FOLIA NEUROPATHOL. 1994, 32, 3
ISSN 0028-3894

BARBARA GAJKOWSKA, MIROSŁAW J. MOSSAKOWSKI

ISCHEMIA INHIBITS GABAERGIC NEURONS OF THE RAT THALAMIC RETICULAR NUCLEUS. AN IMMUNOCYTOCHEMICAL STUDY

Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, Warszawa

GABA-immunoreactivity in the thalamic reticular nucleus was studied in rats subjected to 10 min global cerebral ischemia, due to experimentally induced cardiac arrest. The studies were performed in different postischemic periods (10 min, 1 h and 24 h after ischemia) with postembedding immuno-gold technique for electron microscopy, applying antisera raised against protein-gamma amino butyric acid-conjugates. Transient reduction of gold particles content, indicating GABA appearance and distribution in neuronal perikarya and synaptic terminals, was noticed 10 min and 1 h after ischemia. Reduction of immunoreactivity accompanied ultrastructural abnormalities involving both neurons and synapses and taking the form of severe swelling and disorganization of organelles arrangement. Immunocytochemical abnormalities concerning neuronal perikarya appeared earlier and were more severe. At 24th h after ischemia immunoreactivity of most of the neurons and synapses was similar to this in normal control animals. Morphologically unchanged asymmetric synapses were present in all experimental groups. The presented data confirm high vulnerability of GABAergic neuronal population of the thalamic reticular nucleus to ischemia and suggest transient nature of postischemic GABAergic insufficiency.

Key words: *global cerebral ischemia, thalamic reticular nucleus, GABA-immunoreactivity, immuno-gold method*

For many years, GABAergic neurons have been considered as resistant to the ischemic injury. This opinion was based on the reaction of GABAergic interneurons of the CA_1 hippocampal sector to the short-term forebrain ischemia in rats and gerbils (Johansen et al. 1983, 1989; Schlander et al. 1988; Nitsch et al. 1989). However, studies of other structures of the central nervous system convincingly showed that GABAergic neuronal populations in the striatum, cerebellar cortex, *pars reticulata* of the substantia nigra and the thalamic reticular nucleus are highly ischemia-sensitive (Pulsinelli et al. 1982; Smith et al. 1984; Ross, Duhaime 1989; Schmidt-Kostner, Freund 1991; Kawai et al. 1992). In this respect observations of Kawai et al. (1992) seem to be of a special interest, as they demonstrated unusually early and severe damage of the great proportion of neuronal population of the thalamic reticular nucleus, thought to be mostly of GABAergic nature. Searching for explanation of this striking difference in the reaction to ischemia presented by GABAergic neurons in different brain structures, it was noticed that ischemia-sensitive GABAergic neurons belong to long projecting nerve cells, while ischemia-resistant hippocampal interneurons represent a group of local circuit neurons. Schmidt-Kostner and Freund (1991) suggested that a major difference in vulnerability to ischemia existing between projecting cells and interneurons, depends on functional and metabolic distinctions between these populations.

In our earlier studies (Gajkowska et al. 1989) we had observed remarkable ultrastructural abnormalities of interneurons in CA_1 hippocampal sector in gerbils appearing in very early postischemic period (6-12 h after bilateral ligation of common carotid artery). They consisted in reversible acute cellular swelling, totally normalizing in later postischemic periods. These changes suggested a possibility that early abnormalities in GABAergic interneurons may provide a period of neuronal disinhibition and thus contribute to an excitotoxic damage of selectively vulnerable CA_1 pyramidal neurons. Our observations were then confirmed by the immunocytochemical studies with immuno-gold technique, in which we demonstrated significantly decreased GABA-immunoreactivity in interneurons and their synaptic terminals in CA_1 hippocampal sector in gerbils after short-term forebrain ischemia (Gajkowska et al. 1994).

This inclined us to perform a series of studies on the subcellular distribution of GABA in neurons and synapses of the thalamic reticular nucleus in the condition of global cerebral ischemia, resulting from

experimentally induced cardiac arrest, with the use of antisera raised against protein-gamma amino butyric acid-conjugates. Thalamic reticular nucleus is known to be a selectively vulnerable structure in which prevailing GABAergic neuronal population is in the conditions of brain ischemia exposed to the excitotoxic action of glutamate, although they have mostly non-NMDA receptor system (Ross, Duhaime 1989).

Material and methods

The experiments were performed on adult, male albino rats, in which clinical death was induced according to the method described originally by Korpachev et al. (1982). Compression of the heart vascular bundle by a special hook inserted into the thorax led in the course of 2.0–3.5 min to cardiac arrest and cessation of respiratory function lasting until resuscitatory management was undertaken. This was done after 10 min of complete cessation of the brain bioelectric activity, which appeared usually 20-25 sec after compression of the heart vascular bundle. Following resuscitation, which included external heart massage and artificial ventilation, the experimental animals survived in groups of 3 for 10 and 60 min and 24 h. Three animals subjected only to sham operation, consisting of intrathoracic hook insertion without any further experimental procedure served as a control group. Detailed characteristics of the experimental procedure and basic pathophysiological observations were presented in the previously published papers (Mossakowski et al. 1986; Kapuściński 1987).

Control and experimental animals were sacrificed by transcardiac perfusion with a solution consisting of 2.5% glutaraldehyde and 1% paraformaldehyde in sodium phosphate buffer, pH 7.4. This was preceded by a short (1 min) rinse of the vascular system with heparinized physiological saline solution. The perfusion carried out under the hydrostatic pressure of 100 mm Hg lasted 15 min. Brains removed from the skull were immersed for 24 h in the above perfusion solution and cut frontally into the slices, 1 mm thick. Tissue blocks, containing several regions of the reticular thalamic nucleus were taken with the use of thick transfusion needle (1 mm in diameter). They were rinsed overnight in the sodium phosphate buffer, pH 7.4, postfixed in 1% osmium tetraoxide for 1 h, dehydrated in series of graded alcohol solution and embedded in Epon.

Ultrathin sections were treated according to postembedding immuno-gold procedure. They were mounted on formvar-coated gold grids and floated for 10 min on 10% H_2O_2. This was followed by 15 min rinsing in phosphate buffered saline (PBS). Then they were exposed for 30 min to 5% bovine serum albumin (BSA) in PBS. Subsequently they were floated for 2 h at 37°C on polyclonal rabbit antibody to gamma amino butyric acid – GABA (Biogenesis Ltd, UK) diluted: 1:400 in Tris phosphate buffered saline. Afterwards the grids were washed with PBS for 30 min and exposed for 45 min to anti-rabbit IgG-conjugated to colloidal gold particles 15 nm in diameter (Janssen Pharmaceutica, Belgium) diluted 1:20 in PBS. This procedure was carried out in darkness. After incubation the grids were washed in PBS and distilled water and air-dried. Subsequently they were counterstained with uranyl acetate 4.7% in distilled water for 20 min and with lead citrate for 2 min. Controls for the immunocytochemical reaction were carried out replacing the incubation with rabbit antibody by an incubation with normal rabbit serum diluted 1:5 in PBS.

