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PAPER

PATHOLOGY/BIOLOGY

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Myocardial Hypertrophy Induces Carotid Body Hyperplasia

ABSTRACT: The carotid bodies tend to enlarge after long-standing cardiopulmonary disease. Our objective was to investigate whether cardiac hypertrophy is associated with carotid body hyperplasia. Fifteen autopsy cases with combined left and right ventricular hypertrophy were examined and compared with two control groups (16 cases). The study involved a meticulous dissection of carotid bifurcations, thin serial sections, and morphometric analysis of carotid body volume and cell types (progenitor, dark, light, and sustentacular). There was a significant increase in sustentacular cells in all individuals with cardiac hypertrophy, which was not drug-induced, and accompanied by a similar increase in carotid body volume. Dark or light cell accumulation was detected focally and only in three instances. It appears that the generalized sustentacular cell hyperplasia is the result of long-standing hypoxia, while a superimposed focal prominence of dark or light cells may be proliferative or metaplastic in nature and attributed to short-term hypoxia.

KEYWORDS: forensic science, forensic pathology, myocardial hypertrophy, carotid body hyperplasia, sustentacular cells

The human carotid body is not an inert organ as it is generally thought, because specific morphological changes have been described in relation to aging (1,2) and, indeed, a variety of diseases including bronchial asthma (3), chronic bronchitis and emphysema (4), cirrhosis (5,6), and systemic hypertension and chronic hypoxia (7,8). In addition, several peptides have been shown to be expressed by the organ indicating a dynamic intervention to the physiological function of the human body (9–11). We report here our evaluation of the changes noted in the human carotid body in response to a long-standing combined left and right myocardial hypertrophy.

Methods

The series comprised 15 subjects with a long-standing history of systemic hypertension, confirmed at autopsy by an enlarged heart involving both ventricles (combined heart weight over 420 g) and subsequent histological examination (left and right ventricular hypertrophy; Table 1).

To ensure that cardiac enlargement was not an adaptive response to drugs, i.e., cocaine (12,13), a control group of six individuals with cardiac hypertrophy (as defined earlier) and drug-free urine and blood samples was included (control group A; Table 2)—a toxicological screening analysis which was performed by enzyme immunoassay and gas chromatography/mass spectrometry, respectively (14). The study was complemented by 10 age-matched controls (combined heart weight <310 g for men and <290 g for women), with no evidence of cardiac or pulmonary disease (control group B; Table 2, Fig. 1).

After a meticulous dissection of carotid bifurcations, serial sections at 3 mm were taken in planes transverse to the longitudinal axis of the common carotid artery. All tissue specimens, including study cases and controls, were fixed in 10% formalin solution and processed routinely to paraffin wax. They were subsequently cut serially at 3-μm sections and stained with hematoxylin and eosin.

Tables 1 and 2 indicate the age, sex, and the combined heart weight for each subject in the series.

The various cell types of the carotid bodies, previously described by Heath et al. (10), were identified under the light microscope (Nikon, model eclipse E400; Tokyo, Japan), and semi-quantitative differential counts were performed manually. In all cases, the numbers of progenitor, dark, light, and sustentacular cells were counted within two randomly selected high power fields at ×400 magnification. Areas of dense fibrous tissue were excluded from this assessment. The procedure was facilitated by the use of an eyepiece graticule.

The carotid bodies are essentially spherical in shape and, hence, their volume was calculated by using the formula $4/3 \times \pi \times \rho^3$, where $\pi = 3.14$ and ρ = radius. The diameter of the carotid body was measured by means of an ocular micrometer after examining serial hematoxylin and eosin-stained sections.

Statistical analysis was performed using the GraphPad Prism 5.0 (GraphPad Software Inc., San Diego, CA). The unpaired two-tailed *t*-test was used to compare groups with continuous variable data. A *p*-value of <0.05 was used for significance.

Results

The diagnosis of myocardial hypertrophy, suspected at autopsy for both left and right cardiac ventricles, was confirmed by light microscopy.

