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Immunohistological investigations of autopsied carotid bodies and their application to diagnosing strangulation

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Summary. Using immunohistochemical staining, the histological changes and the presence of neuropeptides (enkephalin and VIP) in the carotid body have been investigated in medico-legal autopsy cases, especially asphyxia cases. Only in cases of manual and/or ligature strangulation cases that sustained a force near the carotid body, were the chief cells mainly lightly stained, indicating that they had been "active" cells. Furthermore, these cells and their nuclei were enlarged in comparison to the chief carotid body cells in other autopsy groups. It was thus felt that these changes had resulted from the force that had directly affected the carotid body. Based on these findings, it was concluded that immunohistochemical investigation of the carotid body offers a useful possibility for diagnosing manual asphyxia, especially in autopsy cases involving strangulation.

Key words: Asphyxia – Strangulation – Carotid body – Immunohistochemistry

Zusammenfassung. Mit Hilfe immunhistochemischer Färbemethoden wurden die histologischen Veränderungen und das Vorhandensein von Neuropeptiden (Enkephalin und VIP) im Glomus caroticum bei rechtsmedizinischen Autopsie-Fällen, speziell bei Asphyxie-Fällen, untersucht. Lediglich in Fällen von manueller und werkzeugbedingter Strangulation, in denen die Gewalt in der Nähe des Glomus caroticum erlitten wurde, waren die Hauptzellen hauptsächlich leicht angefärbt, als Hinweis, daß sie „aktive" Zellen darstellten . Weiterhin waren diese Zellen und ihre Kerne vergrößert im Vergleich zu den Hauptzellen des Glomus caroticum in anderen Autopsie-Fällen. Es entstand daher der Eindruck, daß diese Veränderungen resultierten aus der Gewalt, die direkt das Glomus caroticum traf. Aufgrund dieser Befunde wurde geschlossen, daß die immunhistochemische Untersuchung des Glomus caroticum eine nützliche Möglichkeit darstellt, um die manuelle Asphyxie zu diagnostizieren, speziell in Autopsie-Fällen unter Einbeziehung der Strangulation.

Schlüsselwörter: Asphyxie – Strangulation – Glomus caroticum – Immunhistochemie

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Introduction

Autopsy diagnosis of mechanical asphyxia depends on the visual evaluation of injuries, but should also take into account general signs, such as petechial hemorrhages, cyanosis, congestion of organs, pulmonary edema, and the fluidity of blood. It would also be of great value to find evidence indicating a vital reaction to asphyxia.

In necropsy diagnostics, particularly with reference to compressive neck injuries, little attention is given to investigating the carotid body, a small organ the size of a rice grain, situated in the carotid bifurcation fork (Fig. 1) that secretes the following neuropeptides: enkephalin, a vasoactive peptide (VIP) (Heath and Smith 1985), and substance P (Wharton et al. 1980). Therefore, in view of the anatomical position of the carotid body and its physiological functions, we have immunohistochemically studied the carotid body in autopsy cases involving strangulation, to determine whether the findings can assist in achieving a diagnosis of a compressive neck injury.

Materials and methods

Preparation of carotid body tissue section. Carotid bodies were collected from Nagasaki University medico-legal autopsy cases, paraffin embedded and cut into 2.5 μm thick sections. Based on the cause of death, especially the position of the strangulation mark and subcutaneous hemorrhage and/or histological findings of the compressed skin, these carotid bodies were classified into 3 groups. Group A, from 6 cases of manual and/or ligature strangulation with a mechanical force that had been applied to the neck near the carotid body; Group B, from 3 cases of hanging and liga-

