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ORIGINAL ARTICLE

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Immunohistochemical investigation of hypoxic/ischemic brain damage in forensic autopsy cases

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Abstract A neuropathological study of 41 forensic autopsy cases of hypoxic/ischemic brain damage has been undertaken, using immunohistochemical staining to detect the 70-kDa heat shock protein (hsp70) and the status of the glial cells. In cases surviving 2–5 h after hypoxic/ischemic injury, ischemic cell changes were seen whereas glial reactions were not apparent. In cases of longer survival, neuronal necrosis and a loss of neurons were seen, and these changes were accompanied by proliferation of glial fibrillary acidic protein (GFAP), vimentin-positive astrocytes and microglia which transformed into rod cells or lipid-laden macrophages. In cases with a history of hypoxic attacks, GFAP-positive and vimentin-negative astrocytes had proliferated in the CA3 and CA4 regions of hippocampus. The cases of severe hypoxic injury, such as an asthmatic attack and choking, showed no ischemic changes in the hippocampal neurons. On the other hand, the CA1 pyramidal cells showed neuronal necrosis in a patient suffering from tetralogy of Fallot (TOF), who survived for 2 h after a traffic accident. Therefore, it is suggested that even moderate hypoxic injury induces astrocytosis in the CA3 and CA4 regions and may affect the neuronal proteins and the metabolism, and that in cases with a history of hypoxic attacks neuronal damage may be severe even several hours after ischemic injury. The protein hsp70 expression was found in the CA2, CA3 and CA4 regions in cases of long-term survival after severe hypoxic/ischemic injury and in cases of alcoholic intake or toluene abuse just before acute death. Thus, it is suggested that the detection of hsp70 in the hippocampus indicates hypoxic/ischemic injury or other stress prior to death. In forensic practice, immunohistochemical investigation of the hsp70 and glial cell staining can be of great value for diagnosing not only hypoxic/ischemic brain damage during the process of death but also the victim's past history of hypoxic attacks.

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Key words Hypoxic/ischemic brain damage
Immunohistochemistry · Neurons · Heat shock protein
Glial cell

Zusammenfassung Eine neuropathologische Studie von 41 forensischen Autopsie-Fällen mit hypoxischen/ischämischen Hirnschäden wurde durchgeführt, um das 70-kDA Hitzeschock-Protein (hsp70) und den Zustand der Gliazellen zu untersuchen. In Fällen, in denen der hypoxisch-ischämische Schaden 2–5 Stunden überlebt wurde, waren ischämische Schäden erkennbar, während Glia-Reaktionen noch nicht vorhanden waren. In Fällen längerer Überlebenszeit war ein neuronale Nekrose und ein Verlust von Neuronen zu beobachten, und diese Veränderungen waren begleitet von einer Proliferation des glialen fibrillären sauren Proteins (GFAP), der Vimentin-positiven Astrozyten und der Mikro-Glia, welche in stabförmige Zellen oder lipidbeladene Makrophagen transformierte. In Fällen mit einer Anamnese von hypoxischen Attacken war eine Proliferation GFAP-positiver und Vimentin-negativer Astrozyten in der CA3- und CA4-Region des Hippocampus zu beobachten. Die Fälle mit schwerem hypoxschämischen Schaden, wie Asthma-Anfall und Strangulation, zeighten keine ischämischen Veränderungen in den Neuronen des Hippocampus. Andererseits zeigten die CA1-Pyramiden-Zellen bei einem Patienten mit Fallot'scher Tetralogie (TOF), welcher zwei Stunden nach einem Verkehrsunfall starb, eine neuronale Nekrose. Daher wird vermutet, daß auch weniger schwere hypoxische Schäden eine Astrozytose in der CA3- und CA4-Region induzieren und einen Einfluß haben dürften auf die neuronalen Proteine und auf den Metabolismus und daß in Fällen mit einer Anamnese hypoxischer Attacken der neuronale Schaden schwer sein kann, sogar mehrere Stunden nach dem ischämischen Schaden. Das Protein hsp70 wurde in den CA2-, CA3- und CA4-Regionen in Fällen langzeitigen Überlebens nach schweren hypoxischen/ischämischen Schäden gefunden und in Fällen, in denen kurz vor dem Tode eine Alkoholaufnahme oder Toluol-Mißbrauch stattfand. Daher wird vermutet, daß ein Nachweis hsp70 im Hypocampus eine hypoxischen Scha-

den oder einen anderen Streß kurz vor dem Tode anzeigt. In der forensischen Praxis sind die immunhistochemische Untersuchung von hsp70 und Gliazell-Färbungen von großer Bedeutung für die Diagnostik nicht nur des hypoxisch-ischämischen Hirnschadens während des Sterbeprozesses, sondern auch für die Diagnostik der Anamnese des Opfers im Hinblick auf hypoxische Attacken.