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TABLE 1—*Study group. Clinical characteristics and the heart weights of individuals with myocardial hypertrophy.*

Cases	Age (years)	Sex	Heart Weight (g)
1	65	M*	476
2	62	M	450
3	74	M	547
4	65	F†	446
5	58	M	598
6	63	F	512
7	79	F	420
8	67	M	428
9	93	F	502
10	66	M	531
11	50	M	426
12	73	M	440
13	49	M	503
14	76	F	439
15	54	M	565

*Male.
†Female.

TABLE 2—*Control groups. Clinical characteristics and the heart weights of proved drug-free individuals with myocardial hypertrophy (Control A) and individuals with no evidence of cardiopulmonary disease (Control B).*

Cases	Age (years)	Sex	Heart Weight (g)
Control A			
1	45	M*	423
2	72	F†	441
3	65	F	420
4	54	M	462
5	50	M	438
6	81	M	470
Control B			
1	69	F	222
2	76	M	292
3	74	M	305
4	76	M	260
5	55	F	277
6	55	M	290
7	50	M	227
8	62	M	250
9	71	M	287
10	50	M	270

*Male.
†Female.

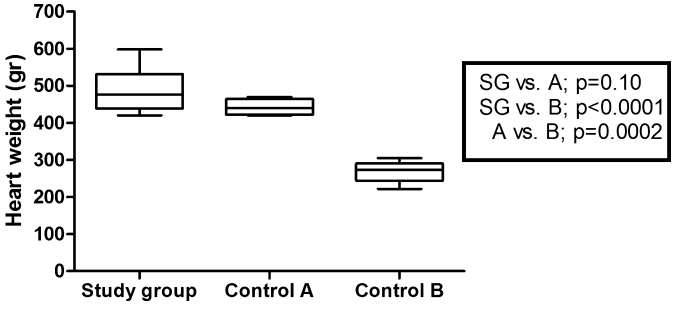

FIG. 1—*Heart weights. Study group: individuals with cardiac hypertrophy; Control group A: proved drug-free individuals with cardiac hypertrophy; and control group B: individuals with no evidence of cardiopulmonary disease.*

With regard to carotid bodies, these, as expected, were composed of glomic tissue arranged in lobules which in turned were organized into discrete cell clusters. The lobules were separated by connective tissue containing capillaries and nerve bundles. The cell clusters, small in size and spherical in shape, contained two main types of glomic cells: the chief (type I) cells and the supportive or sustentacular (type II) cells. As described by Heath et al. (15), the type I cells were made of three variants: the progenitor cell, the dark cell, and the light cell. The chief cells occupied the core of the clusters while the sustentacular cells were preferentially set at the periphery. There was a rich vascular network.

The *progenitor cells* were readily recognized as round cells with a sharp outline, a deep purple cytoplasm, and a compact round eccentrically situated nucleus. The *dark cells* had intensely hematoxyphilic nuclei and purple cytoplasm. The *light cells* were large, with round or slightly oval nuclei, and had an abundant vacuolated cytoplasm with ill-defined borders; they were usually arranged in groups, often giving a syncytial appearance to the cytoplasm. The *sustentacular cells* were elongated with large pale nucleus and indistinct cytoplasmic borders.

At first sight, there was an apparent increase in the size of clusters, as these were crowded by glomic cells. This general impression was subsequently substantiated by differential cell counting which showed an apparent increase in the number of sustentacular cells in both the study and the control group A, relative to control group B (Tables 3 and 4, Figs 2 and 3*a*). This difference in the glomic (sustentacular) cell population between subjects with combined left and right ventricular hypertrophy and those without (control group B) was statistically significant ($p < 0.0001$). It is of note that in 12 cases the sustentacular cell hyperplasia was generalized, but in the remaining three cases, there were carotid body lobules showing focal accumulation of dark (one case) or light cells (two cases) (Fig. 3*b*,*c*). The presence of a rich vascular supply and the abundance of nerve bundles, often hypertrophied, was a common feature.

