ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Acta Neurobiol. Exp. 1994, 54: 219-225

Effects of staphylococcal α-toxin on the ultrastructure of the rat hypothalamo-neurohypophyseal system

Barbara Gajkowska[1], Roman Gadamski[2] and Małgorzata Frontczak-Baniewicz[1]

[1]Laboratory of the Ultrastructure of the Central Neurons System and [2]Department of Neuropathology, Medical Research Centre, Polish Academy of Sciences, 3 Dworkowa St., 00-784 Warsaw, Poland

Abstract. The influence of staphylococcal α-toxin on the ultrastructure of hypothalamo-neurohypophysical system in the brain (nucleus supraopticus, nucleus paraventricularis, neurohypophysis) was studied in the rat. In neurohypophysis, an area lacking blood-brain barrier, α-toxin damaged both neuronal endings and capillary vessels. On the other hand in hypothalamus, where blood-brain barrier is present structural alterations were much less pronounced. Reactive gliosis, accordant with cell damage, was observed in the entire neurosecretory system. Putative mechanisms leading to brain damage after systemic administration of α-toxin, including direct disruption of cell membrane and induction of nitric oxide synthesis, are discussed.

Key words: staphylococcal, α-toxin, nucleus supraopticus, nucleus paraventricularis, neurohypophysis, rat

INTRODUCTION

Staphylococcal α-toxin is a water-soluble 33 kD polypeptide secreted by Staphylococcus aureus. This substance, being lethal for most laboratory animals, has haemolytic and dermonecrotic properties. The toxin has been shown to damage artificial membranes (Tomita et al. 1992) and a variety of mammalian cells including erythrocytes (Cassidy and Harshman 1976), platelets, monocytes (Bhakdi 1990), pulmonary endothelial cells (Suttorp et al. 1985), alveolar macrophages (McGee et al. 1983), and adrenocortical Y1 cells (Thelstam and Blomquist 1984).

It has been established by Füssle et al. (1981) that α-toxin is assembled from monomers to hexamers inserted in the plasma membrane. There is evidence for a causal relationship between hexamer formation and the induction of functional membrane lesions, hexamers forming ion-permeable trans-membrane pores (Ikigai and Nakae 1987).

Besides circulatory disturbances induced by α-toxin, the central nervous system has been considered to be a putative target for its lethal action. The results showing binding the toxin to the myelin with myelin disruption (Harshman et al. 1985), electroencephalographic studies (Edelwein and Jeljaszewicz 1973), and *in vivo* distribution of ^{131}I-labelled toxin (Jeljaszewicz et al. 1963) imply a neurotoxic action of α-toxin. In this study we show that neurotoxic effects of α-toxin in brain hypothalamo-neurohypophyseal system are particularly pronounced in neurohypophysis, a region devoid of blood-brain barrier.

METHODS

The experiments were performed on 10 Wistar rats, females weighing 170 g. Solution of α-toxin was prepared according to Wadstrom (1968) and 0.5 ml of the diluted toxin (1:20) was injected into the pedal vein of 5 animals under anaesthesia of 4% halothane in 30:70 v/v O_2/N_2O mixture. 0.5 ml of physiological saline instead of toxin was injected in 5 control rats. The animals were sacrificed after 48 h and transcardially perfused with 2.5% glutaraldehyde in phosphate buffer (pH 7.4). The brains were isolated and additionally fixed in the same solution. At the end of the postfixation period the tissue samples containing paraventricular and supraoptic nuclei, and the nervous part of the pituitary gland were treated with 2% OsO_4 dissolved in the same buffer. Subsequently, the samples were dehydrated in the increasing concentrations of alcohol and propylene oxide, and embedded in Epon 812. The ultrathin sections were contrasted in aqueous solution of uranyl acetate and lead citrate. The microphotographs were taken in JEM 1200 EX electron microscope.

