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Ultrastructural observation on the rat supraoptic neurons following acute operative stress

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Summary. The response of the SON to various forms of stress is well documented. However, the effect of operative stress, which is a common and important clinical event requiring the mediation of vasopressin, has largely escaped attention. The present report describes the ultrastructural changes in the neurons of the caudal (retrochiasmatic) part of the SON following a deep-seated linear incision on the dorsum of the rat. The observations were confined to the first forty-eight hours after trauma.

At 24-hours post-operatively, a marked depletion of the neurosecretory granules was observed. This was associated with a proliferation of the granular and smooth endoplasmic reticulum, Golgi cisternae and ribosomes. A few of the neurosecretory granules were seen to lie in the close vicinity of the Golgi complexes. At 48-hours after trauma, these features persisted. In addition, an accumulation of neurosecretory granules was conspicuous in some axon pre-terminals.

From the above findings, it is suggested that an increased demand for vasopressin during the early post-operative period is met by the supraoptic neurons by a liberation of their neurosecretory contents. An attempt at replenishment of the latter is evidenced by a proliferation in the membrane components and ribosomes. The pooling of neurosecretory granules in occasional axon pre-terminals may indicate an imbalance in the synthesis-secretion coupling of vasopressin.

Key words: Rat supraoptic neuron, Operative stress, Ultrastructure

Introduction

The supraoptic nucleus (SON) of the mammalian hypothalamus is concerned with elaboration of vasopressin, as confirmed by physiological (Sachs et al., 1971; Cross et al., 1975) and immunohistochemical (Rhodes et al., 1981; Sofroniew et al., 1981; Kawata and Sano, 1982; Ray and Choudhury, 1987) studies. Vasopressin is one of the prime substances concerned with fluid regulation of the body. Accordingly, its synthesis, storage and release undergo a considerable flux under conditions that require adjustments in fluid balance. Such functional changes are reflected in the ultrastructure of the supraoptic neurons, as highlighted by available studies under conditions of fluid deprivation (Zambrano and DeRobertis, 1966; Tweedle and Hatton, 1977; Alonso and Assenmacher, 1979), salt loading (Morris and Dyball, 1974), hypoxia induced by morphine poisoning (Borowicz et al., 1974) and malnutrition (Fercakova, 1977). Among clinical conditions that require continual adjustments to fluid environment, surgical trauma is of considerable relevance. The early postoperative period is particularly associated with changes in the level and distribution of body fluid. The morphological correlations of such changes in the supraoptic neurons were investigated earlier at light microscope level (Choudhury, 1971, 1972). The findings pointed to an enlargement in the magnocellular neurons with a concomittant depletion of the neurosecretory material. The present work has been extended to record ultrastructural changes during the first forty-eight hours, when the machinery for fluid adjustment is operative at its peak. As the supraoptic neurons are known to exhibit zonal variation in morphology (Lafarga et al., 1975; Krisch, 1976), the present investigation was confined to a selected area of the SON.

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Materials and methods

Twelve young male adult Wistar rats (200-250 g) were divided into control (4 animals) and experimental (8 animals) groups. A deep-seated linear incision was made

on the dorsum of the experimental animals under intraperitoneal pentabarbitone (Nembutal; 4mg/100g body weight) anaesthesia. The muscles and skin were apposed in separate layers with interrupted sutures. Control rats received only an appropiate dose of the same anaesthetic. During the post-operative period, the control and experimental animals were caged individually under identical conditions of temperature (70°F), humidity (45%), light/dark cycle (12 hours/12 hours) and cage space. Food and water were given ad libitum.

The experimental rats were sacrificed at 24- and 48-hours after the operation. The control animals were killed at similar time intervals following administration of the anaesthetic.

All animals were perfused under Nembutal anaesthesia (4mg/100g body weight) with 2.5% glutaraldehyde in 0.165 M phosphate buffer according to techniques described elsewhere (Ray and Choudhury, 1985). The brains were removed, grossly trimmed and kept immersed in the above perfusate at 4°C overnight. The next day the blocks of brain tissue containing only the retrochiasmatic part of the SON between the caudal end of the optic chiasma and the beginning of the infundibular recess were dissected out under a stereomicroscope. The paraoptic regions containing the supraoptic neurons were carefully isolated from these blocks and processed for transmission electron microscopy as of routine. Ultrathin sections were stained with uranyl acetate and lead citrate and were examined under a Jeol electron microscope (Jeol 100 CX), operated at 80KV.