As a consequence of the increased cell population, the total carotid body volume was enlarged in all study cases in the series. This was also true for the control group A (individuals with cardiac hypertrophy who were also proved drug-free) but not for control group B (age-matched controls with no evidence of cardiac or pulmonary disease; Fig. 4).

Discussion

The precise physiological role of the human carotid body has not been defined as yet, although a chemoreceptor function stimulated by hypoxia is known for over 80 years (16). Which is the presumptive oxygen sensor or the sensitive site to raised intravascular pressure remains an enigma, with the alleged sites being the type I cells (progenitor, dark, and light), the carotid sinus, and the glomic capillary bed (17). Exposure to chronic hypoxia was reported to induce light cell proliferation in Quechua Indians of the Perubian Andes (18), while analogous response was shown to dark cells in native highlanders from Ladakh (19). In pathological conditions, like chronic bronchitis and emphysema, the most common morphological feature noted in carotid bodies is certainly hyperplasia of the type II sustentacular cells (7). There are indications, both experimental (20) and in humans (21), that dark cell proliferation is a short-term response of the carotid body to hypoxia which later subsides and matures into the light variant (20,21). With regard to the sustentacular cell proliferation, this apparently represents the ultimate reaction of the carotid body to hypoxia, preceding dark cell activity by a long time (20,21).

Changes in the carotid bodies were described throughout the adult life as part of the aging process (2), but also during early life in neonates and infants (15). It was shown that with advancing age

TABLE 3—*Study group. Differential counts of glomic cells, expressed as percentage of the total, in individuals with myocardial hypertrophy. Carotid body diameters and volumes are also indicated.*

	Glomic Cell Variants (%)				Carotid Body	
Cases	Progenitor	Dark	Light	Sustentacular	Diameter (μm)	Volume (μm^3)
1	3	7	32	58	20.0	4186.67
2	3	5	38	54	19.8	4062.32
3	2	4	39	55	23.0	6367.40
4	2	4	41	53	19.0	3589.54
5	2	5	34	59	22.0	5572.45
6	1	3	35	61	21.6	5273.99
7	2	3	32	63	17.5	2804.74
8	2	4	29	65	18.5	3313.55
9	1	5	45	49	21.0	4846.59
10	4	5	40	51	22.4	5881.97
11	3	3	40	54	19.3	3762.27
12	5	4	36	55	19.5	3880.45
13	2	3	38	57	22.0	5572.45
14	2	3	45	50	19.6	3940.46
15	3	5	39	53	23.0	6367.40
Mean (SD)	2.4 (0.96)	4.2 (0.90)	37.5 (4.33)	55.8 (4.52)	20.5 (1.7)	4628 (1143)

TABLE 4—*Control groups. Differential counts of glomic cells, expressed as percentage of the total, in proved drug-free individuals with myocardial hypertrophy (Control A) and individuals with no evidence of cardiopulmonary disease (Control B). Carotid body diameters and volumes are indicated, respectively.*

	Glomic Cell Variants (%)				Carotid body	
Cases	Progenitor	Dark	Light	Sustentacular	Diameter (μm)	Volume (μm^3)
Control A						
1	3	4	40	53	19.5	3880.45
2	2	4	31	63	19.7	4001.08
3	2	3	45	50	17.6	2853.10
4	3	2	40	55	23.0	6367.40
5	3	5	38	54	19.4	3821.06
6	2	5	43	50	22.6	6040.93
Mean (SD)	2.5 (0.54)	3.8 (0.89)	39.5 (4.6)	54.1 (5.8)	20.3 (2.0)	4494 (1390)
Control B						
1	2	4	53	41	7.3	203.59
2	2	5	56	37	8.8	356.64
3	3	6	49	42	12.0	904.32
4	2	5	54	39	8.0	267.95
5	2	5	51	42	8.3	299.24
6	3	5	49	43	11.0	696.56
7	2	4	57	37	7.5	220.78
8	2	6	54	38	7.4	212.07
9	4	6	51	39	8.6	332.87
10	2	4	54	40	8.2	288.55
Mean (SD)	2.4 (0.77)	5.0 (0.66)	52.8 (2.60)	39.8 (2.03)	8.7 (1.5)	378.3 (233.3)