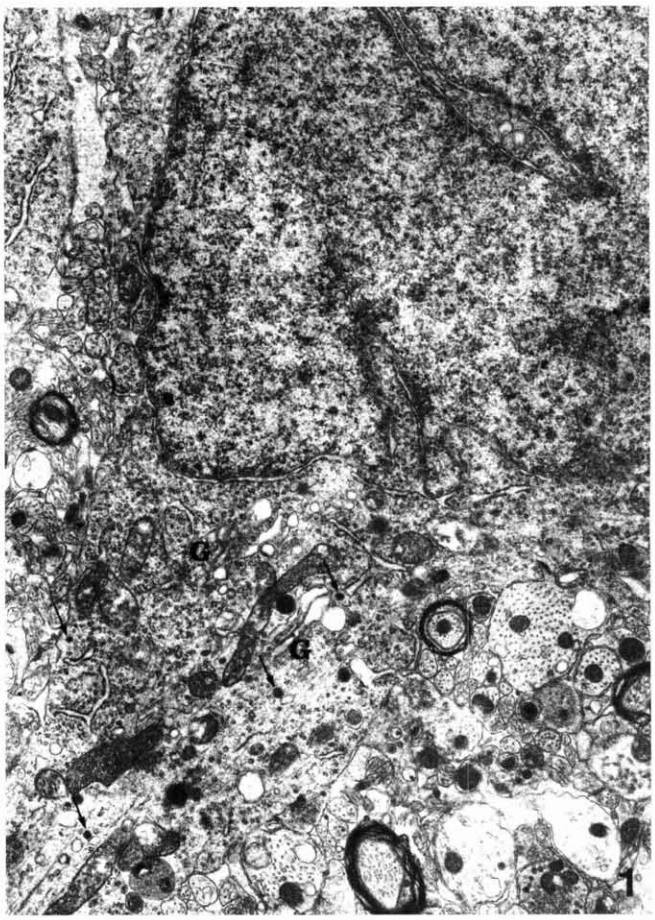

Fig. 1. Nucleus supraopticus, control animal. Fragment of normal neuron with developed Golgi comples (G) and many neurosecretory granules (arrow). x10,000.

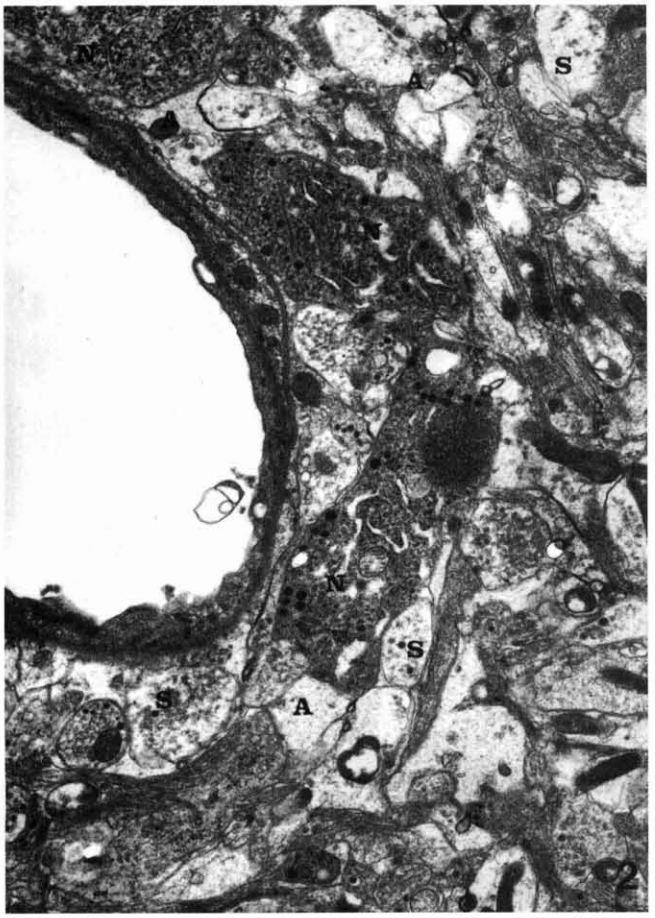

Fig. 2. Nucleus paraventricularis, 48 h after administration of α-toxin. Ultrastructurally normal capillary vessel and fragment of neurosecretory cells (N) with multiple neurosecretory granules in the perivascular area. Note some swollen astrocytic processes (A) and synapses (S). x18,000.