Schlüsselwörter Hypoxisch-ischämischer Hirnschaden Immunohistochemie · Neuron · Hitzeschock-Protein Gliazelle

Introduction

It is well known that CA1 pyramidal cells and neurons in the third, fifth and sixth neocortical layers are most vulnerable to hypoxic/ischemic injury [1, 5, 7, 9–11, 23, 25, 32]. Few neuropathological changes occur in the neurons immediately after hypoxic/ischemic injury, but neuronal ischemic changes begin to appear in human brain tissue after several hours. Furthermore, neuronal necrosis and/or a loss of neurons is found in patients after long-term survival [5, 7, 9–11, 25, 32].

Astrocytes react to hypoxic/ischemic injury by conversion to reactive forms and proliferation in the regions showing neuronal necrosis [3–5, 18, 26, 27, 36]. Microglia are transformed into rod cells that have rod-shaped nuclei and processes in the regions showing neuronal necrosis [3, 5, 17, 21]. In the destroyed tissue, these microglia become rounded and contain fat droplets in their cytoplasm and are termed lipid-laden macrophages or lipid phagocytes [5]. These glial reactions are proportional to the extent of neuronal alterations [3–5, 17, 18, 21, 26, 27].

Exposure of the cells to various forms of stress, such as heat, ischemia and alcohol, induces the synthesis of a heat shock protein species [2, 15, 30, 31], and in an immunohistochemical study, the indication of a 70-kDa heat shock protein (hsp70) was found to be far lower in the CA1 pyramidal cells than in the neurons of the CA3 region. These findings suggest that heat shock proteins, such as hsp70, may play a important role in protection against hypoxic/ischemic injury and other pathological conditions [2, 15, 30, 31, 35].

The present study examined morphological changes in the neurons, the hsp70 expression in the neurons, and the glial reactions in various types of hypoxic/ischemic brain damage which were characterized by duration and extent of the hypoxia and/or ischemia and the survival time. In addition, the neuropathological findings in the brains of subjects with a history of hypoxic attacks were compared to those with no history of hypoxic attacks. For this investigation, immunohistochemical techniques were used for the staining of hsp70, and the glial cells were investigated using glial fibrillary acidic protein (GFAP) and vimentin as markers for astrocytes and the lecting *Recinus Communis* agglutinin-1 (RCA-1) as the microglial marker.

Materials and methods

The autopsy cases and the examined cerebral regions

The samples used for this study came from forensic autopsy cases, and the cases were divided into 3 groups. Group 1 (n = 33): acute death with no history of hypoxic attacks (Table 1); Group 2 (n = 4): long-term survival after hypoxic/ischemic injury with no history of hypoxic attacks (Table 2); Group 3 (n = 4): acute death or long-term survival with a history of hypoxic attacks (Table 2). The cases in groups 1 and 2 were relatively healthy before hypoxic/ischemic injury. In group 3, the patients had no other pathological conditions which might have caused damage to the brain, such as

Table 1 The causes of death in the cases in group 1

Cause of death	Number
Strangulation	8
Drowning	5
Ischemic heart disease	5
Automotive injuries	4
Bleeding	3
Hanging	2
Carbon monoxide intoxication	2
Choking	2
Respiratory failure	1
Traumatic subarachnoidal hemorrhage	1
Total	33

Table 2 Summary of the cases in group 2 (cases 1–4) and in group 3 (cases 5–8)

Case	Age (years)	Sex	Cause of death	Survival time	Past history of hypoxic episodes
1	42	M	Choking due to inhalation of blood	2–4 h	None
2	59	M	Bleeding	5 h	None
3	42	F	Pneumonia Etiology of hypoxia/ischmia: strangulation	18 d	None
4	45	M	Pneumonia Etiology of hypoxia/ischemia: acute respiratory failure	120 d	None
5	29	M	Bronchial asthma	1 h	Bronchial asthma for 10 years
6	41	F	Bronchial asthma	4–5 h	Bronchial asthma for 5 years
7	57	M	Choking due to a foreign body	8–10 h	Total fibrosis of the left lung over 30 years
8	61	M	Cardiorespiratory failure after a traffic accident	2 h	Tetralogy of Fallot

cerebrosvascular disease, congestive heart failure, and anemia. Tissues from the hippocampus and the arterial boundary zone of the parieto-occipital lobe were used for this study.

