Virchows Archiv A Pathol Anat (1992) 420:31–35

Virchows Archiv A
Pathological Anatomy and Histopathology

Post-mortem changes in the normal rat carotid body: possible implications for human histopathology

D.J. Pallot, M. Seker, and A. Abramovici*

Department of Anatomy, University of Leicester, Medical Sciences Building, University Road, Leicester LE1 7RH, UK

Received July 10, 1991 / Received after revision October 8, 1991 / Accepted October 11, 1991

Summary. The carotid bodies in experimental animals contain only one variety of type I cells whilst in the human organ three varieties of this cell type have been described. We have examined the effects of post-mortem change on the structure of the type I cell of the rat carotid body. When the organ is examined immediately after death of the animal all of the type I cells exhibit similar morphology. With increasing delay in fixation of the tissue the type I cells undergo autolytic changes. Within 2 h of death the nuclei become hyperchromatic and the cytoplasm exhibits an increasing eosinophilia. In carotid bodies fixed 4 h post-mortem a further type I cell variant is seen in which the nucleus lacks a chromatin pattern and becomes pyknotic. We believe that previous descriptions of three varieties of type I cells in the human carotid body are based upon a description of post-mortem change. Furthermore, in any study of this highly oxygen dependent tissue it is essential that due account be taken of the delay between death and fixation.

Introduction

The mammalian carotid body consists of groups of specific cells set in a vascular connective tissue stroma containing numerous nerve fibres. The parenchymal cells consists of two varieties, the type I and type II cells. In all experimental animals studied to date a variable number of polygonal type I cells are surrounded by type II cells, the latter being elongated cells with long fine, cytoplasmic processes (Biscoe 1971; Pallot 1987). In the human carotid body a similar arrangement has been described by Smith and his colleagues (Smith et al. 1982) except that they are able to recognise three sub-populations of type I cells on the basis of nuclear morphology; these are termed the light, dark and pyknotic cells.

A number of workers have attempted to identify type I cell sub-types in the rat, rabbit and cat carotid body using a variety of morphometric techniques (McDonald and Mitchell 1975; Verna 1979; Pallot et al. 1986); to date no convincing evidence for sub-classes of type I cells has been produced in these species (Pallot 1987). We were intrigued by this apparent difference in the structure of the human carotid body and that of other mammalian species. As studies of the human carotid body must involve the examination of post-mortem material we examine here the effects of post-mortem changes on the structure of the rat carotid body.

Materials and methods

The experiments were performed using adult Wistar rats of approximately 4 months of age. The animals, obtained from the Unit of Biomedical Services, University of Leicester, were killed by an overdose of pentobarbitone sodium and treated as follows.

1. The carotid bodies were removed at the time of death (0 h; these tissues acted as controls.
2. Carotid bodies were removed at different times after death (2, 4, 8, 16 and 24 h) from animals in which the carcass had been kept at room temperature for 2 h and thereafter at 4° C.
3. Carotid bodies were removed at 4 and 16 h post-mortem from animals in which the carcass had been kept at room temperature.

A total of 24 animals were studied.

The carotid bodies were fixed overnight in 4% neutral buffered formalin, processed by routine techniques for embedding in paraffin wax and stained either with Harris haematoxylin or by the periodic acid-Schiff (PAS) method.