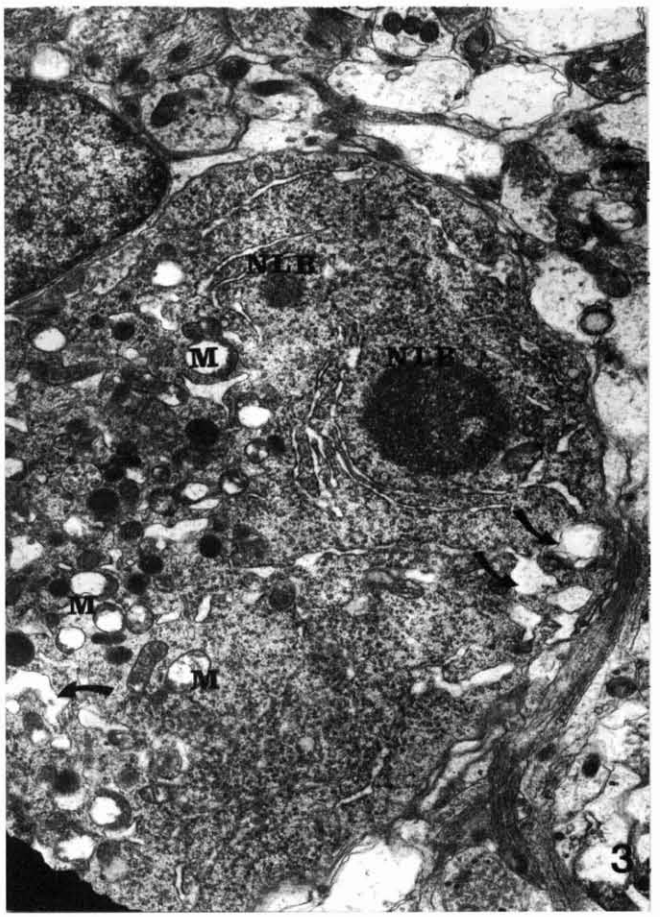

Fig. 3. Nucleus supraopticus, 48 h after administration of α-toxin. Large number of lysosomes, swollen mitochondria (M) and nucleolus-like bodies (NLB) in the cytoplasm. Note abnormal dilation of rough endoplasmic reticulum (arrows). x18,000.

RESULTS

Hypothalamus: nuclei supraopticus (SO) and nucleus paraventricularis (PV) 48 h after administration of α-toxin

Neurones in SO and PV showed similar reaction to α-toxin, and therefore the ultrastructural changes in these two nuclei will be described jointly. Several types of neurones were observed. The most often encountered were ultrastructurally normal neurones, containing giant nuclei with large invaginations, well-developed rough endoplasmic reticulum and Golgi complex, moderate amount of secretory granules, mitochondria, and lysosomes (Fig. 1). In the vicinity of blood vessels, neurones with abundant secretory granules in perikaryon and cell-processes were found (Fig. 2). Neurones with swollen mitochondria (with echolucent matrix and remnants of cristae) were also sporadically encountered. In these cells number of lysosomes was increased. Rough endoplasmic reticulum was well-developed, and in the peripheral areas of the cell it presented inflated channels forming cisterns devoid of ribosomes. The globular aggregates of electron-dense granular material formed "nucleus-like" bodies (Fig. 3). Some other neurons were shrunk and contained dense matrix, abundant amount of lysosomes