Formalin-fixed samples were embedded in paraffin and 5 μm sections were prepared.

Immunohistochemical staining methods

The avidin-biotin complex (ABC) method was used for the immunohistochemical procedure, as described previously [17, 18, 27, 35].

Deparaffinized tissue sections were incubated with the primary antibodies, mouse monoclonal antibody specific for stress-inducible forms of hsp70-related proteins (dilution 1:300), rabbit anti-GFAP polyclonal antibody (dilution 1:300), mouse monoclonal anti-vimentin antibody (dilution 1:25) and biotinylated RCA-1 lectin (dilution 1:200). The sections were incubated with the avitin-biotin complex, using a Vectastain ABC kit. The peroxidase reaction was accomplished by incubation with 3,3′-diaminobenzidine tetrahydrochloride and hydrogen peroxidase, and the negative controls were carried out by omitting the primary antibody or the lectin. Hsp70 immunoreactivity was examined only in the hippocampus. All procedures were carried out at room temperature. All sections were also investigated by hematoxylin and eosin (H & E) and luxol fast blue/cresyl violet staining.

Biotinylated RCA-1 lectin and a Vectastain ABC kit were purchased from Vector Laboratories. Rabbit anti-GFAP antibody and mouse monoclonal anti-vimentin antibody were purchased from DAKO, and the mouse monoclonal antibody specific for stress-inducible forms of hsp70-related proteins from Amersham.

Results

Group 1

Morphological changes in neurons and microglial reactions were not seen in this group. In 4 cases the hippocampus showed an immunoreactivity in the cytoplasm of many neurons and in the nucleus of some neurons in the CA2, CA3 and CA4 regions. These included the cases of alcoholic intake before death and toluene abuse just be-

Table 3 Summary of the findings in the cases of group 2 (cases 1–4) and group 3 (cases 5–8)

Case	Region of brain	Neurons		Astrocytes		Microglia[e]
		Morphological changes[a]	Hsp70[b]	GFAP[c]	Vimentin[d]	
1	CA1	+	–	–	–	–
	CA2	–	+	–	–	–
	CA3/4	–	+	–	–	–
	Neocortex	+	NT	–	–	–
2	CA1	+	–	–	–	–
	CA2	–	+	–	–	–
	CA3/4	–	+	–	–	–
	Neocortex	+	NT	–	–	–
3	CA1	–	–	–	–	–
	CA2	–	+	–	–	–
	CA3/4	–	+	++	–	–
	Neocortex	++	NT	+	+	+
4	CA1	+++	–	++	++	++
	CA2	+++	–	++	+	++
	CA3/4	+++	–	++	+	++
	Neocortex	+++	NT	++	++	++
5	CA1	–	–	–	–	–
	CA2	–	–	–	–	–
	CA3/4	–	–	+	–	–
	Neocortex	+	NT	–	–	–
6	CA1	–	–	–	–	–
	CA2	–	+	–	–	–
	CA3/4	–	+	+	–	–
	Neocortex	+	NT	–	–	–
7	CA1	–	–	–	–	–
	CA2	–	+	–	–	–
	CA3/4	–	+	++	–	–
	Neocortex	+	NT	–	–	–
8	CA1	++	–	–	–	–
	CA2	–	–	–	–	–
	CA3/4	–	+	+	–	–
	Neocortex	+	NT	–	–	–

[a] Morphological changes, – no change; + mild; ++ severe, +++ subtotal or total loss of neurons

[b] Hsp70, – negative or weakly positive in some neurons; + prominently positive in many neurons

[c] GFAP, – no reaction; + mild proliferation; ++ severe proliferation

[d] Vimentin, – negative; + mild proliferation; ++ severe proliferation

[e] Microglia, – no reaction; + rod cells; ++ lipid-laden macrophages

NT, not tested

fore a traffic accident. In the remaining 29 cases, there was weak or no hsp70 immunoreactivity in the hippocampus.