Sections were examined and photographed in JEOL 1200 Ex electron microscope.

Results

In the control animals GABA-like immunoreactivity was found in most of the neurons of the thalamic reticular nucleus, numerous synaptic contacts, mostly of symmetric type and in some glial elements, first of all astrocytes. High concentrations of colloidal gold particles, indicating sites of GABA-immunoreactivity were found in the neuronal cell bodies and both axonal and dendritic profiles. Gold particles were visible over nuclei and cytoplasm of the neurons. In the latter they seemed to be located mostly over Golgi complex, rough endoplasmic reticulum and mitochondria. The other cytoplasmic organelles were rather unlabeled (Fig. 1). Axon terminals forming symmetric synapses with dendritic shafts or cell bodies exhibited high concentration of gold particles. Their accumulation, although evident, was weaker in postsynaptic parts of these synapses. In presynaptic bags gold particles were mostly located over synaptic vesicles and small mitochondria. In postsynaptic parts their association with rough endoplasmic reticulum and mitochondria prevailed (Fig. 2). Relatively low immunoreactivity was observed in the cytoplasm of glial cells and their processes.

In animals subjected to ischemic injury cellular distribution of GABA-like immunoreactivity varied depending on the stage of postischemic period.

Ten minutes after ischemia distribution of GABA-immunoreactivity was in general similar to this found in the control animals. Great proportion of neurons and nerve terminals revealed no remarkable ultrastructural changes. Relatively high concentra-

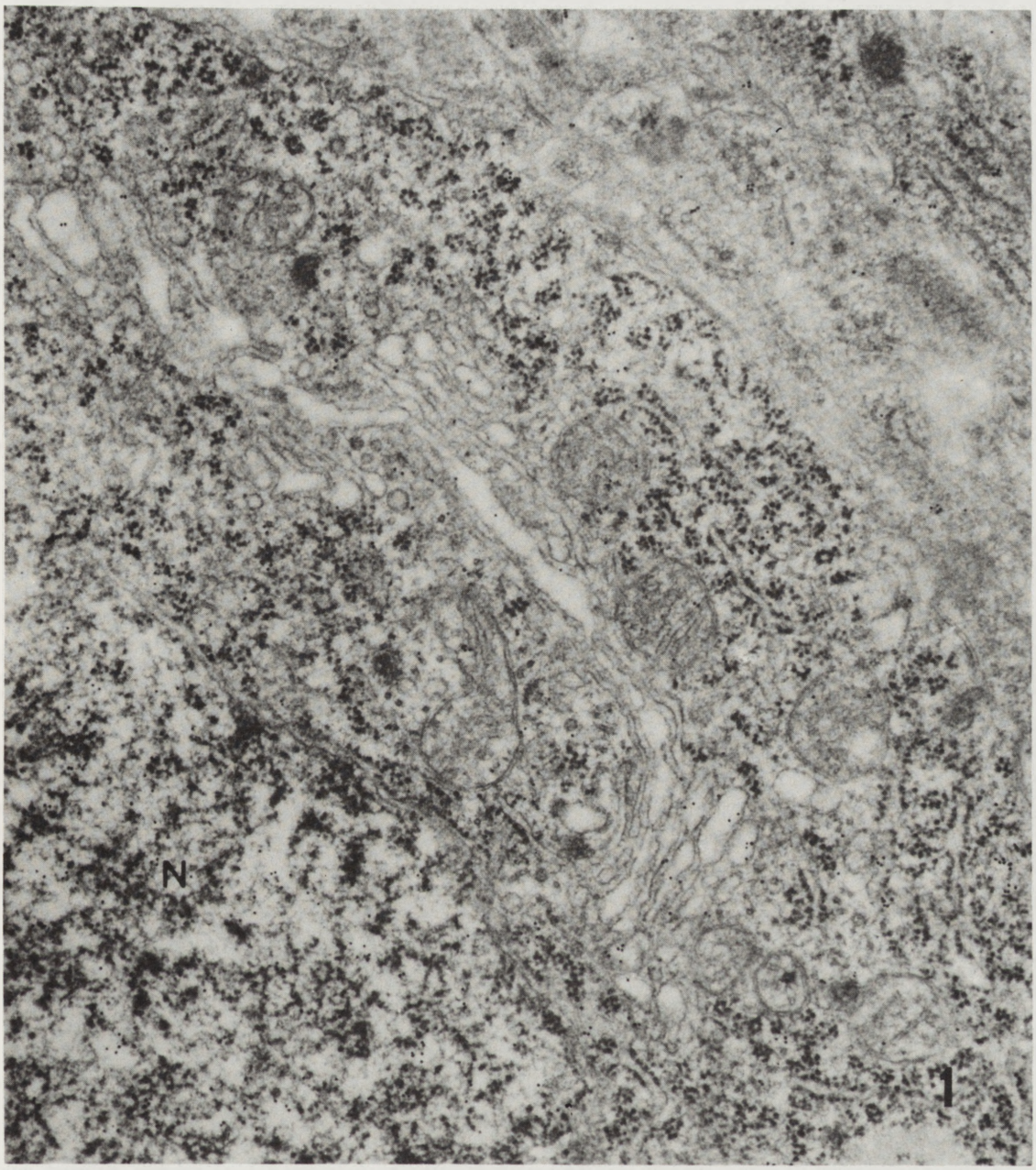

Fig. 1. Control animal. Reticular thalamic nucleus. Neuron (N) heavily loaded with gold particles, expressing sites of GABA-immunoreactivity. In adjacent neuropil only slight traces of labeling are visible. × 36 000.

tion of gold particles was labeling unchanged axon terminals (Fig. 3). However, already at this period some neurons and nerve terminals forming symmetric synapses revealed some swelling, accompanied by less intense gold particle labeling (Fig. 4). The same concerned swollen glial cells.

Sixty minutes after ischemia numerous neurons of the reticular thalamic nucleus and synapses visible in the neuropil revealed ultrastructural abnormalities. Most of the GABAergic neurons were swollen, with remarkable widening of channels and cisterns of Golgi complex and rough endoplasmic reticulum (Fig. 5). Remarkably swollen were GABAergic synapses, showing uneven dispersion of the synaptic vesicles (Fig. 6). In striking contrast to this unlabeled asymmetric synapses were ultrastructurally normal. The condensation of gold particles both over neuronal perikarya and symmetric synapses was much lower than in the control animals (Fig. 5, 6). In a noticeable way the gold particles over presynaptic parts were condensed in places of persistent aggregations of the synaptic vesicles (Fig. 6). Great proportion of glial cells and their processes were swollen and the intensity of their labeling was much lower when compared to control animals.