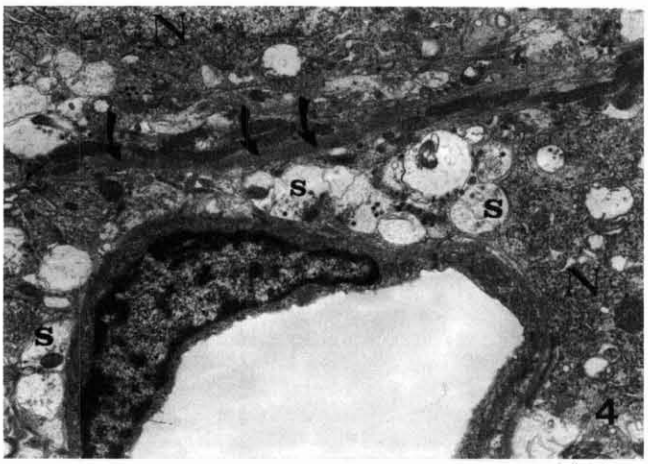

Fig. 4. Nucleus supraopticus, 48 h after administration of α-toxin. Two slightly changed secretory neurones (N) in the perivascular area, swollen synapses (S) and an astrocytic process with large number of gliofibrilles (arrows). x8,500.

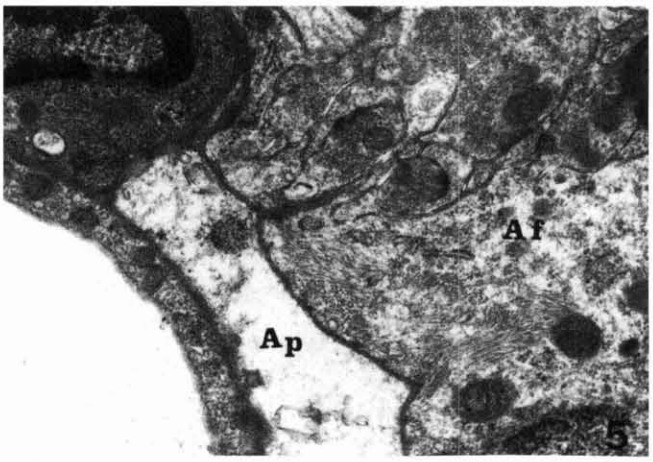

Fig. 5. Nucleus paraventricularis, 48 h after administration of α-toxin. Fragment of a capillary vessel with ultrastructurally unchanged endothelial cell and pericyte. Markedly swollen protoplasmic astrocytic process (Ap) and fibrillar astrocytic process with abundant gliofilaments (Af). x10,000.

and dense bodies (so-called dark neurones). Microglial cells were always found in their neighbourhood. Synapses with the ultrastructurally-abnormal neurones were swollen in their presynaptic parts, and contained echolucent cytoplasm, small amount of synaptic vesicles in bizarre configurations, and mitochondria.

Structurally-abnormal astrocytes were encountered neuropil surrounding PV and SO neurones. In the majority of fibrillar astrocytes there was excessive amount of gliofibrils in the perikaryon and processes. The perivascular processes of protoplasmic astrocytes were swollen, contained echolucent cytoplasm and remnant organelles (Fig. 4). Glia surrounding capillaries was often abnormal; some glial processes were swollen, the others having excessive amount of gliofibrils (Figs. 2, 4 and 5).

Endothelium, pericytes and basement membranes of capillary vessels did not show significant ultrastructural abnormalities.

Neurohypophysis (PN) - 48 hours after administration of α-toxin

Normal ultrastructure of neurohypophysis is showed in Fig. 6. In the experimental group critical abnormalities in the ultrastructure of all tissue elements in neurohypophysis were found (Fig. 7). The most significant changes were observed in the perivascular area. Many axonal fibres were severely damaged, their plasma membrane was broken and organelles translocated into the dilated perivascular space. Many fibres contained small amount of neurosecretory granules, but instead increased number

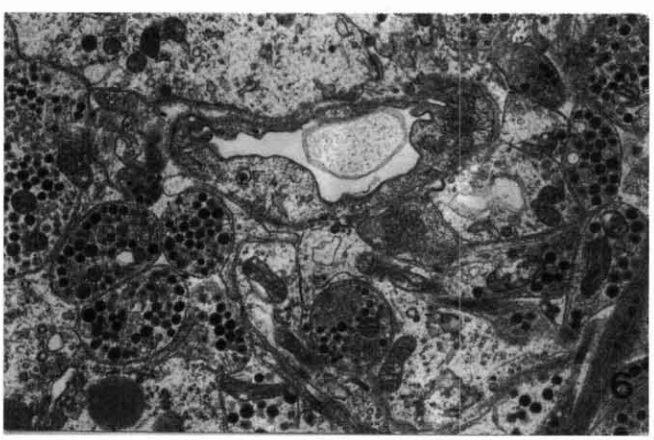

Fig. 6. Neurohypophysis, control animal. Ultrastructurally unchanges axon profiles containing neurosecretory granules, mitochondria, microvesicles, and neurotubules, remaining in a close contact with unchanged pituicytes and capillaries. x10,000.