Results

In the control animals, the neurons in the SON exhibited large vesicular nucleus with prominent nucleolus. Indentation of the nuclear membrane was frequently observed. Cell to cell apposition over wide areas without intervention of any glial processes was also noticed. Well defining Golgi cisternae and mitrochondria characterised the paranuclear zone, while granular endoplasmic reticulum occupied a relatively peripheral part of the cytoplasm. Electron-dense neurosecretory granules varying in size from 1000 Å to 3000 Å were sparse and widely scattered throughout the cytoplasm. Some of these granules were closely associated with the membrane system. Lysosome-like dense bodies were also a consistent finding. The above features are presented in Fig. 1.

At 24-hours following operation, a marked proliferation in the membrane system was observed. Extensive stacks of endoplasmic reticulum studded with ribosomes characterised the peripheral part of the cytoplasm. The lamellae in the above stacks were arranged parallel to each other (Figs. 2-4). An abundance of polyribosomes in the form of rosettes was also apparent (Fig. 3). In many neurons, the endoplasmic reticulum showed wide dilatation, giving the tubules a circular profile on transverse section (Fig. 5). Proliferation of the Golgi complex was a consistent observation. The Golgi cisternae showed a close association with neurosecretory granules and dense-coated vesicles (Figs. 2, 3, 5). Another conspicuous feature at this stage was a marked depletion of neurosecretory granules. The few granules that were seen, were scattered in the cytoplasm and appeared smaller. At 48 hours after operation, many of the features seen at 24 hours post-operatively, also persisted. Thus, a proliferation of the endoplasmic reticulum and of the Golgi cisternae was characteristic. This was associated with a depletion of neurosecretory granules in the neuronal perikarya (Fig. 6). However, amongst neuronal profiles with depleted neurosecretory granules, an accumulation of the latter in occasional axon pre-terminals, was seen (Fig. 7).

Fig. 1. A supraoptic neuron from a control rat. A large vesicular nucleus (N) with a prominent nucleolus (nu) is evident. A Golgi zone (G) is seen in the paranuclear region. The cytoplasm is also characterized by peripheral granular endoplasmic reticulum (r) and an assortment of neurosecretory granules (arrows). Some of the latter are clearly seen in close relationship to the membrane system. A few dense bodies (db) of larger sizes are also visible. × 10,850

Fig. 2. A survey micrograph of supraoptic neurons from a 24-hour post-operative rat. A neuron with its initial segment is seen separated from an adjacent neuron by a neuropil containing axonal and dendritic profiles. A proliferation of Golgi cisternae (G) is noticeable in both the neurons. Some neurosecretory granules (arrows) lie in close association with the Golgi cisternae. The neuron on the right exhibits an abundance of granular endoplasmic reticulum (r). A depletion of neurosecretory granules (arrows) is evident in the neuronal perikarya. × 5,480