In the latest postischemic phase studied (24 h after ischemia) quite a proportion of visible GABAergic neurons and symmetric synapses appeared ultrastructurally normal. However, the condensation of gold particles labeling them varied to a great extend. Alongside with cells and axon terminals poorly labeled, there were some cells and synapses visible

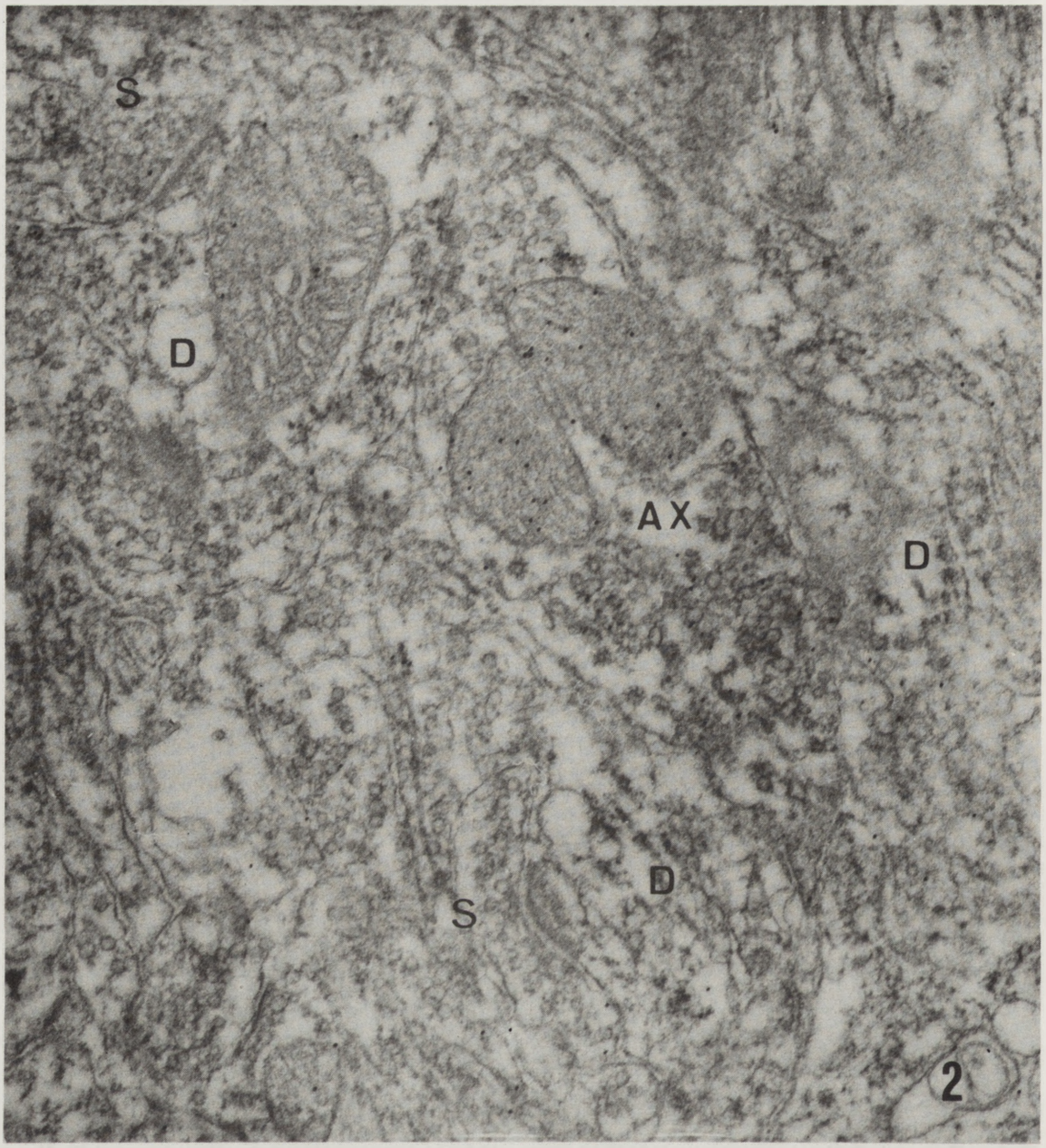

Fig. 2. Control animal. Reticular thalamic nucleus. GABA-immunoreactive axon terminal (Ax) exhibits stronger gold particle accumulation as compared with postsynaptic dendrite (D). Axonal terminals participating in the formation of asymmetric synaptic contact (S) contain less gold particles. × 45

which seemed to be enriched in gold particles indicating GABA-immunoreactivity as compared with these in the control material (Fig. 7, 8). Weaker labeling of postsynaptic parts in comparison with presynaptic axon terminals was striking. Labeling of swollen glial processes was very weak (Fig. 8).

Discussion

The reticular nucleus of the thalamus consists in a great proportion of a population of GABAergic neurons (Houser et al. 1980). Great variety of synaptic inputs can influence this neuronal population. Neurons of the reticular nucleus receive afferent inputs from the collaterals of axons forming thalamocortical and corticothalamic pathways. These represent the most important excitatory input to the neurons of the nucleus. There are good indications that both afferent pathways are mediated by amino acid excitatory neurotransmitters, mostly glutamate. In return, two main sources of GABAergic terminals have been demonstrated in the thalamic reticular nucleus, one deriving from the axonal collaterals of its own GABAergic neurons (Yen et al. 1985) and the second one originating from the external part of globus pallidus as well as from the most of the thalamic nuclei (Mulle et al. 1986; Spreafico et al. 1988).

The reticular nucleus of the thalamus is also densely innervated by cholinergic afferents, deriving mostly from the tegmentopedunculopontine nucleus and from the nucleus basalis of Meynert (Wo-