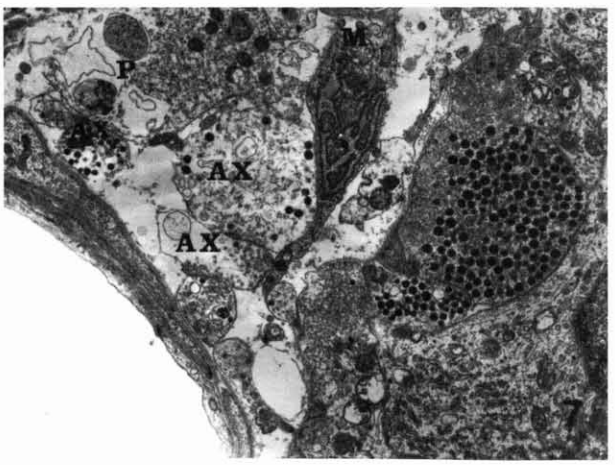

Fig. 7. Neurohypophysis, 48 h after administration of α-toxin. Axon profiles (Ax) and pituicytes (P) exhibiting signs of degeneration with interrupted cell membrane bilayer and extruded organelles. Note presence of microglial cells (M) near the perivascular area. x8,500.

of lysosomes, dense bodies, myelin figures, and abnormal mitochondria. In the neighbourhood of the damaged fibres, microglial cells with the signs of activation (wide channels of rough endoplasmic reticulum filled with a electron-dense substance, large amount of ogranelles) were observed (Fig. 7). Some pituicytes were structurally abnormal with increased number of lipid droplets and big, irregular, electron-lucent vacuoles. These cells contained fragments of axonal fibres.

The majority of vessels were structurally abnormal and showed features of considerable damage. Endothalium was thin, the individual endothelial cells contained dilated or opened tigh junctions, with deficiencies in the continuity of basement membrane. The perivascular space was dilated, electron-lucent, and occasionally contained remnants of organelles, such as neurosecretory granules, microvesicles, and fragments of collagen fibres (Fig. 8).

DISCUSSION

The analysis of ultrastructural changes in hypothalamo-neurohypophyseal system revealed that especially the neurohypophysis is susceptible to the