Swelling of GFAP-positive astrocytes was noted in the subpial and perivascular regions in 16 cases, whereas 10 cases showed no changes in the astrocytes. In the remaining 7 cases, which included 4 heavy alcoholics, a toluene abuser, an epilepsy patient, and a victim with a history of self-administration of antipsychotic drugs, a proliferation of GFAP-positive astrocytes was seen in the CA3 and CA4 regions. In this group, vimentin-positive astrocytes were seen only occasionally in the subpial region.

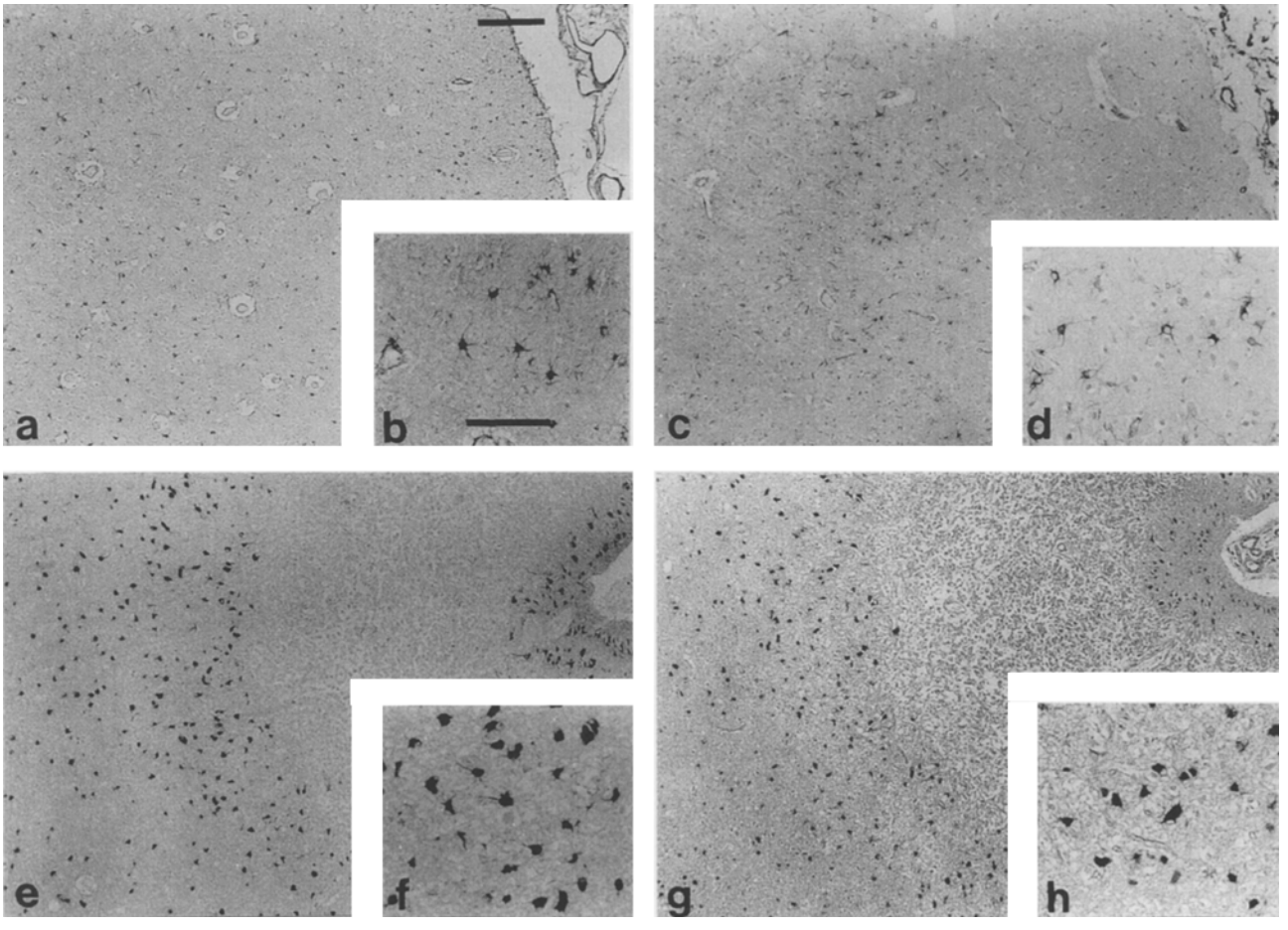
Group 2 (Table 3)