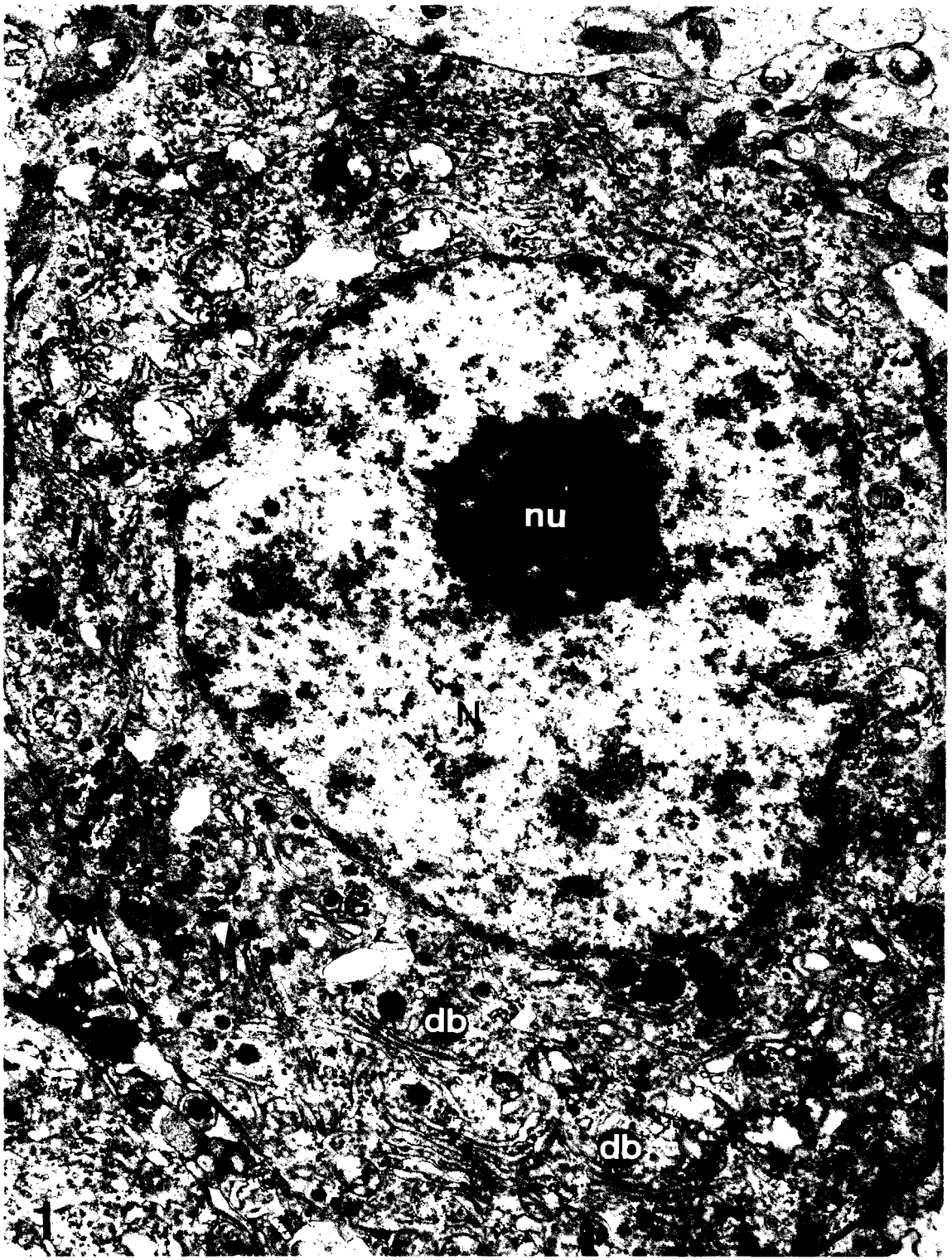
Fig. 3. Three adjoining supraoptic neurons from a 24-hour post-operative rat. A marked proliferation of granular endoplasmic reticulum (r) arranged in parallel stacks is evident. Polyribosomal rosettes (p) are seen scattered throughout the cytoplasm. The neuronal perikarya exhibit an overall reduction in the number of neurosecretory granules (arrows). A few lie in close proximity of an extensive Golgi complex (G) in the neuron at the top left. A large number of mitochondria (m) and a few dense bodies (db) populate the perikarya. Cell to cell contacts (arrowheads) are conspicuous. × 11,200

Fig. 4. Part of a supraoptic neuron surrounded by neuropil taken from a rat 24-hours after operation. Extensive rough endoplasmic reticulum (r) and numerous polyribosomal rosettes (p) are characteristic. An almost total lack of neurosecretory granules is noteworthy. A large number of mitochondria (m) and a few dense bodies (db) are also visible. × 11,428

Fig. 5. A marked proliferation and gross dilatation of tubules of endoplasmic reticulum (t) as seen in a 24-hour post-operative rat. A prominent Golgi complex (G) with some neurosecretory granules (arrows) in its close vicinity is apparent at upper left corner. A large population of electron-dense bodies of varying sizes (db) and neurosecretory granules (arrows) characterise the cytoplasm. A nucleolus-like body (nb) is seen on the right. × 14,870