Table 1. Summary of the cases

Case no.	Age (years)	Sex	Cause of death	Post mortem duration
1	63	F	Manual strangulation	10 h
2	3	F	Manual strangulation	13 h
3	1	F	Ligature strangulation	24 h
4	84	F	Ligature and manual strangulation	4 h
5	36	F	Ligature strangulation	7 h
6	17	F	Manual strangulation	24 h
7	20	F	Ligature strangulation	24 h
8	42[a]	F	Ligature and manual strangulation	8 h
9	47	F	Atypical hanging	20 h
10	42	M	Smothering	16 h
11	57	M	Choking	7 h
12	35	F	Drowning	36 h
13	67	M	Drowning	24 h
14	50	M	Acute heart failure	18 h
15	41	M	Acute heart failure	7 h
16	27	F	Bleeding	15 h
17	21	F	Drug intoxication	16 h
18	51	F	Hypertensive cerebellum bleeding	23 h

[a] 18-day survival after strangulation

Table 2. Histopathological findings of the chief cells

Group	Case no.	Main type of the chief cells	Size (μm)		Anti-enkephalin	Anti-VIP
			Cell	Nucleus		
A	1	Light	33.8 ± 8.2	14.0 ± 1.5	++	++
	2	Light	10.6 ± 1.0	6.4 ± 0.9	++	+
	3	Light	14.8 ± 3.0	8.6 ± 1.5	++	++
	4	Light	12.2 ± 1.9	8.1 ± 1.4	++	+
	5	Light	27.9 ± 6.3	13.0 ± 1.7	++	++
	6	Light and dark	12.0 ± 1.4	6.4 ± 0.5	++	++
B	7	Light and dark	8.3 ± 1.7	5.8 ± 1.2	++	++
	8	Light and dark	9.2 ± 1.6	6.2 ± 0.4	++	++
	9	Light and dark	8.0 ± 0.7	4.8 ± 0.4	+	+
C	10	Dark	9.6 ± 0.8	5.6 ± 0.5	++	++
	11	Light	10.2 ± 1.2	6.0 ± 0.6	++	++
	12	Dark	8.0 ± 0.7	4.3 ± 0.4	++	++
	13	Dark	9.0 ± 0.6	5.7 ± 0.5	++	++
D	14	Light and dark	10.9 ± 1.9	5.6 ± 0.5	++	+
	15	Dark	10.8 ± 1.2	5.8 ± 0.8	++	++
	16	Dark	7.2 ± 0.8	5.5 ± 1.0	++	+
	17	Dark	8.8 ± 1.0	4.8 ± 1.2	+	+
	18	Light and dark	9.0 ± 1.3	5.5 ± 0.5	+	+

ture strangulation without the application of a mechanical force near the carotid body and one case in which the patient died 18 days after strangulation, and Group C, from 4 cases of other types of asphyxia, i.e., smothering, choking or drowning. Group D were carotid body controls, collected from 5 cases of sudden, unexpected death. For details, see Table 1.

Immunohistochemical staining. Anti-human enkephalin antisera (Bioproducts, UK) and anti-human VIP antisera (Biomedia, USA) were used (dilution; 500×) and immunohistochemical staining was performed by the avidin-biotin peroxidase complex (ABC) method (Vecstain ABC Kit, USA).

Method to classify the histopathological findings. The appearance of the chief cells in the carotid body after staining was the basis for classification. If more than 60% of the cells had stained only lightly, then the cells classification was the "light" type; if only 40–60% appeared to be lightly stained, then the classification was

Fig. 1. Macroscopic view of the carotid body

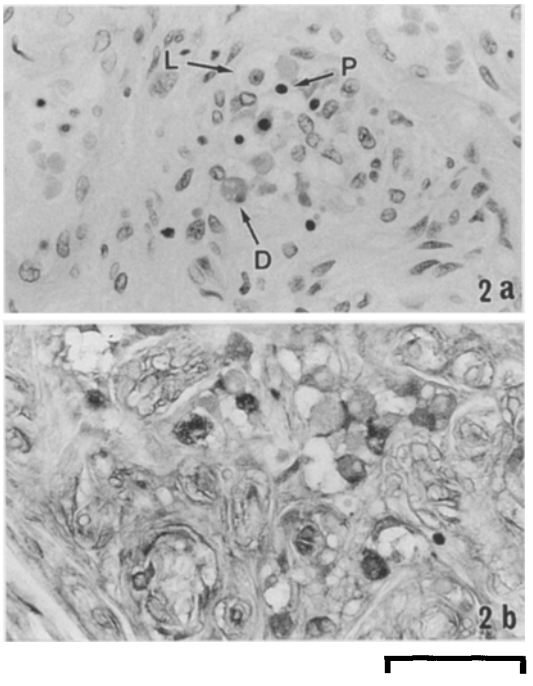

Fig. 2a, b. Carotid body from Case 14 (Group D). **a** Hematoxylin and eosin (×100). **b** Immunohistochemical staining for enkephalin (×100) 50 μm