In the 2–5 h survival period (cases 1 and 2), some neurons in the neocortex and the CA1 region showed ischemic cell changes, i.e. shrinkage of the cell bodies, eosinophilia, or dark staining of the cytoplasm with luxol fast blue, and deformities and pyknosis of the nuclei. In one case of 18 days survival (case 3), no neurons of the hippocampus showed ischemic change, although laminar necrosis was seen in the third, fifth and sixth cortical layers. In a case of 120 days survival (case 4) a severe subtotal loss of neurons was seen in both the hippocampus and the second to sixth layers of the neocortex.

In the case of 18 days survival, the cell bodies of many neurons showed a hsp70-positive staining, as did some nuclei in the CA2, CA3 and CA4 regions. However, there was weak or no hsp70 immunoreactivity in the CA1 pyramidal cells.

In cases of 2–5 h survival (cases 1 and 2), swelling of the astrocytes was observed. After 18 days survival (case 3), GFAP-positive astrocytes were numerous in the neocortex (Fig. 1 a and b) and vimentin-positive cells proliferated in the regions showing neuronal necrosis (Fig. 1 c and d). In one case of 120 days survival (case 4), astrocytosis in the neocortex was more marked than in case 3 (Fig. 1 e–h) and GFAP-positive cells had proliferated throughout the hippocampus. Vimentin-positive cells were numerous in the CA1 sector but there were fewer in the CA2, CA3 and CA4 regions.

Fig. 1 Astrocytic reactions: immunohistochemical staining with GFAP (*left;* **a, b, e, f**) and vimentin (*right;* **c, d, g, h**). Proliferation of GFAP-positive cells throughout the neocortex (**a, b**) and vimentin-positive cells proliferation confined to the region of neuronal necrosis (**c, d**) in a case surviving for 18 days after strangulation (case 3). Pronounced proliferation of GFAP- (**e, f**) and vimentin- (**g, h**) positive cells around the region of neuronal loss in a case surviving for 120 days after acute respiratory failure (case 4). The astrocytes in case 4 (**f, h**) had larger cell bodies and processes than those of case 3. *Bar* = 200 μm (**a, c, e, g**); *bar* = 100 μm (**b, d, f, h**)



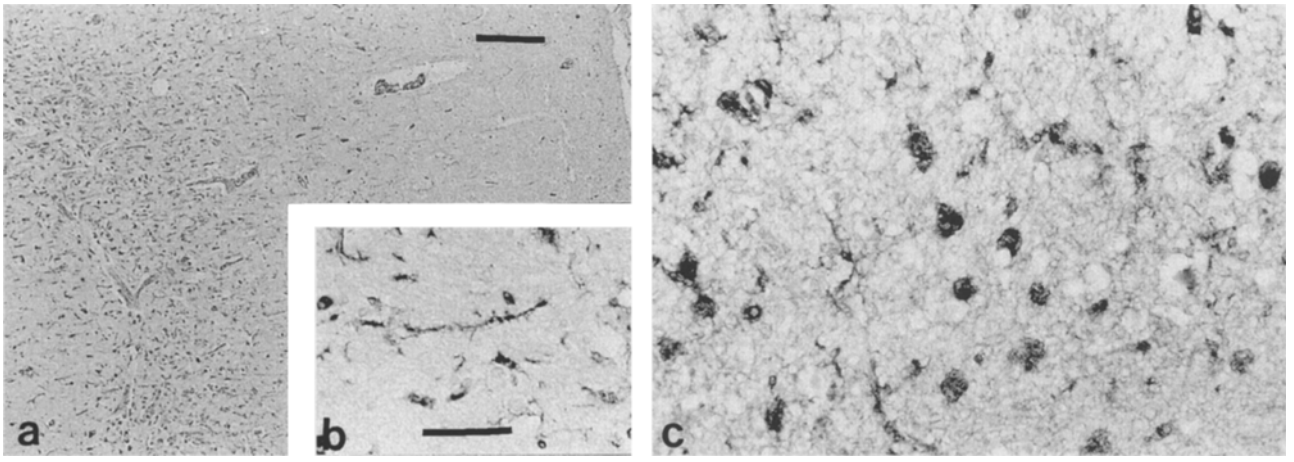


Fig. 2 Microglial reactions with RCA-1 staining. A proliferation of microglia including rod cells (**a, b**) in the neocortex of a case after 18 days survival (case 3). A proliferation of lipid-laden macrophages (**c**) in the case surviving for 120 days (case 4). *Bar* = 200 μm (**a**); *bar* = 50 μm (**b, c**)