Fig. 6. Initial segment of a supraoptic neuron taken from a 48-hour post-operative rat. A large stack of granular endoplasmic reticulum (r) is seen at the top. A Golgi complex (G) associated with dense-coated vesicles (v) and a neurosecretory granule (arrow) is noticeable. The segment contains numerous mitochondria (m) and neurotubules (nt) and is largely devoid of neurosecretory granules. × 12,957

Fig. 7. An axon pre-terminal in the SON abutting a perikaryon. 48-hour post-operative rat. The pre-terminal contains an assortment of neurosecretory granules (arrows), dense bodies (db) and mitochondria (m). × 17,467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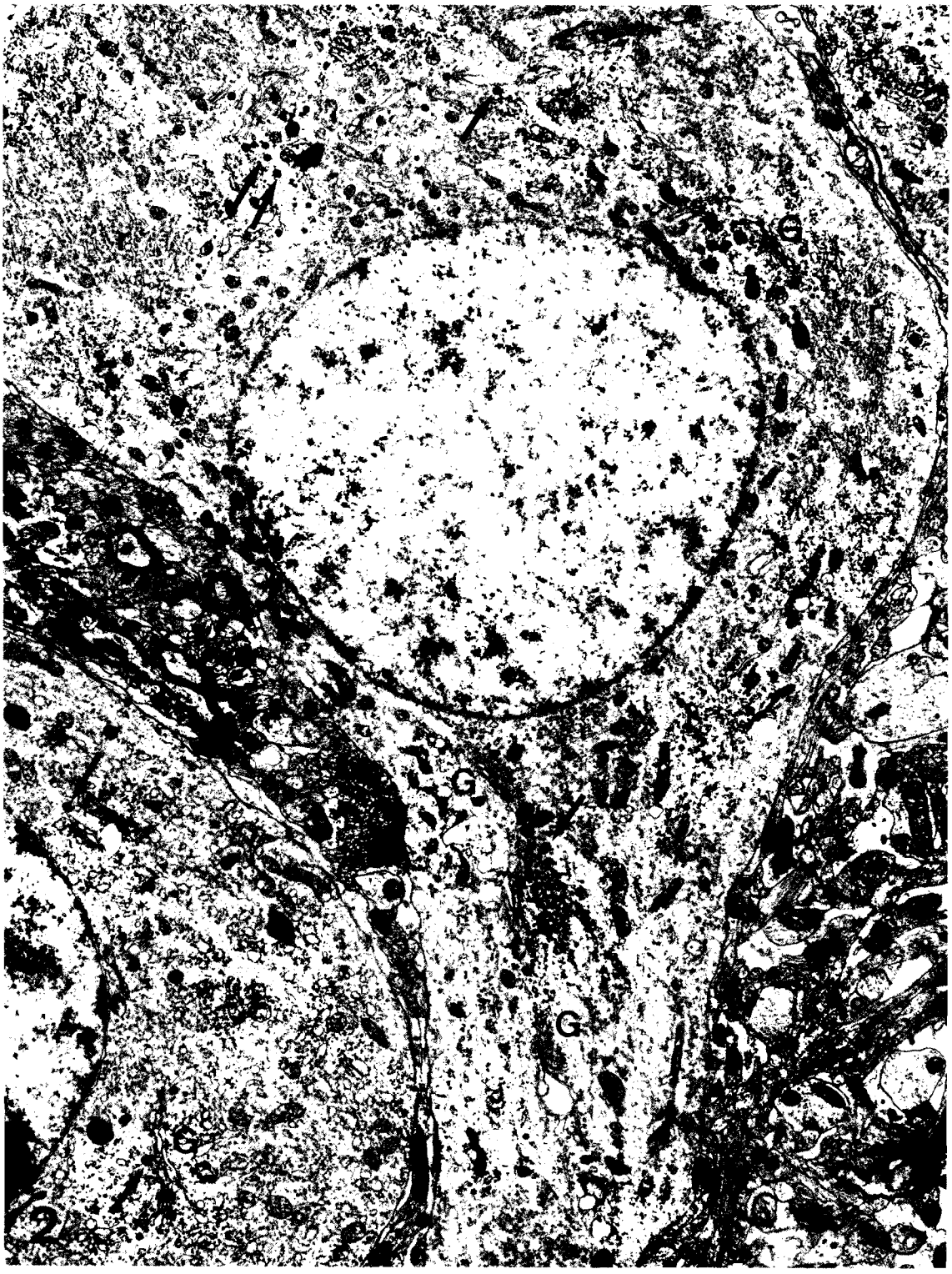
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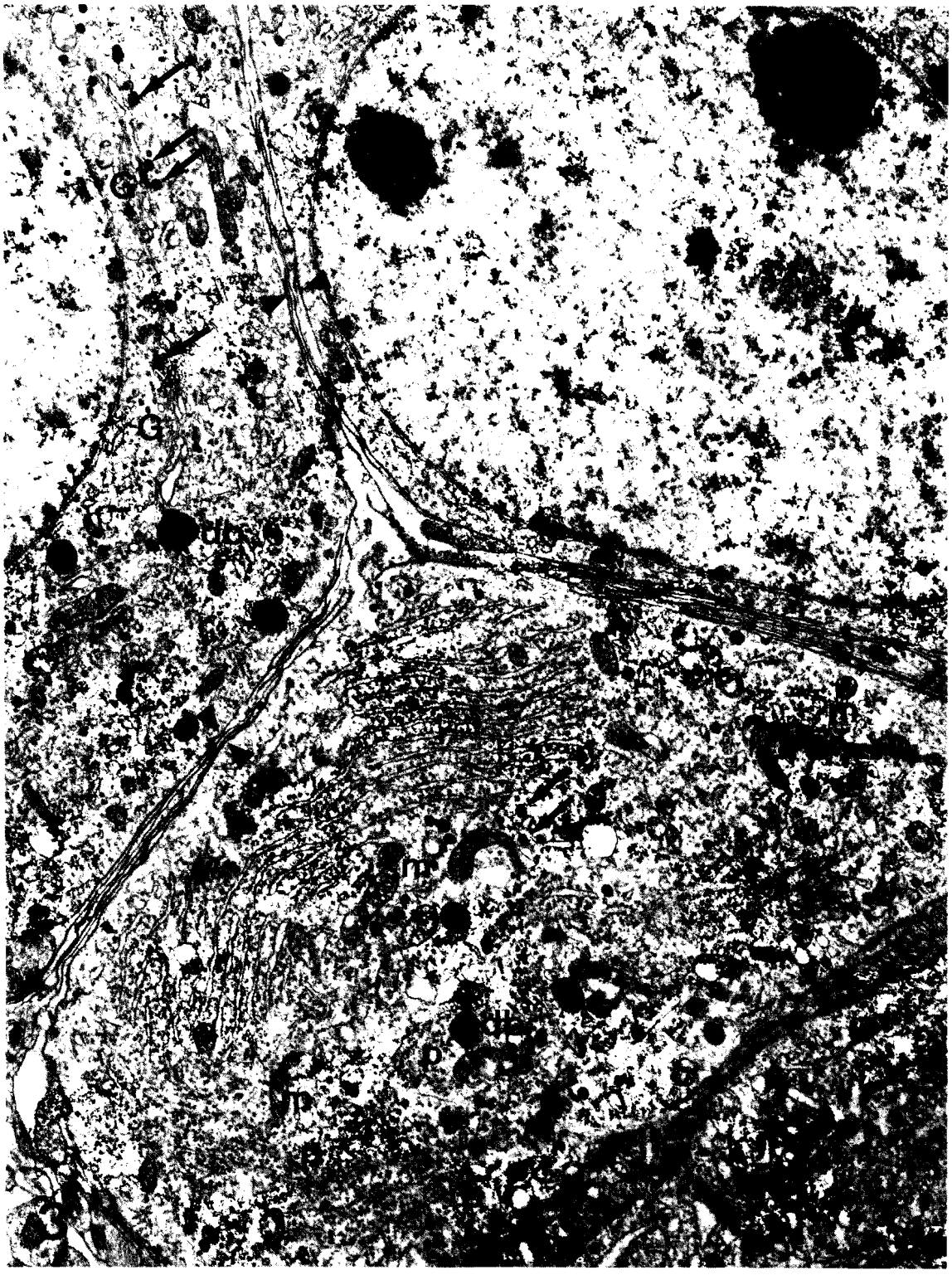