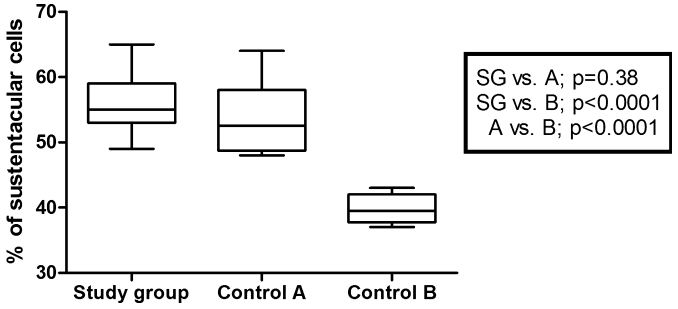

FIG. 2—*Sustentacular cells expressed as percentage of the total. Study group: individuals with cardiac hypertrophy; Control group A: proved drug-free individuals with cardiac hypertrophy; and control group B: individuals with no evidence of cardiopulmonary disease.*

there was a gradual decrease in glomic tissue, with progressive fibrosis and focal lymphocytic infiltrates (2). In the postnatal period, changes include dark cell proliferation in response to sustained hypoxia, i.e., bronchiolitis and sudden infant death syndrome with infection (15) indicating immaturity of the carotid body (22).

The dark variant of the type I cells was also associated with residency in high altitude (23), bronchial asthma (3), and in subacute reversal of a congenital intracardiac shunt (21). In these conditions, the increased numbers of dark cells were attributed to the effects of relatively mild or brief hypoxia (24). The dark cell prominence noted in the carotid bodies of subjects with cirrhosis (1,5,6) was ascribed to mechanisms other than hypoxia, namely the secretion of a natriuretic peptide in response to the hyperaldosteronism and sodium retention of cirrhosis (6,10). It is interesting, however, that

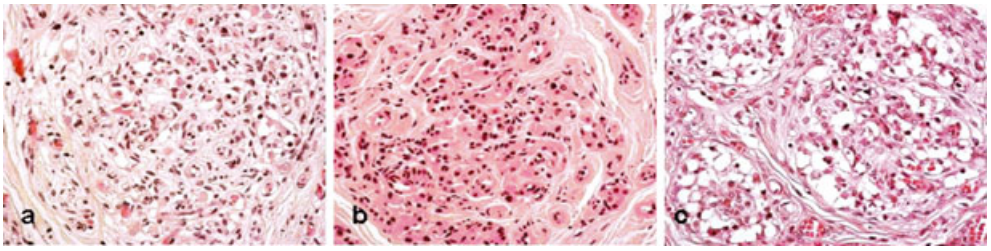

FIG. 3—*(a) Sustentacular cell hyperplasia in carotid body (hematoxylin and eosin stain, 10 × 10). (b) Carotid body lobule showing focal accumulation of dark cells (hematoxylin and eosin stain, 10 × 10). (c) Carotid body lobule showing focal accumulation of light cells (hematoxylin and eosin stain, 10 × 10).*

