be a dendrite. 1 y postdiabetes. Bar, 0.5 μm.

Fig. 9. An abnormal dendrite (D1) emanates from a seemingly normal soma (S). Another abnormal electron-dense dendrite (D2) is in close proximity to D1 and a blood vessel (BV). Note the presence of several other electron-dense degenerating neuronal profiles in the neuropil. 1 y postdiabetes. Bar, 1 μm.

Fig. 10. Differences in cell size (mean cross-sectional area in μm^2) of SON neurons in the control and diabetic rats at different time intervals (3 d, 1, 3, 6, 9, 12 months). Each value is mean ± S.D. Control vs diabetes: ** $P < 0.001$.

Fig. 11. Mean cross-sectional nuclear area (μm^2) of SON neurons in control and diabetic rats at different time intervals (3 d, 1, 3, 6, 9, 12 months). Each value is mean ± S.D. Control vs diabetic rats: * $P < 0.001$; ** $P < 0.001$.

Table. *Number of abnormal neuronal profiles (mean ± S.D.) in the SON of diabetic and control rats*

Survival period	Abnormal axonal profiles (per 1000 axons)	Abnormal dendrites (per 1000 dendrites)	Abnormal somata (per 100 nucleated somata)
3 d			
Control	0	0	0
Diabetic	1.3±0.4**	1.5±1.1**	0
1 mth			
Control	0	0	0
Diabetic	0	0	2+1*
3 mths			
Control	0	0	0
Diabetic	5±1**	0	5±1*
6 mths			
Control	2±1	0	1±1
Diabetic	8±1**	0	5±2*
9 mths			
Control	12±3	21±8	2±1
Diabetic	37±9*	77±8*	4±1
12 mths			
Control	28±6	49±7	2±1
Diabetic	158±22**	329±37**	3±1

Each value is mean ± S.D.
Control vs diabetic, * $P < 0.01$; ** $P < 0.001$. There were 4 control and 4 diabetic rats for each survival period.

cation since these profiles show an increased electron density of the background matrix of the cytoplasm. Vacuolated neuronal somata have also been encountered in these survival periods, but in smaller numbers. Macrophages containing electron-dense debris in their cytoplasm were also present.

Morphometric analysis. The morphometric study revealed significant increases in cross-sectional cell area (Fig. 10) and cross-sectional nuclear area (Fig. 11) of the SON at 3 d and 1, 3, 6, 9 and 12 months after streptozotocin treatment. From the histograms it was evident that at all the time intervals studied, the SON neurons of diabetic rats were markedly hypertrophied in comparison with age-matched controls injected with normal saline.

Quantitation of abnormal neuronal profiles

Abnormal somata were characterised by the presence of electron-lucent vacuoles throughout the cytoplasm and swollen cellular organelles in electron-dense cytoplasm. Although numerous unidentifiable highly vacuolated profiles were observed in the diabetic rats, only nucleated neurons were counted. Abnormal axonal profiles were characterised by electron-dense axoplasm containing clustered agranular vesicles, swollen mitochondria and swollen neurosecretory granules. Abnormal dendrites were characterised by electron-dense cytoplasm, swollen mitochondria and dilated ER. The numbers of abnormal neuronal profiles of both control and diabetic rats are shown in the Table. The quantitative data showed the significant increase of abnormal axonal profiles at 3 d and 3–12 months of diabetes, abnormal dendrites at 3 d and 9 and 12 months of diabetes and abnormal somata at 1–12 months of diabetes in comparison with age-matched controls injected with normal saline. There was also an increase in the number of abnormal neuronal profiles in the controls after 6 months. However, the increase of abnormal neuronal profiles in the diabetic rats is clearly greater than in the controls at all the time intervals studied.

DISCUSSION

In the present study, diabetes was induced in male Wistar rats using streptozotocin which selectively destroys the pancreatic β cells (Junod et al. 1967; Dulin & Soret, 1978). Various degrees of ultrastructural changes in the axonal profiles, dendrites and neuronal somata of the SON of diabetic rats were observed at 3 d and 1–12 months after diabetes induction. The ultrastructural lesions observed in the present study are considered to be a consequence of streptozotocin-induced diabetes since a toxic effect of streptozotocin on the central nervous system has been excluded (Tay & Wong, 1991*b*). Moreover, some of these lesions are believed to be an indication of neuronal degradation since degenerate material was being removed by macrophages.