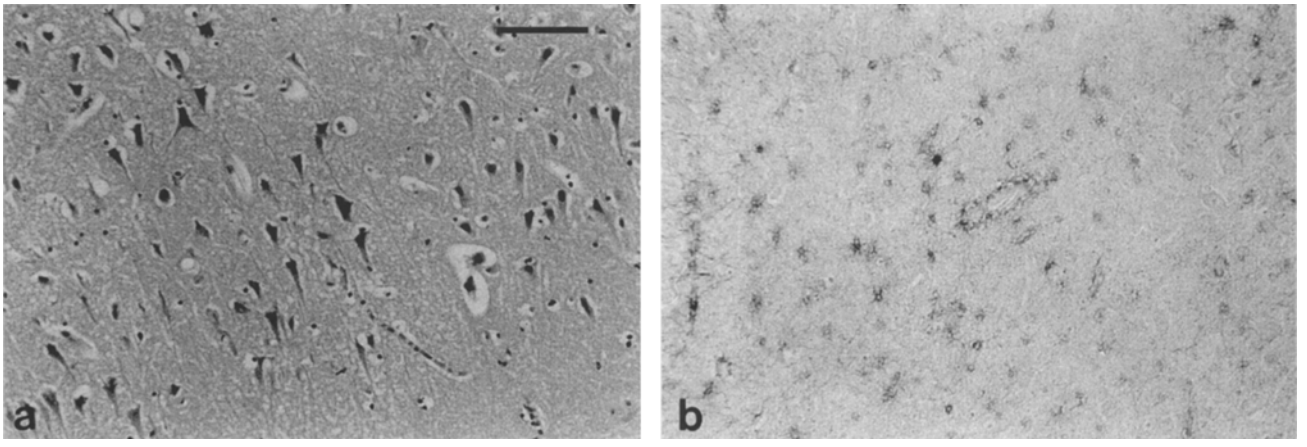


Fig. 3 Neuronal necrosis in the CA1 sector, stained with H & E (**a**) and a proliferation of GFAP-positive astrocytes in the CA3 and CA4 regions (**b**) in a case of tetralogy of Fallot, surviving 2 h after a traffic accident (case 4). *Bar* = 100 μm (**a, b**)

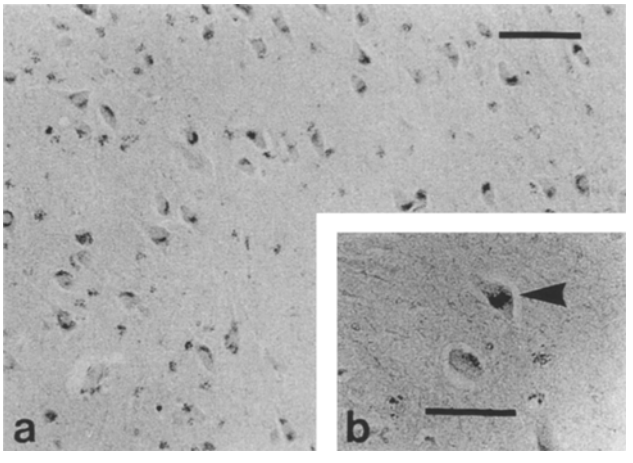


Fig. 4 Hsp70 immunohistochemistry. Strongly positive neurons in the CA3 and CA4 regions (**a**) in a case surviving for 8–10 h after aspiration of a foreign body (case 7). In these neurons (**b**), most immunoreactivity occurred in the cytoplasm and occasional immunostaining in the nucleus *(arrow)*. *Bar* = 100 μm (**a**); *bar* = 50 μm (**b**)

In cases 1 and 2, no microglial reaction was found in the brain. In case 3, a proliferation of microglia was observed in the neocortex manifesting neuronal necrosis (Fig. 2 a). In this case, many of the microglia were transformed into rod cells (reactive microglia) with bipolar processes and rod-shape nuclei (Fig. 2 b), and other microglia manifested short processes and roundish cell bodies. Numerous microglia showed lipid-laden cells, i.e. macrophages in the brain after 120 days survival period (Fig. 2 c).