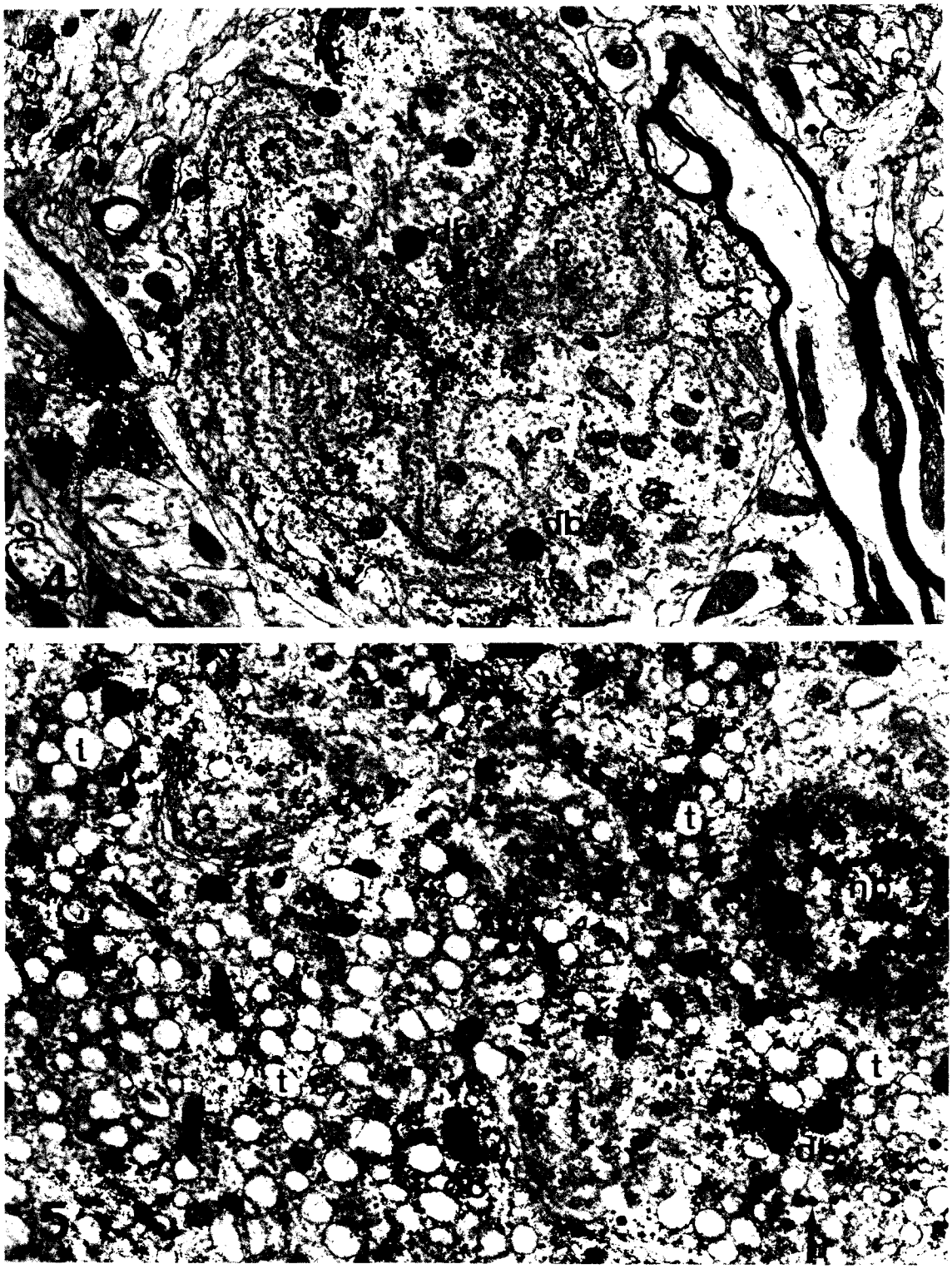
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