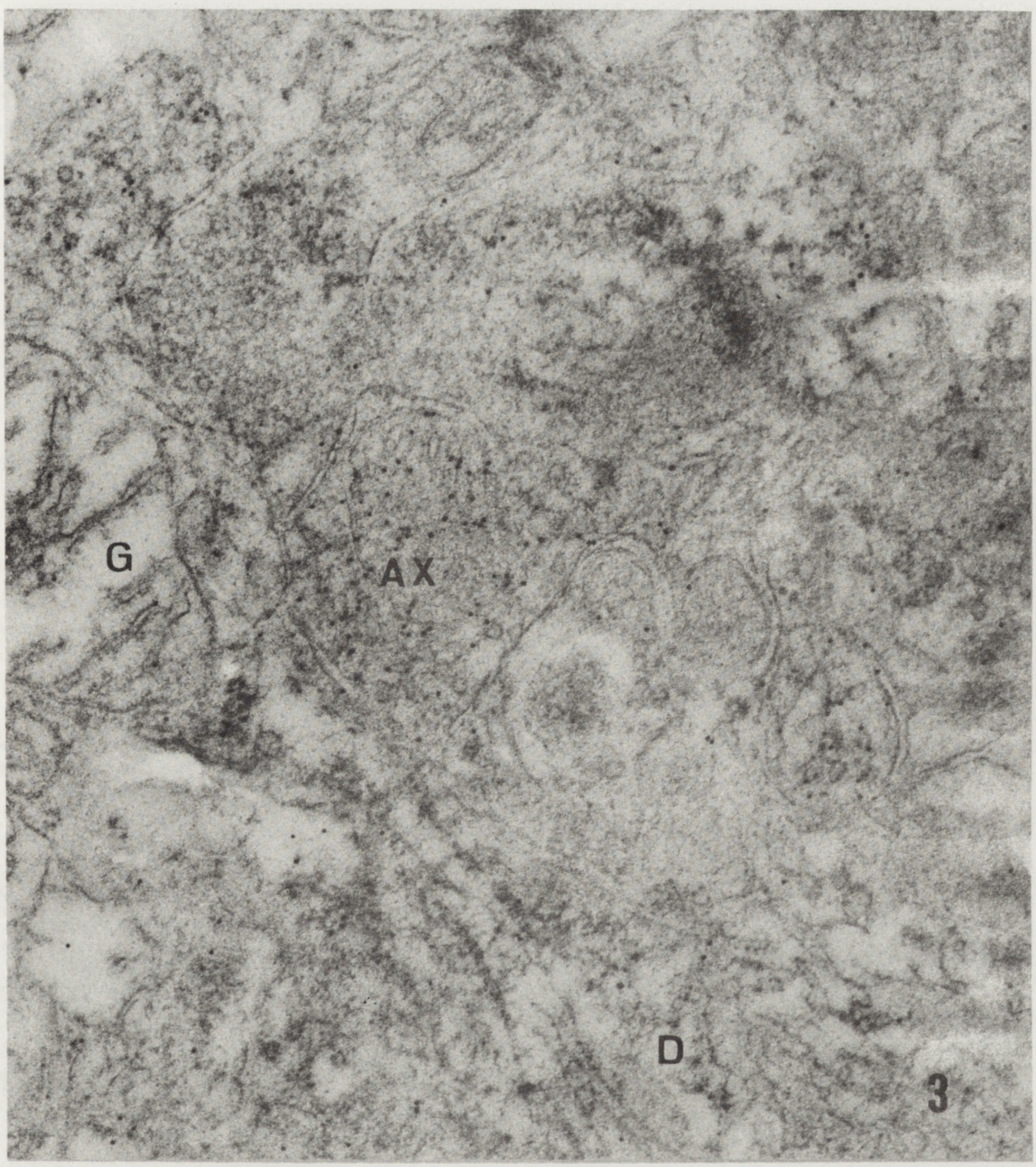

Fig. 3. Experimental animal, 10 min survival after ischemia. Reticular thalamic nucleus. Fragment of neuropil demonstrating relatively high concentration of gold particles over axons (Ax) forming symmetric synaptic contact. Accumulation of gold particles over other elements of neuropil such as dendrites (D) and glial processes is much lower. × 60 000

olf, Butcher, 1986; Hallanger et al. 1987). The noradrenergic and serotoninergic innervations of the nucleus derive from locus ceruleus and from the dorsal raphe nuclei, respectively. On the basis of this connectivity the reticular nucleus of the thalamus can be considered as an inhibitory system, that could regulate or modulate on a state-dependent manner the coupling between thalamic and cortical activities. This implicates the pathophysiological importance of its selective damage resulting from even mild cerebral ischemia (Kawai et al. 1992).

The above presented afferentation of the neuronal population of the thalamic reticular nucleus clearly points out to two different sources of its GABAergic synaptic endings, one group of which is connected with ischemia-sensitive autochtonous GABAergic neurons, the other one derives from brain formation less sensitive to ischemia, such as outer part of globus pallidus or other thalamic nuclei. This may reflect on different reaction of different synaptic groups to ischemic injury. It is obvious that any distinction between GABAergic synapses deriving from various sources in the condition of our experimental model is totally impossible. However, it seems to be worth mentioning that synaptic immunocytochemical changes appeared later than in neuronal perikarya and were less intense. Even at the time of most advanced ultrastructural and immunocytochemical abnormalities (1 h after ischemia) some symmetric synapses revealed relatively