Group 3 (Table 3)

In 3 cases (cases 5, 6 and 7), ischemic cell changes were seen in the neocortex, whereas no neurons showed any ischemic changes in the hippocampus. In one case with tetralogy of Fallot (TOF), the CA1 pyramidal cells showed neuronal necrosis (Fig. 3 a) and ischemic cell changes were seen in the neocortex.

In this group, the GFAP-positive astrocytes had proliferated in the CA3 and CA4 regions (Fig. 3b), and these proliferating cells showed no vimentin immunoreactivity. No microglial reactions were seen in this group.

In 3 cases (cases 6, 7 and 8) with more than 2 h survival, hsp70 immunoreactivity was found in the CA2, CA3 and CA4 neurons (Fig. 4a and b).

Discussion

In this study, no ischemic cell changes were found in the brain tissue following acute death, although swelling of astrocytes was seen in 16 cases. In the early stages of hypoxic/ischemic injury, astrocytes showed a swelling of their cell bodies, which is a significant sign for the diagnosis of hypoxic/ischemic injury [6]. However, such swelling also occurs during post-mortem autolysis. Because of a lack of detailed information about the duration and severity of the hypoxic/ischemic injury, it was not clear whether these 16 cases differed from the other 10 cases showing no astrocytic change. Therefore, GFAP immunostaining was unable to distinguish whether this astrocytic swelling was due to the hypoxic/ischemic injury or to post-mortem changes.

After 2–5 h survival (cases 1 and 2, group 2) ischemic cell changes were seen in the brain tissue. Severe neuronal necrosis and a loss of neurons associated with glial reactions occurred in the brain of a patient surviving for 120 days (case 4, group 2) and in the neocortex after 18 days survival (case 3, group 2). However, there were no neuronal changes in the hippocampus of case 3. In humans surviving for 4–12 h after cardiac arrest, the neocortex showed ischemic cell changes and neuronal changes in the hippocampus were invariable. Severe neuronal necrosis or a loss of neurons is accompanied by a glial reaction in the brains of long-term survival after cardiac arrest [1, 5, 7]. In contrast to a deprivation of the blood supply such as in cardiac arrest, a reduction in the cerebral blood flow induces neuronal changes in the neocortex, especially in the arterial boundary zone, but this does not necessarily damage the hippocampus [5]. Since the arterial boundary zone of the parieto-occipital lobe is most remote from the origin of each major artery, it is most vulnerable to a reduction in cerebral blood flow. In this study, the results found in the cases of long-term survival with no history of hypoxic attacks were relatively consistent with those of previous studies.

Ischemia includes several pathophysiological conditions, i.e., hypoxia, hypercapnia, acidosis, and a reduction in the energy supply [5, 14, 20]. However, the mechanisms of neuronal changes due to ischemia are not entirely clear. Furthermore, it is not clear how and whether hypoxia, which induces the release of amino acids, calcium uptake, and activation of several proteases, causes ischemic cell changes [14, 20]. Based on observations from human and animal brain tissue, it has been suggested that hypoxia (anoxia) causes no ischemic cell change, and that morphological changes in the neurons may be a consequence of circulatory failure due to hypoxemia (anoxemia) [5, 14, 20, 29, 34]. In this study, the hippocampus showed no neuronal changes in the victims of an asthma attack (cases 5, 6 and 7, group 3). Therefore, in the hippocampus of these cases, the damage due to a reduction in cerebral blood flow after the hypoxic injury might have been less severe. In a patient suffering from TOF, who survived for 2 h after a traffic accident (case 8, group 3), the hippocampus showed severe necrosis of the CA1 pyramidal cells, and these change were more advanced than the neuronal alterations seen in the 2 cases (cases 1 and 2, group 2) surviving for 2–5 h. It seems that the neuronal changes appeared after the traffic accident, since the immunohistochemical staining did not detect an astrocytic or microglial reaction to neuronal necrosis in the CA1 region, and a few days prior to death this patient did not complain of any problems or showed any clinical symptoms. It also has been reported that even 10 min of anoxia induces a decrease in the immunoreactivity to the microtubule-associated protein 2 (MAP2), a cytoskeletal protein, in the hippocampus [14]. Therefore, these findings suggest that repeated hypoxic attacks, such as asthma attacks, may impair some neuronal proteins to some extent, and that the neurons which are vulnerable to ischemia, such as the CA1 pyramidal cells, may show more advanced alterations after being subjected to severe stress as in this patient with a history of hypoxic attacks.

