HSP70 and c-Fos expression of brain stem hypoglossal nucleus in drowning

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Abstract: The brain stem hypoglossal nucleus (HN) is the center of nerves innervating the upper respiratory tract and is related to control of mastication, deglutition, speech and respiration. To elucidate the relationship between asphyxia and the HN, we investigated the change of hypoglossal neurons in cases of hanging, strangulation, smothering, choking, drowning and respiratory failure. Using immunohistochemical techniques, we observed the brain stem HN with antibodies against microtubule-associated protein 2 (MAP2), muscarinic acetylcholine receptor (mAChR), *c-fos* gene product (c-Fos) and 72 kD heat-shock protein (HSP70). MAP2, a cytoskeletal protein of the neuron, is a marker of neuronal damage. Muscarinic AChR was used as a marker of neuronal membrane and ACh signaling. We employed both HSP70 and c-Fos as markers of stress- or damage-related events. We measured the percentage of immunopositive neurons in total neurons of HN. Drowning produced higher expression of HSP70 and c-Fos than other causes of asphyxia, suggesting that drowning induces more severe damage in HN neurons. Furthermore, it was suspected that neuronal changes in drowning might relate to functions of the HN. These observations indicate that immunohistochemical examination of the brain stem HN could provide useful information for determining the cause of asphyxia.
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Key words : *neuropathology, hypoglossal nucleus, asphyxia, neuronal damage, immunohistochemistry*

INTRODUCTION

Several nuclei in the brain stem are known to play important roles in supporting human life. However, the relationship between neuronal damage to the nuclei of the brain stem and the cause of death is not yet fully understood. We studied nuclei of the brain stem in forensic autopsy cases using immunohistochemical techniques (1, 2). Neuronal changes in the hypoglossal nucleus (HN) were observed especially in asphyxia. To elucidate the relationship between asphyxia and the HN, we investigated changes of hypoglossal neurons immunohistochemically and statistically.

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MATERIALS AND METHODS

Twenty-seven forensic autopsy cases were divided into 4 groups based on the cause of death. Group A included cases of hanging, ligature strangulation and manual strangulation (8 cases) ; Group B, smothering and choking (6 cases) ; Group C, drowning (10 cases) ; Group D, respiratory failure (3 cases) including asthma, pleurisy and pulmonary thromboembolism.

The brain was fixed with phosphate-buffered formalin, and the brain stem was horizontally dissected at the level of the obex, then embedded in paraffin, and sectioned at 4 μm. The sections were stained

with antibodies against microtubule-associated protein 2 (MAP2) (Amersham, GB), muscarinic acetylcholine receptor (mAChR) (Transduction Laboratories, USA), *c-fos* gene product (c-Fos) (Medac, GFR) or 72 kD heat-shock protein (HSP70) (Amersham, GB). Immunoreactions were then detected by the streptoavidin-biotin method (LSAB kit, Dako, Denmark). In addition, conventional stains such as hematoxylin-eosin (HE) and Klüver-Barrera were carried out to examine the morphological changes in the neurons.

To obviate the effects of postmortem changes, cases more than 24 hours postmortem duration showing no or little MAP2 immunoreactivity were excluded (3).

Immunoreactivities of neurons in the hypoglossal nucleus were examined. The percentage of immunopositive neurons in total neurons was determined by counting neurons using a light microscope. Differences among percentages were analyzed by a non-parametric two-way ANOVA.

RESULTS

Significant morphological changes in neurons, such as shrinkage and loss, were not observed in any of the 24 cases. There was no correlation between the rate of immunoreactivity in cells and amount of time postmortem or survival duration in our cases.

The percentage of MAP2-positive neurons was more than 78% in each group (Table 1). Immunoreactivity to mAChR was more than 80% of neurons (Table 1).

The number of HSP70-positive neurons in drowning victims was significantly higher than other

Table 1. The percentage of MAP2 and mAChR immunopositive neurons

Group	Percentage (mean±S.E.)	
	MAP 2	mAChR
A	79.9±4.6	80.3±4.2
B	78.9±6.4	80.5±4.5
C	91.4±3.3	90.8±1.3
D	78.5±2.0	90.7±4.6

A : hanging, ligature strangulation and manual strangulation
B : smothering and choking
C : drowning
D : respiratory failure