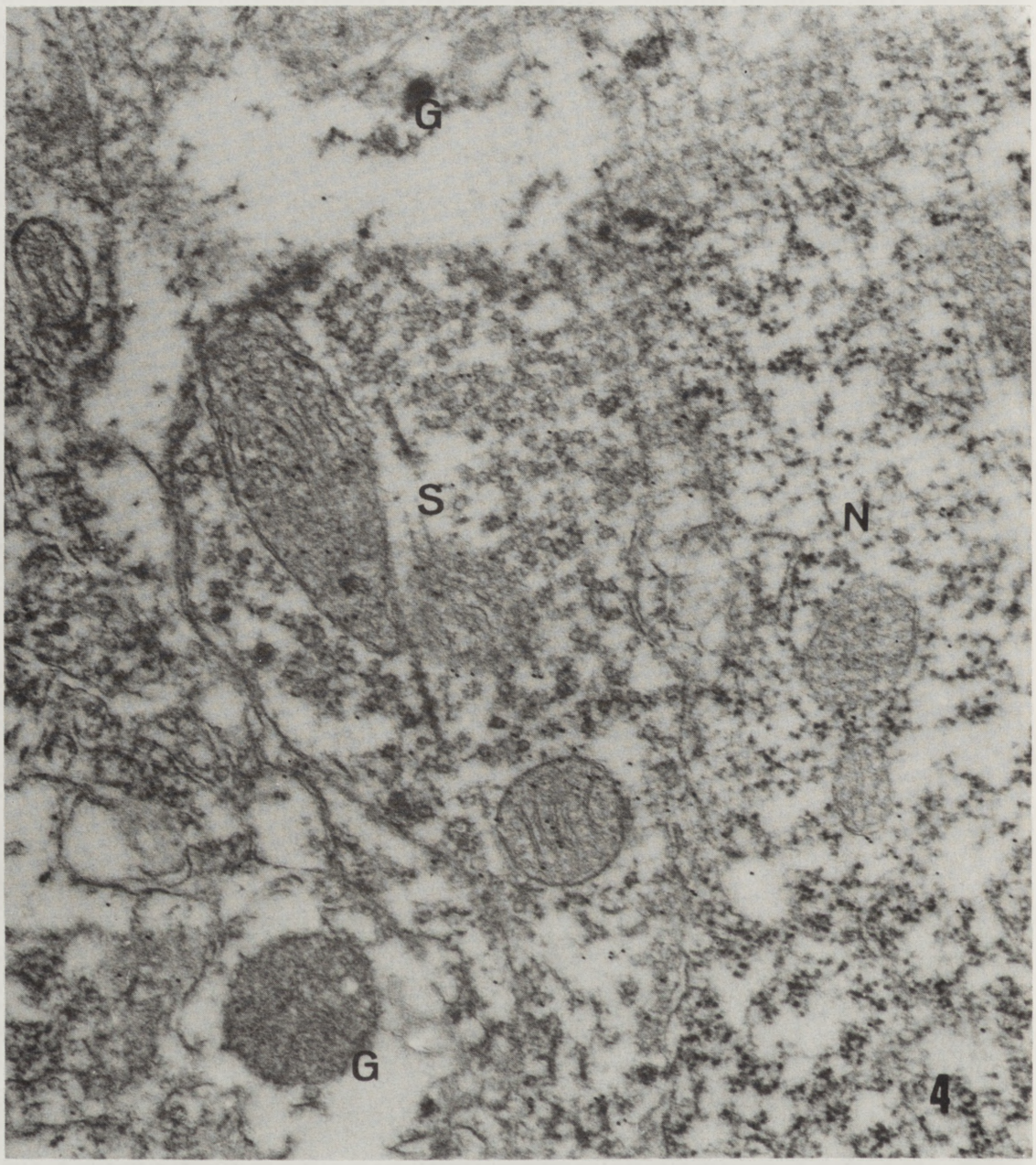

Fig. 4. Experimental animal, 10 min survival after ischemia. Reticular thalamic nucleus. GABAergic neuron (N) with swollen cytoplasm and injured cytoplasmic organelles reveales relatively poor aggregation of gold particles. Axonal terminal forming symmetric synaptic contact (S) is covered by more numerous particles. Hardly any gold particles are visible over greatly swollen glial processes (G). × 45 000

high condensation of gold particles over persistent synaptic vesicles.

Another striking phenomenon of our observations consists in relatively normal appearance of asymmetric synapses, which with all probability correspond to excitatory ones. This was found in all experimental groups, indicating lack of time dependence on the survival period after ischemia. Similar picture of well preserved synapses was found in our previous studies on ultrastructure of postischemic changes in hippocampal CA_1 sector in gerbils (Mossakowski et al. 1989; Gajkowska et al. 1989, 1992). Most of the synapses localized in different layers of the CA_1 sector remained normal even at the stage of severe damage or disintegration of the pyramidal neurons. Recently Kirino (1993) demonstrated a good ultrastructural preservation of presynaptic terminals in hippocampal gliosis following transient ischemia in Mongolian gerbils.

Immunocytochemical abnormalities concerning perikarya of GABAergic neurons in the thalamic reticular nucleus appeared in very early postischemic period. They were already noticeable 10 min after ischemia, it means at the time when ultrastructure of neurons was almost normal, showing only slight features of cytoplasmic swelling. They became more advanced when ultrastructure of neurons was obviously abnormal (1 h after ischemia). This was

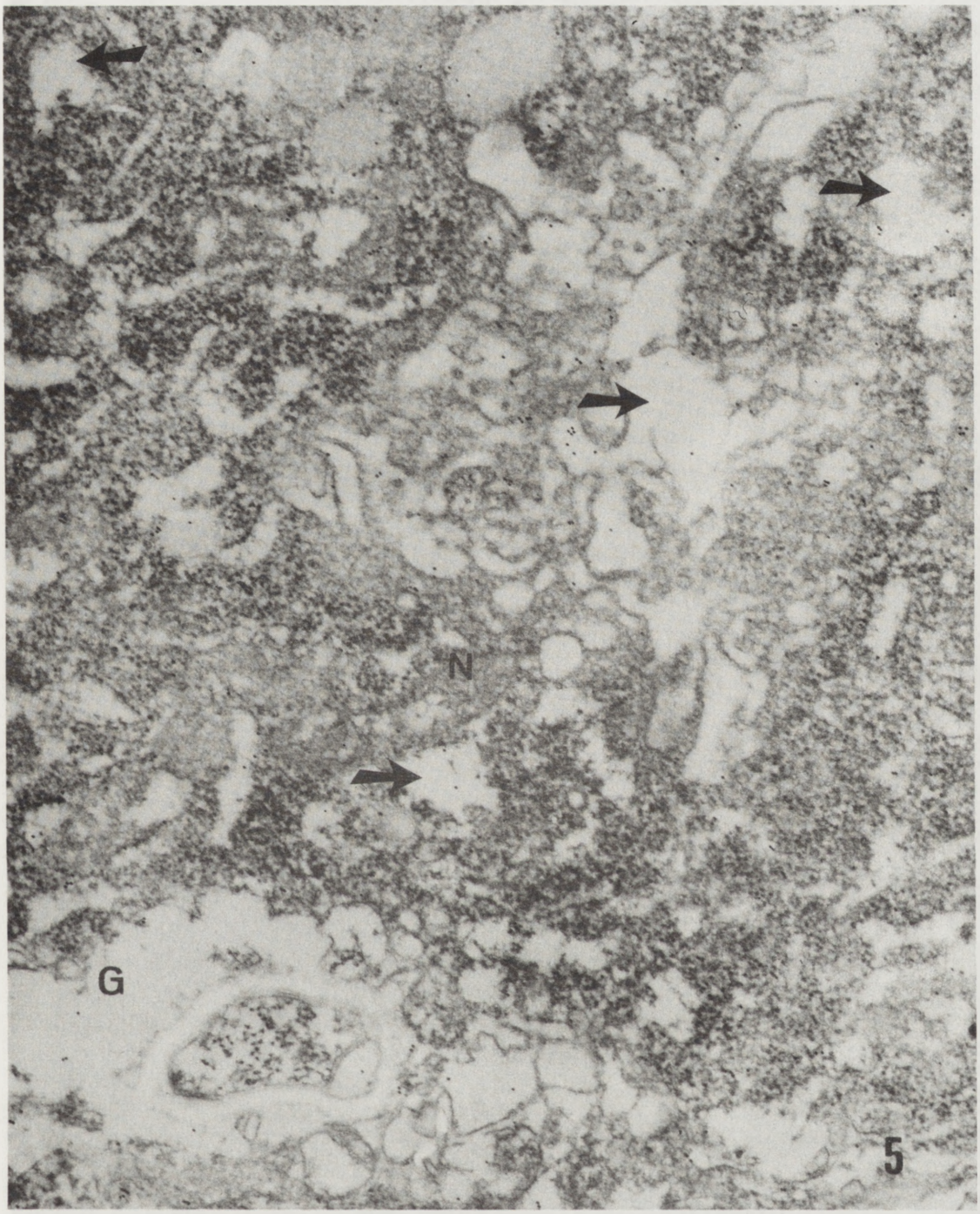

Fig. 5. Experimental animal, 1 h survival after ischemia. Reticular thalamic nucleus. Injured GABAergic neuron (N) with greatly widened Golgi and rough endoplasmic reticulum channels and cisterns. Gold particles are dispersed over damaged cytoplasmic structures. They are less numerous than in the control material. Single particles are also seen over greatly swollen glial process (G). × 36 000

concurrent with cytological changes observed in this postischemic stage in studies of Kawai et al. (1992) carried out on the same experimental model of cerebral ischemia. This what does not fit well in these two series of experiments concerns later sequences of neuronal changes. In our experiments both ultrastructural and immunocytochemical alterations were reversible. They were not observed in great proportion of GABAergic neurons 24 h after ischemia, contrary to the cytological picture described by Kawai (1992) who showed further progress of pathological process at that postischemic period. Perhaps this discrepancy depends on the fact that progression of cytological pathology was visible only in the particular parts of nucleus reticularis, its other portions remaining relatively normal.