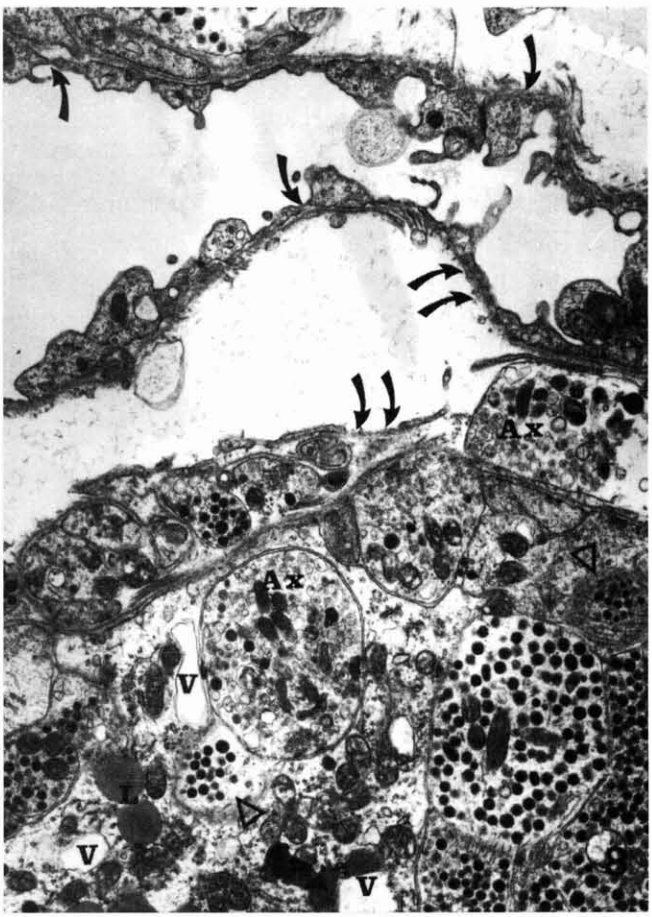

Fig. 8. Neurohypophysis, 48 h after administration of α-toxin. Ultrastructurally changed capillary vessel with flattened endothalial cells, open tight junctions, and interrupted basement membrane (arrows). Abnormal axon fobres (Ax) and pituicyte (P) near a dilated perivascular space. Note multiple electron-lucent vacuoles (V), liposomes (L), and disintegrated axonal profiles (arrowheads) in the cytoplasm of a pituicyte. x10,000.

injury imposed by the systemic administration of staphylococcal α-toxin. In SO and PV only a minor fraction of neurones showed features of upregulation of neurosecretory granule biosynthesis. Some of cells was ultrastructurally damaged with characteristics suggesting a mild retardation and inhibition of neurosecretion. In the axons and endings of PV and SO neurones, forming the neural portion of the pituitary gland, the ultrastructural picture suggested a severe impairment of the process of neurosecretion. It is thus conceivable that the action of α-toxin reduces secretion of the hormones into the circulation.

Microglia in SO, PV and neurohypophysis showed morphological features of activation, being in a close surface communication with the structurally changed neurones and synapses. This, together with an apparent activation of fibrillar astrocytes and pituicytes suggests an activation of macrophage properties of glia as a response to cell damage triggered by α-toxin. Activated fibrillar astrocytes are immunologically competent cells functionally similar to macrophages and microglia (Zaręba-Kowalska et al. 1983, Malipiero et al. 1990). Reactive gliosis, such as observed in this study, is a typical response to injury within the territory of the central nervous system (Giulian 1993). It has been established that astrocytes may regulate synapse formation in the neurosecretory system; glial retraction and associated synaptogenesis have been proposed to synchronise cellular activity which is an indispensable phenomenon for a proper pulsate hormone release (Hatton et al. 1984). Our results may thus suggest that astroglial and microglial cells are involved in central neuronal survival in SO and PV.

The exact mechanism of the deleterious action of α-toxin in the central nervous system has not been fully elucidated. This toxin may directly damage cell membranes as described by Freer and Arbuthnott (1983), but an indirect action via nitric oxide (NO) is also possible. It has been recently showed that α-toxin stimulates NO synthetase in endothelial cells (Suttorp et al. 1993), and NO in the central nervous system is considered to be a neurotoxic agent (reviewed by Bredt and Snyder 1992). NO is synthesised mainly in the endothelium and in the post-synaptic neural elements, and diffuses freely into other cells (Garthwaite 1991).

The finding of only minor, ultrastructural abnormalities in capillary vessels in SO and PV is in accordance with previous observations that α-toxin does not cross blood-brain barrier in this area (Blumquist et al. 1987). In contrast, in neurohypophysis which lacks blood-brain barrier, we observed major ultrastructural alterations, exemplified by presence of wide, irregular perivascular spaces, membrane lesions, and degeneration of a few axonal endings and pituicytes. These changes may be attributed to a free penetration of the toxin through the capillary wall.

In conclusion, this study provides evidence for the deleterious action of staphylococcal α-toxin in the hypothalamo-neurohypophyseal system in brain. It seems likely, that the damage triggered by the toxin depends on the patency of blood-brain barrier.

REFERENCES

Bhakdi S., Muhly M., Korom S., Schmidt G. (1990) Effect of Escherichia coli haemolysin on human gonocytes: cytocidal action and stimulation of interleukin 1 release. J. Clin. Invest. 85: 1746-1751.