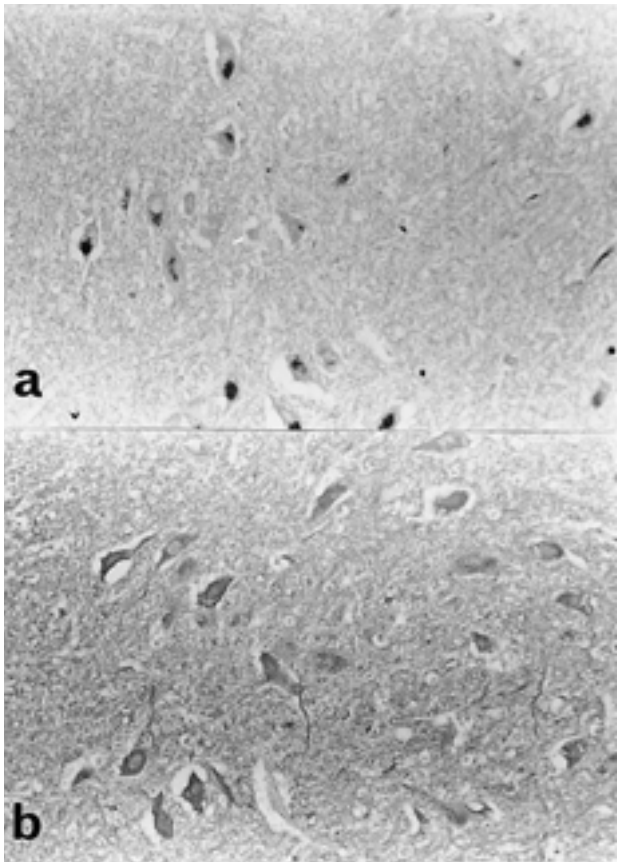

Fig.1. HSP70 and c-Fos expression in drowning
a : HSP70, b : c-Fos (×200)

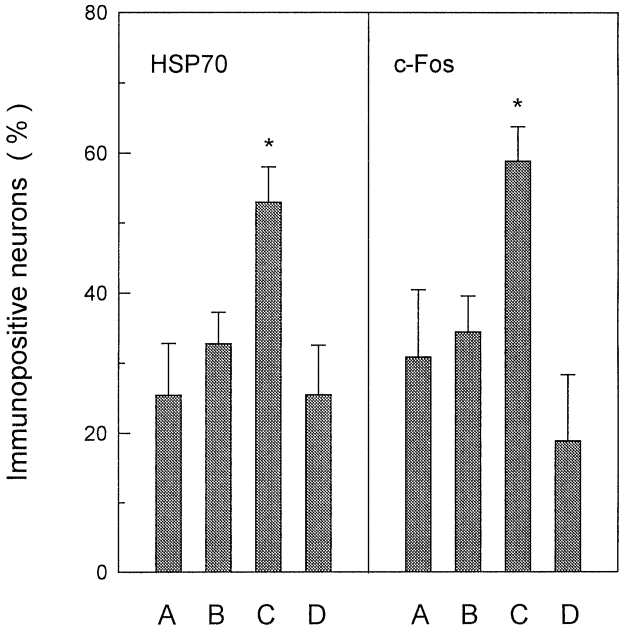

Fig.2. The percentage of HSP72 and c-Fos immunopositive neurons
A : hanging, ligature strangulation and manual strangulation
B : smothering and choking
C : drowning
D : respiratory failure
* $p<0.05$

groups (53.0±5.1%) (Figs. 1a and 2). A high rate of c-Fos expression was also observed in drowning (58.8±5.0%) (Figs. 1b and 2).

DISCUSSION

MAP2, a cytoskeletal protein of the neuron, is a marker of neuronal damage, and alteration in the immunostaining of this protein was generally observed prior to morphological changes (4, 5). Muscarinic AChR is localized on the cell membrane and mediates the action of neurotransmitter ACh. In this study, mAChR was used as a marker of the neuronal membrane and ACh signaling.

HSP70 is known to be expressed in neuronal cells after various stresses, such as heat, trauma and ischemia, thereby protecting neurons against stress-induced damage (6-10). Similar to HSP70, c-Fos is also considered to be a cellular marker of transcriptional activity in the stress-related circuitry (6-14). While the functional significance of either HSP70 or c-Fos expression is unclear, it is evident that they are linked to events that either promote cellular recovery or lead to cell death. Thus, we employed both HSP70 and c-Fos as markers of stress- or damage-related events.

Morphological observation showed no significant changes of neurons in the HN. There was no correlation between the rate of immunoreactivity in cells and length of postmortem period or survival duration in our cases. Therefore, we examined the relationship between immunohistochemical changes and cause of death.

In the present study, causes of respiratory failure (Group D) were internal, such as asthma, pleurisy and pulmonary thromboembolism. These cases showed high immunoreactivity to MAP2 and mAChR and less expression of HSP70 and c-Fos. It is possible that the HN might not have been damaged in our cases.

Based on these results, we investigated the asphyxia groups (Group A, B and C). Although there were no significant differences of MAP2 and mAChR immunostaining in any of the 4 groups, drowning produced significantly higher expression of HSP70 and c-Fos ($p<0.05$). Since high rates of HSP70 and c-Fos expression in the HN were observed only in drowning, it was considered that drowning might induce neuronal changes or the HN damage. The HN is the center of nerves innervating the upper respiratory tract and is related to control of mastication, deglutition, speech and respiration (15, 16). During the course of drowning, respiration is sometimes spasmodic (17), and it is conceivable that mastication, swallowing, respiration and speech are also disturbed. It is suspected that neuronal changes seen in drowning victims might be related to those functions of the HN.

These observations indicate that immunohistochemical examination of brain stem nuclei can provide useful information for determining the cause of death.

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