The time sequences of immunocytochemical abnormalities observed in reticular nucleus of thalamus were in general similar to these in CA_1 hip-

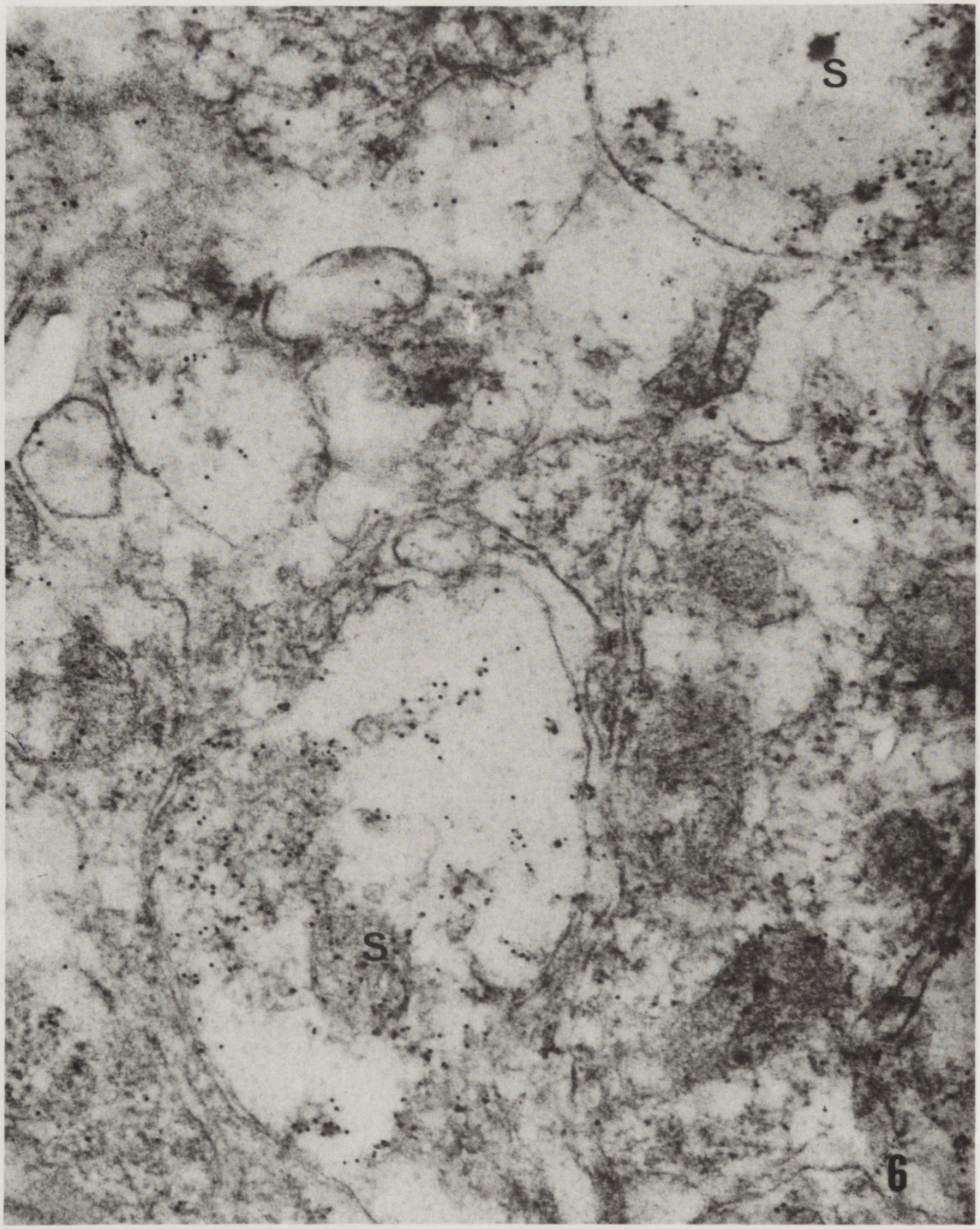

Fig. 6. Experimental animal, 1 h survival after ischemia. Reticular thalamic nucleus.Two symmetric synapses (S) greatly swollen with lower labeling with gold particles as compared with the control material. Note that gold particles are mostly covering the remaining synaptic vesicles. × 60 000

pocampal sector, described in our previous studies (Gajkowska et al. 1994) despite of essential differences of experimental models. So was outcome of the process, expressed by normalization of the immunocytochemical picture within the course of 24 postischemic hours. However, it seems that temporary decrease of GABAergic activity in the early postischemic period may be responsible for inhibitory disinhibition, enhancing damaging effect of excitotoxic neurotransmitters.

Niedokrwienie hamuje neurony GABAergiczne w jądrze siatkowatym wzgórza u szczurów. Badania immunocytochemiczne

Streszczenie

Przeprowadzono immunocytochemiczną analizę rozmieszczenia GABA w jądrze siatkowatym wzgórza u szczurów poddanych całkowitemu niedokrwieniu mózgu, towarzyszącemu doświadczalnie wywołanemu zatrzymaniu akcji serca. Badania wykonano po