Blomquist L., Appelgren L.E., Thelestam M. (1987) Distribution of ^{3}H-labelled staphylococcal α-toxin and toxin fragment in mice. Infect. Immunol. 55: 1906-1913.

Bredt D.S., Snyder C.H. (1992) Nitric oxide, a novel neuronal messenger. Neuron 8: 3-11.

Cassidy P., Harshman S. (1976) Studies on the binding of staphylococcal ^{125}I-labelled α-toxin to rabbit erythrocytes. Biochemistry 15: 2348-2355.

Edelwein Z., Jeljaszewicz J. (1993) Influence of staphylococcal α hemolysin on cortical sensory evoked potentials. Toxicol. Appl. Pharmacol. 25: 1-6.

Freer J., Arbuthnatt J.P. (1983) Toxins of Staphylococcus Aureus. Pharmacol. Ther. 19: 55-76.

Füssle R., Bhakdi S., Sziegaleit A., Tranum-Ensen J., Kranz T., Wellensick H.-J. (1981) On the mechanism of membrane damage by Staphylococcus aureus α-toxin. J. Cell Bol. 91: 83-94.

Garthwaite J. (1991) Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurol. Sci. 14: 60-67.

Giulian D. (1993) Reactive glia as rivals in regulating neuronal survival. Glia 7: 102-110.

Harshman S., Burt A.M., Robinson J.P., Bankenship M., Harshman D.L. (1985) Disruption of myelin sheaths in mouse brain in vitro and in vivo by staphylococcal α-toxin. Toxicon 23: 801-806.

Hatton G.J., Perlmutter L.S., Salm A.K., Tweedle C.D. (1984) Dynamic neuron-glial interactions in hypothalamus and pituitary: implications for hormone synthesis and release. Peptides 5: 121-138.

Ikigai H., Nakae T. (1987) Assembly of the α-toxin hexamer of Staphylococcus aureus in the liposome membrane. J. Biol. Chem. 262: 2150-2155.

Jeljaszewicz J., Szmigielski S., Zak C. (1968) Distribution of ^{131}I-labelled Staphylococcal α hemolysin in the rabbit. Zentbl. Bakt. Parasitkde. 209: 310-315.

Malipiero U.V., Frei K., Fontana A. (1990) Production of hemopoietic colony-stimulating factors by astrocytes. J. Immunol. 144: 3816-3812.

McGee M., Kreger A., Leake E.S., Harshman S. (1983) Toxicity of staphylococcal α toxin for rabbit alveolar macrophages. Infect. Immunol. 39: 439-444.

Suttorp N., Fuhrmann M., Tannertotto S., Brimminger F., Bhakdi S. (1993). Pore-forming bacterial toxic potently induce release of nitric oxide in porcine endothelial cells. J. Exp. Med. 178: 337-341.

Suttorp N., Seeger W., Dewein E., Bhakdi S., Roka L. (1985) Staphylococcal α-toxin-induced PGI_2 production in endothelial cells: role of calcium. Am. J. Physiol. 248: C127-C132.

Thelestam M., Blomquist L. (1984) Intoxication of cultured mouse adrenocortical tumor cells by staphylococcal α-toxin. In: Bacterial protein toxins (Eds. J.E. Alouf, F.J. Fehrenbach, J.H. Ereer and J. Jeljaszewicz). Academic Press, London, p. 279-285.

Tomita T., Watanabe M., Yasuda T. (1992) Influence of membrane fluidity on the assembly of Staphylococcus aureus α-toxin, a channel-forming protein, in liposome membrane. J. Biol. Chem. 267: 13391-13397.

Wadstrom T. (1968) Studies on extracellular proteins from Staphylococcus aureus. IV. Separation of α-toxin by electric focusing. Biochem. Biophys. Acta 168: 228-242.

Zaręba-Kowalska M., Renkawek K., Gajkowska B. (1983) Ultrastructural study of hypophysial neural lobe of newborn rats in tissue culture. Cell Tissue Res. 230: 463-468.

Received 24 January 1994, accepted 22 March 1994