the "light and dark" type, and less than 40% of cells were lightly stained this was considered the "dark" type. The diameter of the cell and nucleus of the chief cells was measured.

Results

Histopathological findings of carotid bodies are shown in Table 2. The immunohistopathological staining results against enkephalin and VIP in the cytoplasm of the chief carotid body cells were specific. This method provided

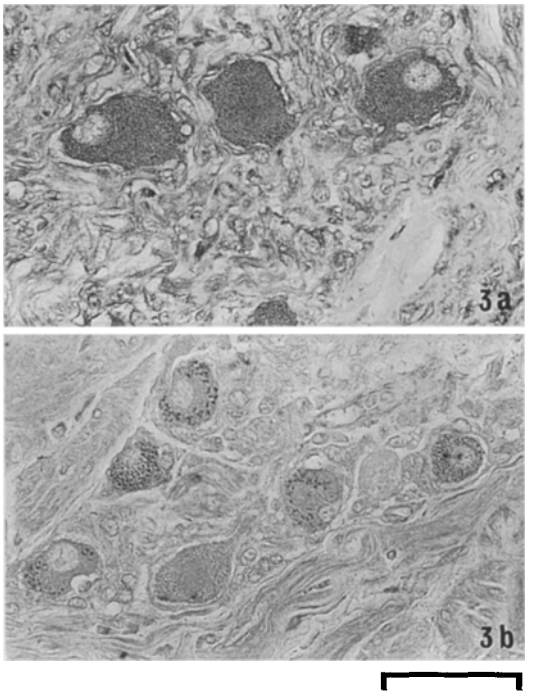

Fig. 3a, b. Immunohistochemical staining for enkephalin. **a** Case 1 (×100). **b** Case 5 (×100) 50 μm

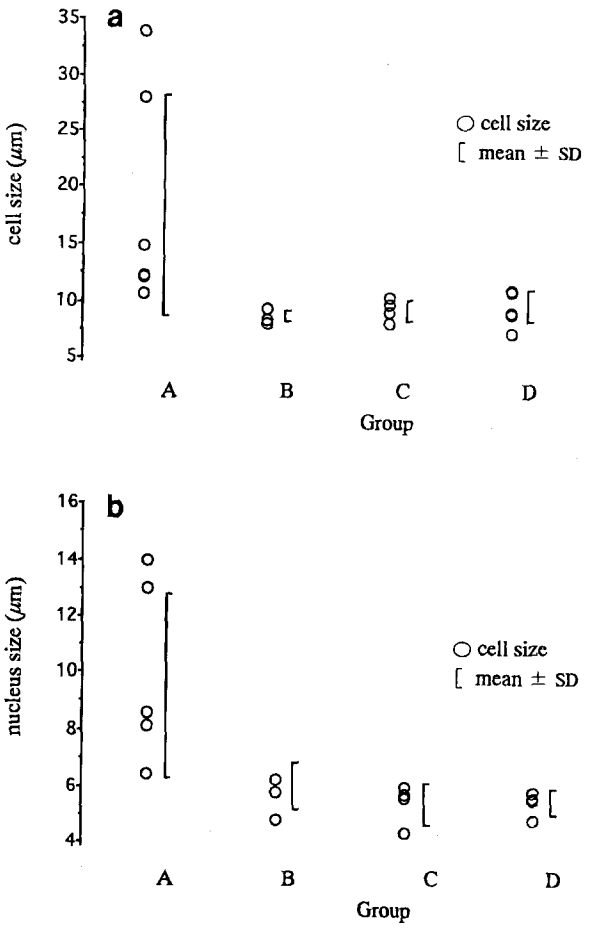

Fig. 4a, b. Scattergrams of cell and nucleus size. **a** Scattergram of cell size. **b** Scattergram of nucleus size

clearer evidence than the usual method of hematoxylin and eosin staining (Fig. 2), with enkephalin showing a stronger reactivity than VIP (Table 2).