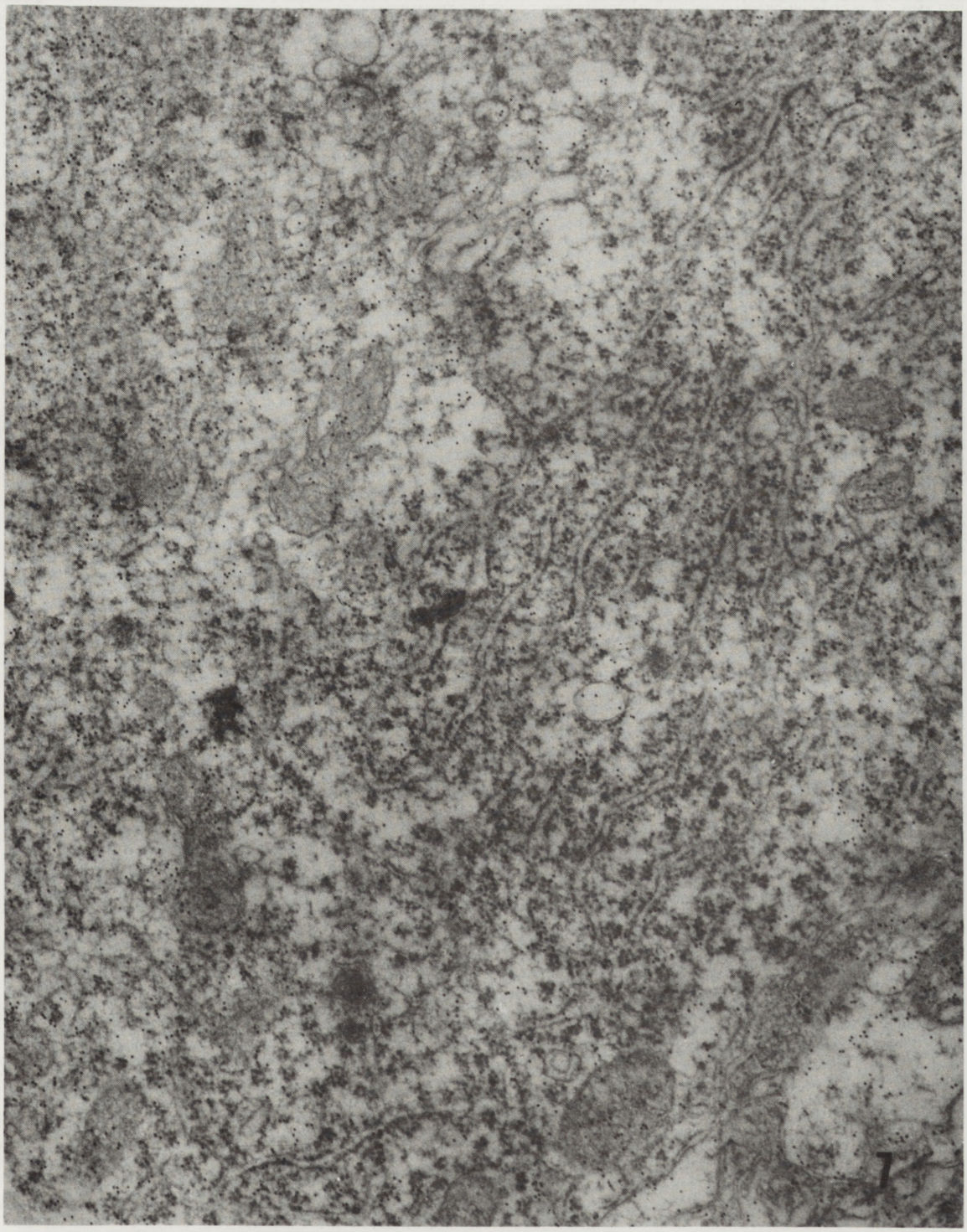

Fig. 7. Experimental animal, 24 h survival after ischemia. Reticular thalamic nucleus. Fragment of neuron with apparently normal ultrastructure, revealing strong labeling with gold particles, comparable with that observed in normal animals. × 36 000

upływie 10 min oraz 1 godz i 24 godz po niedokrwieniu, przy zastosowaniu techniki immunocytochemicznej z użyciem złota koloidalnego, wykorzystując surowice z przeciwciałami skierowanymi przeciwko konjugatom GABA z białkiem. Przejściowy spadek zawartości cząsteczek złota, wyznaczających rozmieszczenie GABA w perykarionach komórek nerwowych i w zakończeniach synaptycznych stwierdzono w okresie 10 min i 1 godz po niedokrwieniu. Spadek zawartości cząsteczek złota towarzyszył nieprawidłowościom obrazu ultrastrukturalnego zarówno neuronów jak i synaps. Nieprawidłowości te najczęściej przyjmowały postać obrzmienia o różnym nasileniu oraz dezorganizacji układu organelli cytoplazmatycznych. Nieprawidłowości immunocytochemiczne dotyczące perykarionów neuronalnych występowały wcześniej i były bardziej nasilone. Po upływie 24 godz immunoreaktywność większości komórek nerwowych i synaps przypominała obraz typowy dla zwierząt kontrolnych. We wszystkich grupach doświadczalnych obraz ultrastrukturalny niesymetrycznych synaps nie odbiegał od normy.

Uzyskane dane potwierdzają wysoką wrażliwość na niedokrwienie neuronów GABAergicznych jądra siatkowatego wzgórza i sugerują przejściowy charakter GABAergicznej niewydolności w następstwie niedokrwienia mózgu.