In the rat hippocampus after ischemia, GFAP-positive and vimentin-positive astrocytes appeared only in the CA1 region showing neuronal necrosis, whereas GFAP-positive and vimentin-negative cells were seen not only in the CA1 region but also in the CA3 region which showed neuronal viability [17, 21]. Based on these findings and the results of this study, vimentin could be a useful marker for neuronal necrosis. The stimuli which provoke an astrocytic reaction in a non-damaged area may differ from the stimuli that affect the damaged area, as reviewed by Petito et al. [27], since many factors have been identified in vivo or in vitro, including cytokines [19], growth factors [16], steroids [24, 33], protein kinase C and prostaglandin [28]. In this study, a proliferation of GFAP and vimentin-positive astrocytes were found in the regions of severe neuronal changes. On the other hand, GFAP-negative and vimentin-positive cells had proliferated in the CA3 and CA4 regions in a case surviving for 18 days and the cases with a history of hypoxic attacks. Astrocytosis was also seen in the cases of acute death, including heavy alcoholics, a toluene abuser, an epilepsy patient and victims with a history of self-administration of antipsychotic drugs. These findings would indicate that a relatively mild hypoxic episode can induce the proliferation of GFAP-positive and vimentin-negative astrocytes, and such astrocytosis may be induced by epilepsy or toxic disorders.

In this study, microglial reactions were found in the areas showing neuronal necrosis in the cases of long-term survival. However, there were no microglial reactions in the CA3 and CA4 regions in which GFAP-positive cells had proliferated. Thus, it is possible that microglia do not

react to mild hypoxic/ischemic injury, whereas astrocytes do react.

In the brains of long-term survival patients (cases 1, 2, 3, 6, 7 and 8), neurons in the CA2, CA3 and CA4 regions showed a definite hsp70 immunoreactivity, whereas weak or no hsp70 immunostaining was seen in the CA1 sector. In the cases of alcoholic intake or toluene abuse just before death, the hsp70 distribution resembled the pattern seen in the hippocampus of long-term survivors. In other studies, the induction of species of heat shock protein was seen after exposure to various types of stress, such as heat, ischemia, and alcohol [2, 15, 30, 31, 35]. The significance of hsp70 expression is not clear. After a transient ischemia, weak or no hsp70 immunoreactivity was induced in the CA1 pyramidal cells, whereas a significant increase in hsp70 immunostaining was noted in the CA3 region [12]. The hsp70 immunoreactivity in CA1 neurons increased after a 2 min period of ischemia and a further increase in hsp70 immunoreactivity was seen in the CA1 pyramidal cells after a second period of ischemia [22]. Furthermore, an early recovery of protein synthesis was noted in the CA1 region. It has also been reported that transient ischemia, which is in itself not lethal to neurons, induces a tolerance to subsequent ischemic injury [8, 13]. These studies seem to indicate that ischemic stress, which is lethal to the CA1 neurons, impairs the cellular metabolism, including the hsp70 expression in the CA1 sector, and that such stress induces the presence of hsp70 in other regions of the hippocampus. Therefore, it is suggested that the hsp70 expression in the hippocampus found in this study reflected a hypoxic/ischemic injury and other stress prior to death.

In summary, ischemic cell changes in the hippocampal neurons were not obvious in brains damaged by hypoxic injury. However, it is suggested that even a moderate hypoxia, which may affect the neuronal proteins and metabolism, induces astrocytosis in the CA3 and CA4 regions, and that in patients with a history of hypoxic attacks neuronal damage may be severe even several hours after ischemic injury. Furthermore, hsp70 expression was found in the CA2, CA3 and CA4 regions after long-term survival of severe hypoxic/ischemic injury. In forensic practice, detailed information about the duration and extent of a hypoxic/ischemic injury is often unavailable, so that immunohistochemical detection of hsp70 and glial cell staining can be of great value in diagnosing not only the hypoxic/ischemic injury during the process of death but also the victim's past history of hypoxic attacks.

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