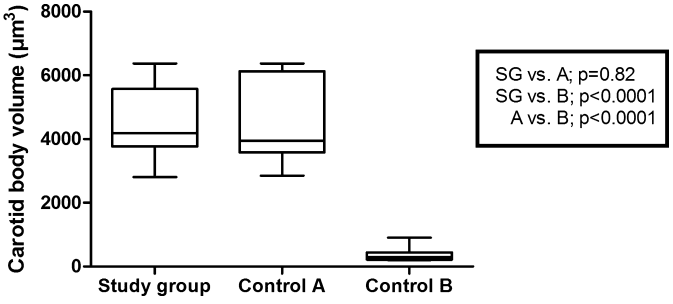

FIG. 4—*Carotid body volume. Study group: individuals with cardiac hypertrophy; Control group A: proved drug-free individuals with cardiac hypertrophy; and control group B: individuals with no evidence of cardiopulmonary disease.*

a number of peptides residing in the dark cells of the human carotid body, including methionine and leucine enkephalins (9), bombesin and neurotensin, substance P, and the vasoactive intestinal peptide (10,11,25) are of obscure functional significance. Other investigators demonstrated the frequent co-expression of serotonin with synaptophysin and the protein gene product 9.5 in type I cells of the human carotid bodies, usually in close proximity to capillaries (26).

Carotid body hyperplasia secondary to myocardial hypertrophy was described by Edwards et al. in the 1970s (4) and the early 1980s (1) but not since. They reported an increase in the number of sustentacular cells with a corresponding reduction in the number of light cells, while the mean percentage of both dark and progenitor cells remained low (1).

In our study, carotid body hyperplasia was a constant feature in all subjects with biventricular hypertrophy, including those who were proved drug-free. At first sight, and under low magnification (×10 optical fields), the carotid body lobules appeared enlarged, compared to age-matched controls, having larger clusters crowded with glomic cells—an observation which was further confirmed by measuring carotid body volume. At closer inspection, the most characteristic change in the carotid bodies associated with myocardial hypertrophy was an increase in sustentacular cells which usually was accompanied by a relative increase in their size (hypertrophy). Smith et al. (1) regarded a sustentacular cell count in excess of 47% as indicative of carotid body hyperplasia, and this figure was well reached in our material. Indeed, the mean sustentacular cell count was almost 56% in the cardiac hypertrophy cases (study group), but only 40% in controls free of cardiopulmonary disease (control group B). A statistically significant observation which, apparently, was not drug-induced since it occurred, with an almost equal frequency (54%), in individuals with hypertrophy who were also proved drug-free (control group A). This type of hyperplasia, however, may not be truly representative of the carotid body cell population. An electron microscopy study, performed on carotid body hyperplasia secondary to sustained systemic hypertension, indicated that the so-called sustentacular cell hyperplasia involved both sustentacular and Schwann cells (27). The former were located closer to the central core, whereas the latter adopted a more peripheral position in the cell cluster. The two cell types are, in essence, similar and cannot be distinguished with certainty from each other (27,28). In the above ultrastructural study, few fibroblasts were recognized, but the process, on the whole, was not that of fibrosis (27,28).

In any case, proliferation of sustentacular cells was also reported in bronchial asthma, particularly in long-standing cases (3), chronic bronchitis and emphysema (4), and in systemic hypertension (26). They were also shown in connection with heroin addiction, where the increase in sustentacular cells was ascribed to glomic hypoxia, while the accompanied decrease in the light cell population was thought to be a direct effect of the opiates (29). In the majority of cases, however, the reduced tissue oxygen levels is a result of progressive replacement of the type I cells by sustentacular cells leading to an impaired carotid body function (3,4,26,30).

In light of the above evidence, it would be reasonable to assume that the generalized sustentacular cell hyperplasia occurs as a chronic response to hypoxia (31,32), while any superimposed focal proliferation of light or dark cells may be related to short-term episodes of hypoxia. Whether the increased sustentacular cell numbers are simply a supportive cell population or represent precursors for glomus cells remains unclear (33). It is also debatable whether the focal accumulation of dark cells described represents a true proliferation of cells (hyperplasia) or an oncocytic metaplasia similar to that seen in spinal paragangliomas (34). Further, and indeed larger, studies are obviously needed to substantiate our findings and elucidate these questions.

Conflict of interest: The authors have no relevant conflicts of interest to declare.

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