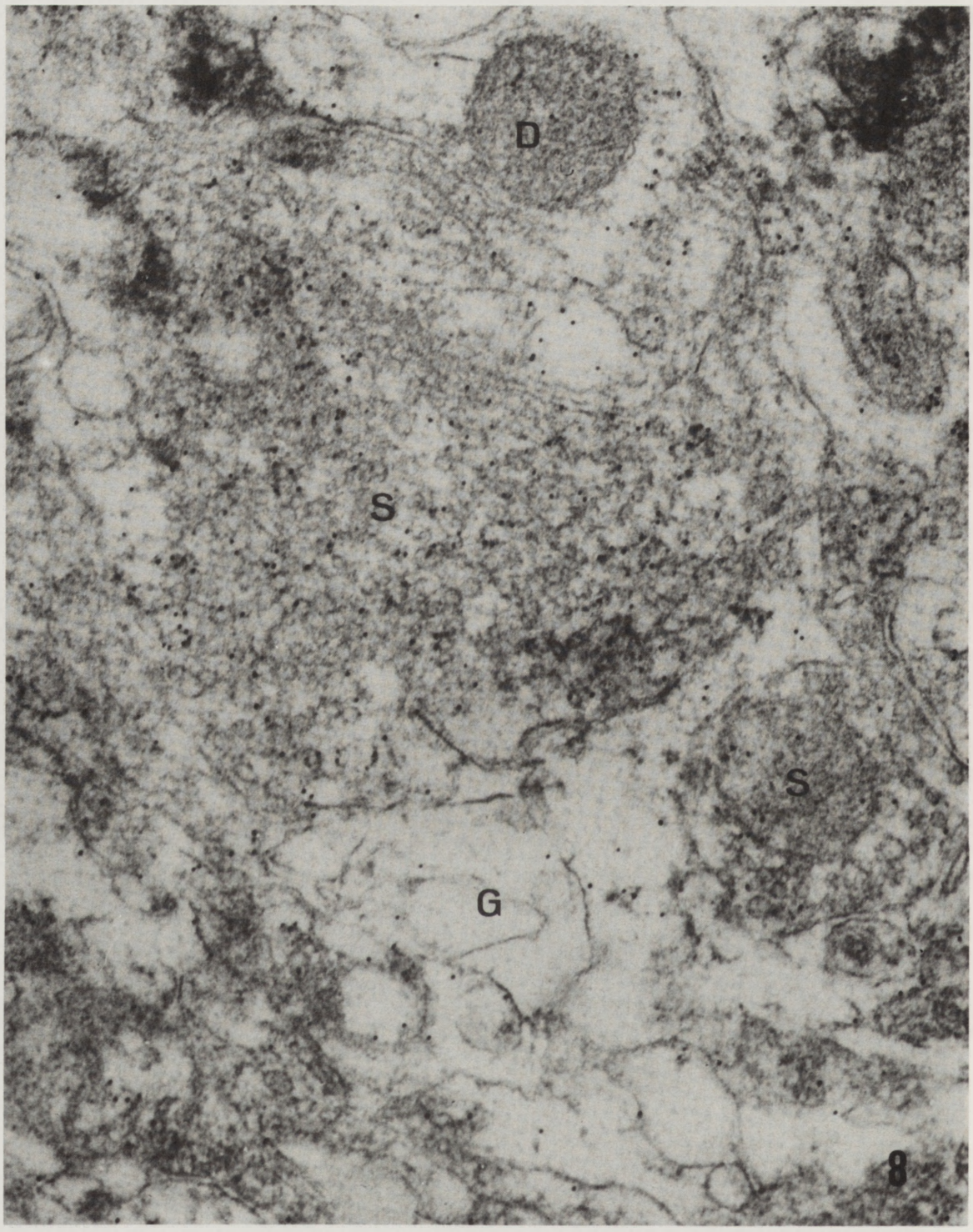

Fig. 8. Experimental animal, 24 h survival after ischemia. Reticular thalamic nucleus. High density of gold particles over axonal terminals participating in formation of symmetric synapses (S). Much lower particles density over dendrites (D) and swollen glial processes (G). × 60 000

References

1. Gajkowska B, Gadamski R, Mossakowski MJ: Influence of short-term ischemia on the ultrastructure of hippocampal gyrus in Mongolian gerbils. Part II. Electron microscope picture of synapses in early postischemic period. Neuropatol Pol, 1989, 27, 339–366.
2. Gajkowska B, Gadamski R, Mossakowski MJ: Influence of short-term ischemia on the ultrastructure of hippocampal gyrus in Mongolian gerbil. III. Synapses in late stage of the pathological process. Neuropatol Pol, 1992, 30, 13–28.
3. Gajkowska B, Gadamski R, Mossakowski MJ: Immunoelectron microscopic demonstration of GABA and glutamate synapses in Mongolian gerbil hippocampus after ischemia. Neuropatol Pol, 1994, 32 (in press)
4. Hallanger AE, Levey AJ, Lee HJ, Rye DB, Wainer RH: The origin of cholinergic and other subcortical afferents to the thalamus in the rat. J Comp Neurol, 1987, 262, 105–124.
5. Houser CR, Vaughn JE, Barber RP, Robertis E: GABA neurons are the major cell type of the nucleus reticularis thalami. Brain Res, 1980, 200, 341–354.

6. Johansen FF, Jorgensen MB, Diemer NH: Resistance of hippocampal CA_1 interneurons to 20 min of transient ischemia in the rat. Acta Neuropathol (Berl), 1983, 61, 135–140.
7. Johansen FF, Lin CT, Schousboe A, Wu J-Y: Immunocytochemical investigation of l-glutamic acid decarboxylase in rat hippocampal formation; the influence of transient cerebral ischemia. J Comp Neurol, 1989, 281, 40–53.
8. Kapuściński A: Cerebral blood flow in experimental model of clinical death (in Polish). Neuropatol Pol, 1987, 25, 287–298.
9. Kawai K, Nitecka L, Reutzler CA, Nagashima G, Joó F, Mies G, Nowak TS, Saito N, Lohr JM, Klatzo I: Global cerebral ischemia associated with cardiac arrest in the rat: I. Dynamics of early neuronal changes. Cereb Blood Flow Metab, 1992, 12, 238–249.
10. Kirino T: Presynaptic terminals in hippocampal gliosis following transient ischemia in Mongolian gerbil. In: Progress in Brain Research 96. Eds: K. Kogure, K-A Hossmann, BK Siesjö. Elsevier, Amsterdam, New York, 1993, pp 261–270.
11. Korpachev G, Lysenkow SP, Tiel LZ: Modielirowanje kliniczeskoj smierti i postreanimationnoj bolezni u krys. Patol Fizjol Eks Ter, 1982, 3, 78–80.
12. Mossakowski MJ, Gajkowska B, Tsitsishvili A: Ultrastructure of neurons from the CA_1 sector of Ammon's horn in short-term cerebral ischemia in Mongolian gerbils. Neuropatol Pol, 1989, 27, 39–53.
13. Mossakowski MJ, Hilgier W, Januszewski S: Pathomorphology of the central nervous system in experimental postresuscitation syndrome (in Polish). Neuropatol Pol, 1986, 24, 471–489.
14. Mulle C, Madariaga A, Deschênes M: Morphology and electrophysiological properties of reticularis thalamic neurons in cat. J Neurosci, 1986, 6, 2134–2145.
15. Nitsch C, Goping G, Klatzo I: Preservation of GABAergic perikarya and boutons after transient ischemia in the gerbil hippocampal CA_1 field. Brain Res, 1989, 495, 243–252.
16. Pulsinelli WA, Brierley JB, Plum F: Temporal profile of neuronal damage in a model of transient forebrain ischemia. Ann Neurol, 1982, 11, 491–498.
17. Ross DT, Duhaime AC: Degeneration of neurons in the thalamic reticular nucleus following transient ischemia due to raised intracranial pressure: excitotoxic degeneration mediated via non-NMDA receptors? Brain Res, 1989, 501, 129–143.
18. Schlander M, Hoyer S, Frotscher M: Glutamate decarboxylase-immuneoractive neurons in the aging rat hippocampus are more resistant to ischemia than CA_1 pyramidal cells. Neurosci Lett, 1988, 241–246.
19. Schmidt-Kostner R, Freund TF: Selective vulnerability of the hippocampus in brain ischemia. Neurosci, 1991, 40 599–636.
20. Smith M-L, Auer RN, Siesjö BK: The density and distribution of ischemic brain injury in the rat following 2–10 min of forebrain ischemia. Acta Neuropathol (Berl), 1984, 64, 319–332.
21. Spreafico F, De Curtis M, Frassoni C, Avanzini G: Electrophysiological characteristics of morphologically identified reticular thalamic neurons from rat brain slices. Neurosci, 1988, 27, 629–638.
22. Yen CT, Conley M, Hendry SHC, Jones EG: The morphology of physiologically identified GABAergic neurons in the somatic sensory part of the thalamic reticular nucleus in the cat. J Neurosci, 1985, 5, 2254–2268.
23. Woolf NJ, Butcher LL: Cholinergic projection of the rat brain. III. Projections from the pontomesencephalic tegmentum to the thalamus, tectum, basal ganglia and basal forebrain. Brain Res Bull, 1986, 16, 603–637.

Authors' address: Medical Research Centre, Polish Academy of Sciences, Dworkowa 3 St., 00-784 Warszawa