In Group A, most of the chief cells were the "light" type, with only a small number of dark and pyknotic cells. In contrast, the number of dark cells was greater in Groups B, C, and D. Furthermore, the diameter of the chief cells and their nuclei were enlarged only in Group A (Table 2, Fig. 3).

Scattergrams of the cell and the nucleus size for each group are shown in Fig. 4. The mean size of the chief cells in Group A was 18.6 ± 9.8 μm, and the mean nucleus size was 9.4 ± 3.3 μm. In Groups B, C, and D, the mean size of the chief cells was 8.5 ± 0.6 μm, 9.2 ± 0.9 μm, and 9.3 ± 1.5 μm, respectively, and the mean nucleus size was 5.6 ± 0.7 μm, 5.4 ± 0.8 μm, and 5.4 ± 0.4 μm, respectively.

Discussion

As seen in Fig. 1, the carotid body, an organ approximately the size of a rice grain, is situated in the fork of the carotid bifurcation. It has a mean weight of about 18.0 mg (Smith et al. 1982), and is known to secrete the neuropeptides, enkephalin, VIP, and substance P (Wharton et al. 1980).

Because of the anatomical position and its physiological function of the carotid body, this immunohistochemical study was undertaken to determine whether the findings in autopsied carotid body specimens would assist the diagnosis of compressive neck injuries. As stated previously, immunohistochemical staining against enkephalin and VIP in the autopsied specimens provided a clearer picture of the histopathological findings than customary hematoxylin and eosin staining, with enkephalin showing a stronger immunoreactivity than VIP (Table 2). Thus, anti-enkephalin is more useful than anti-VIP for observations on the chief carotid body cells. However from the results of immunohistochemical staining of many cases, it should be noted that because of autolysis, the immunoreactivities of these chief cells decreased significantly 1–2 days after death.

The chief cells consisted of 3 types: light, dark, or pyknotic, so designated from the cell's appearance on reacting to hematoxylin and eosin staining. Light cells are considered to be "active" cells, and both the dark and the pyknotic cells are considered to be inactive cells. The relationship of dark cells to light cells is still uncertain (Heath and Smith 1985).

Among the chief cells of the carotid body that were investigated, the main type seen in Group A was the light cell type, whereas the main type seen in Groups B, C, and D was the dark type (Table 2). Since our findings have revealed the presence of light or "active" cells in Group A, this indicates that the chief cells in the carotid body of these cases had synthesized and secreted neuropeptides. Measurement of the diameters of these chief cells and their nuclei revealed that the cells and nuclei were enlarged only in Group A (Table 2, Figs. 3 and 4). Moreover, results of an unpaired t-value calculation between Group D (the controls) and Groups A, B, and C, respectively, confirmed that in Group A both the chief cell size ($P = 0.0693$) and nucleus size ($P = 0.0262$) were significantly enlarged, and that all of these enlarged cells were the light type (Fig. 3). There was no correlation between these chief cell findings and age, and sex, respectively.

Generally, neurotransmitters are secreted and synthesized immediately after stimulation. The large light cell is in keeping with intense synthetic activity and the presumed secretion (Heath and Smith 1985). Ill-defined borders of light cells shows activity for secretion, and also shows changeability of the cell shape. Thus, the larger cytoplasm and nucleus of these chief cells in Group A, the majority being the light type, are thought to be induced by a direct force that affected the carotid body, so that these changes in the chief cells can be a direct reaction to strangulation.

Conclusion

Therefore, based on the findings of this study, immunohistochemical staining of the carotid body has a very useful possibility for a necropsy diagnosis, since it provides a method to detect evidence of mechanical asphyxia in suspected cases of manual and/or ligature strangulation.

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