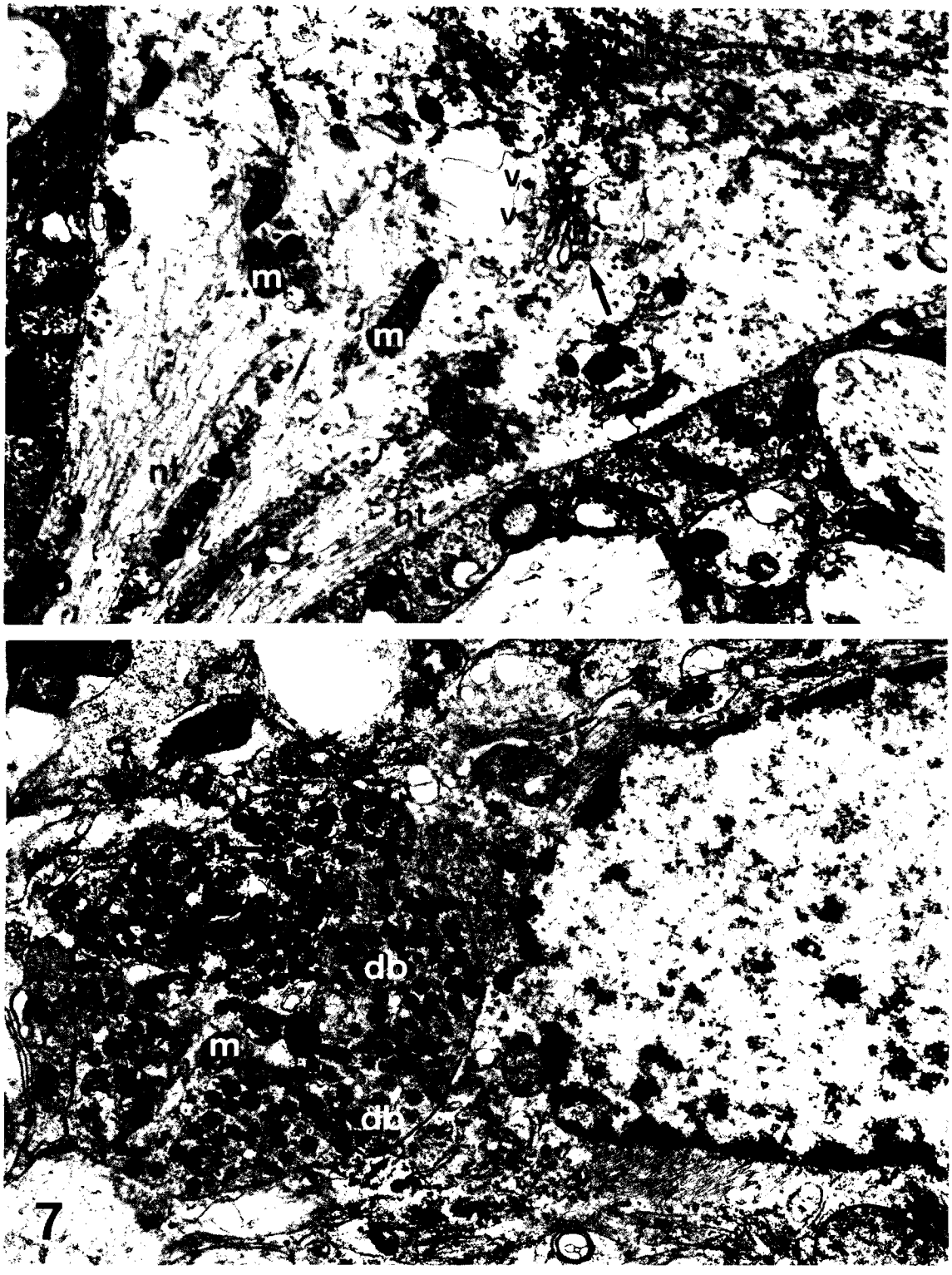
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Discussion