The vacuolated neuronal profiles observed in the present study are comparable with those obtained by Lincoln et al. (1989) after 8 wk of diabetes. However, the degenerating electron-dense neuronal profiles observed in the present study at 3 d and 3–12 months of diabetes have not been reported previously. At all time intervals studied, the neuronal populations show significant increases in cross-sectional soma and nuclear areas in comparison with age-matched control rats. Similar hypertrophy of somata has been observed in the SON of diabetes insipidus rats (Brattleboro strain) (Sokol & Valtin, 1965) and NaCl administered rats (Hillarp, 1949). Sokol & Valtin (1965) suggested that the cellular hypertrophy probably reflects increased protein synthesis as well as greater electrical activity. Increment of nuclear area has also been reported in these neurosecretory neurons during pregnancy and lactation, which produce an increased demand of oxytocin (Russell, 1980), in rats drinking hypertonic solutions, to create a chronic increase in

plasma osmolality, which results in an increase in vasopressin production (Enestrom, 1967; Paterson & Leblond, 1977) and in hypophysectomised rats (Crespo et al. 1990). As nuclear size is an index of neuronal protein synthesis, the increment observed in nuclear size, in comparison with control animals, could represent an increase in the nuclear mRNA synthesis activity of neurohormone precursors (Crespo et al. 1990).

The neuronal somata in the present study contained numerous electron-lucent globular vacuoles throughout the cytoplasm which probably originated from fragmented and dilated rER. The rER distension has previously been reported in the SON neurons of osmotically stimulated rats and castrated rats (Zambrano & deRobertis, 1966, 1968) and in the lumbar sympathetic ganglia of diabetic patients (Kott et al. 1974). It has been attributed to hypersecretion of neurohormones in the SON, although extreme cases of vacuolation may indicate exhaustion (Kalimo, 1975). Distension of rER cisternae was also observed in SON neurons of control rats, but the distended rER in the controls did not fuse with one another as observed in SON neurons of diabetics. Dellmann (1973) reported in his review article that the appearance of vacuoles and tubulovesicular elements reflects hyperactivity of the hypothalamoneurohypophyseal complex. These findings seem to be more plausible in view of the observed increase in hypothalamic levels of arginine vasopressin and oxytocin (neurohormones of SON) in male rats 10 d after streptozotocin treatment (Fernstrom et al. 1990).

Experimentally induced diabetic rats have been shown to exhibit serum hyperosmolality (Fernstrom et al., 1990), plasma hyperosmolality and hyperosmotic dehydration (Young, 1969). In the present study, all of the streptozotocin-induced diabetic rats displayed polyuria and were probably in a state of dehydration. Hence, hypertrophy of the somata and rER distension observed in the present study may partly or wholly be due to osmotic pressure caused by hyperglycaemia since similar features have been reported in SON neurons of diabetes insipidus rats and osmotically stimulated rats (Sokol & Valtin, 1965; Zambrano & deRobertis, 1966).

Electron-dense neuronal profiles undergoing necrosis were also observed in the present study. The appearance of electron-dense cytoplasm may indicate cellular degeneration (Raisman, 1973). These abnormal neuronal profiles were markedly increased in diabetics after 9 months. Some similar lesions have been reported in SON neurons of hypophysectomised rats (Raisman, 1973) and in the gracile nucleus, nucleus ventralis posterolateralis (Tay & Wong, 1991 *a*, *b*, 1992) and autonomic nervous system (Yagihashi & Sima, 1986) of diabetic rats. Osmotic stress caused by chronic dehydration in the diabetic rats could also have caused these abnormal electron-dense neuronal profiles. However, the electron-dense neuronal profiles have never been reported in the SON of dehydrated animals.

Most of the ultrastructural changes observed in the present study were not only specific for diabetes, but have also been encountered in control rats at later survival periods (i.e. 6–12 months). However, the abnormal neuronal profiles were significantly higher in diabetic rats than in controls. These abnormal neuronal profiles did not occur in the SON of controls at the early survival periods (i.e. 3 d–3 months). Since the abnormal neuronal profiles were observed in the SON of control rats after 6 months, it is possible that these ultrastructural changes may be related to ageing phenomena and that SON neurons in the diabetic rats may be more susceptible to ageing. However, systematic experimentation is needed to correlate both ageing and diabetes. On the other hand, it has recently been hypothesised that some unknown trophic factors may have been altered after the onset of diabetes resulting in dysmetabolism of the cell bodies, thereby affecting the axonal profiles and dendrites (Tay & Wong, 1991 *b*).

Insulin may play a role in brain growth and maturation, neurotransmission and neuromodulation (Hendricks et al. 1984; Raizada et al. 1987). Recently specific insulin binding sites have been localized in the rat SON (Corp et al. 1986): therefore we suggest that the hyperglycaemic condition of the diabetic rat could have altered the configuration of the insulin receptors, resulting in metabolic dysfunction thereby contributing to the ultrastructural changes seen in the present study. Further studies, probably at the molecular level, may throw some light on the involvement of insulin as well as other causative factors associated with the diabetic changes observed in the SON of the streptozotocin-induced diabetic rat.

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