It is well known that the neurons of the SON do not present a structurally homogeneous population (Zambrano and Mordoh, 1966; Bardaranayaka, 1971; Leranth et al., 1975; Tweedle and Hatton, 1977). Neurons in the differrent zones of the nucleus vary in their morphological appearances and in their neurosecretory content. However, in any given zone, the neurons are reported to have a comparable structure, since they represent a similar functional state (Lafarga et al., 1975). In the present work, therefore, only the caudal (retrochiasmatic) part of the SON was investigated to obviate structural asynchrony. Also the neurons in this zone are known to be more responsive to stress than those in its rostral counterpart (Krisch, 1976).

The ulstrastructure of the supraoptic neurons in the control animals essentially conforms to those reported by earlier workers (Sloper and Bateson, 1965; Zambrano and DeRobertis, 1966; Eneström, 1967; Clattenberg, 1974; Kalimo, 1975). It is noteworthy that in the rat, the amount of neurosecretory granules in the neuronal perikaryon in rather scanty. The axon and the axon terminals within the SON also contain only a small number of neurosecretory granules. As mentioned earlier, an extensive nuclear infolding was observed in many neurons. The significance of this finding is ill-understood, except as a mechanism to increase the surface area of the nuclear membrane. This has been interpreted as an attempt at increasing the cytoplasmic-nuclear interface by some workers (Zambrano and DeRobertis, 1966; Clattenberg, 1974). In our study, areas of cell to cell contact without intervention of any glial processes were noted. Similar sites of contact within the SON have been reported by other workers both in normal and in experimental animals (Lafarga et al., 1975; Tweedle and Hatton, 1976). It is speculated that such sites act as areas of intercommunication between cells to bring them under identical morphofunctional states.

The almost total depletion of the neurosecretory granules at twenty-four hours following trauma points to their possible release in the neurohypophyseal circulation due to an increased demand for vasopressin. Such depletion coupled with proliferation of the Golgi complex and of the granular endoplasmic reticulum reflect synthetic activity. The association of the newly-formed neurosecretory granules and dense-coated vesicles with the Golgi cisternae is corroborative in this regard. Furthermore, the membrane system shows dilated tubular profiles on transverse and oblique sections. Similar dilatation has also been reported by several authors under various stressful conditions (Zambrano and DeRobertis, 1967; Morris and Dyball, 1974; Kalimo, 1975; Krisch, 1976). While Zambrano and De Robertis, (1967) and Morris and Dyball (1974) suggested that the dilatation represented a greater involvement of the neurons in the synthetic part of a synthesis-storage cycle, Kalimo (1975) ascribed such changes to an exhaustion phenomenon due to an intense stimulus for hormone synthesis. Thus, there appears to be a general agreement that the cisternal dilatation ensues from an attempt at augmenting protein synthesis. It is interesting to note that Alonso and Assenmacher (1979) suggested that stress induces a transformation in the configuration of the peripheral endoplasmic reticulum from a resting lamellar to an active tubular form. In the present investigation, however, no loss in lamellar arrangement was noted in the experimental animals. Pari passu with this, profiles of dilated tubules were also encountered.

The above features of increased neuronal activity also persist at forty-eight hours. Though essentially most of the neurons are still depleted of their neurosecretory granules, an accumulation of the latter is observed in some neurons. In these cells, the granules are seen traversing the axons and are also pooled at axon pre-terminals. Regarding the origin of the neurosecretory granules, it is pertinent to point out that these arise not only from the novo synthesis at the neuronal perikaryon, but may also be derived through an active uptake mechanism from the axon terminals in a retrograde manner (Alonso and Assenmacher, 1983). The pooling of the neurosecretory granules probably indicates the levelling off of an increased demand for vasopressin. Alternatively, this may be regarded as a pointer to an imbalance in the synthesis-secretion coupling of this hormone. The finding of axon terminals containing neurosecretory granules within the SON merits further consideration. Though the neurohypophysis is considered to be the principal destination of the supraoptic axons, an appreciable number of the latter are known to terminate within the SON (Léránth et al. 1975). Based on her immunohistochemical studies on vasopressin-containing axons in the caudal supraoptic neurons in thirsting rats, Krisch (1976) entertained the interesting possibility of vasopressin release within the SON.

The present findings of proliferation of the granular and smooth endoplasmic reticulum and Golgi cisternae, associated with depletion of neurosecretory granules, compare well with those described under various stressful conditions mentioned earlier (vide introduction). In summary, the supraoptic neurons respond to operative stress by releasing their neurosecretory contents. This is immediately followed by an attempt at enhanced synthesis as manifested by a proliferation of membrane components and of ribosomes. This appears to be the almost universal and predictable response of the supraoptic neurosns to any stressful conditions requiring vasopressin release.

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