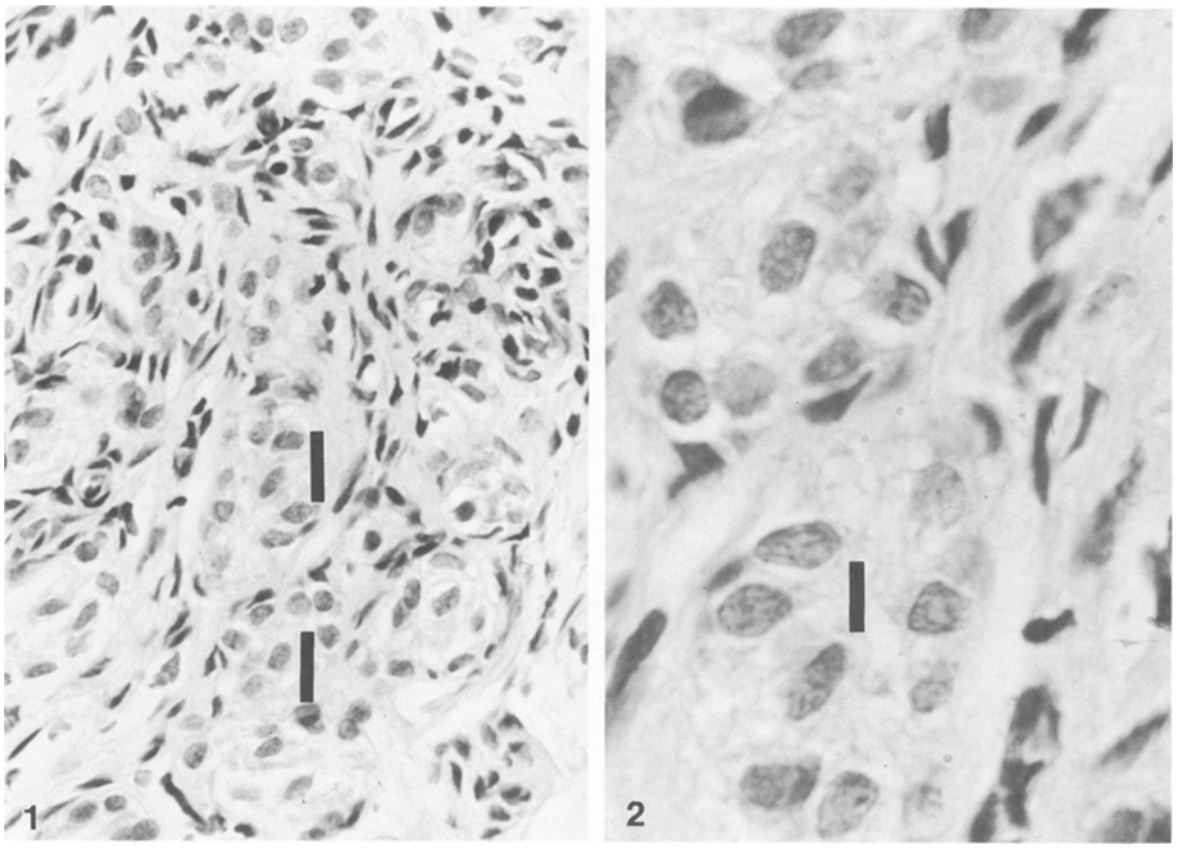
The number of type I cells with either light, dark or pyknotic nuclei was counted in random sections of carotid bodies with a 40× objective; averages of at least four such fields were counted for the quantitative studies.

Results

The control carotid bodies (0 h group) showed the typical clustering of polygonal type I cells surrounded by

* On leave from the Laboratory of Development Pathology, Department of Pathology, Sackler School of Medicine, University of Tel Aviv, Tel Aviv 69978, Israel

Offprint requests to: D.J. Pallot



Figs. 1, 2. Low and high power views of a carotid body removed from a rat immediately after the overdose of anaesthetic. Note how all of the type I cells (*I*) have a similar nuclear morphology and that their cytoplasm lacks eosinophilia

elongated type II cells (Fig. 1). The type I cells were of similar size and shape and possessed a light eosinophilic cytoplasm and large round or ovoid nuclei (Fig. 2). Some type I cells possessed a slightly hyperchromatic nucleus but lacked the eosinophilic cytoplasm at this time. The type II cells had less definite cell borders and the nuclei were elongated and hyperchromatic (Figs. 1, 2). Few congested capillaries and very few pyknotic cells were seen.

Changes in type I cell morphology were more frequent within 2 h of death and became prominent after 4 h at 4° C (Fig. 3). There was an increasing vacuolation of some type I cells and the appearance of more cells with hyperchromatic nuclei; some of these latter cells possessed smaller, eccentrically placed nuclei with a cap of intensely eosinophilic cytoplasm (Fig. 4); these cells are similar to the dark cells described in the normal human carotid body by Smith et al. (1982). A third variant of type I cell became prominent by 4 h. This pyknotic variant was characterised by a small, compact, darkly stained nucleus with a narrow peripheral rim of cytoplasm (Fig. 5). Similar periods of delay induced different pathological findings depending on whether the carcass had been kept at room temperature or at 4° C. The degree of change from the normal structure was much greater at room temperature where even after 4 h nuclear vesiculation and loss of cell margins could be seen.

In contrast to type I cells, the type II cells appeared resistant to autolytic change. However, by 16 h some type II cells had also developed pyknotic nuclei.

Marked vascular dilatation and congestion was observed in all specimens studied at post-mortem periods greater than 2 h and the magnitude of the congestion seemed to increase with increasing post-mortem delay. No leucocytic infiltration was found in any of the carotid bodies examined.

Figure 6 illustrates quantitative data on type I cell variants. The number of light cells decrease almost linearly up to 4 h post-mortem and this decrease is paralleled by an increase in the number of pyknotic and dark cells. Between 4 and 24 h fixation delay there is a further fall in the percentage of clear cells and rise in the number of pyknotic cells. It is also noteworthy that when large numbers of cells are examined, in sections from different levels of the carotid body, that there are a small number of pyknotic cells can be found at time zero.

Discussion

The present observations clearly indicate that three variants of type I cells, the clear, dark and pyknotic cells, do not represent a stable population but rather that their numbers fluctuate in relation to the delay between death of the animal and fixation of the tissue.

It is noteworthy that isolated autolytic changes were

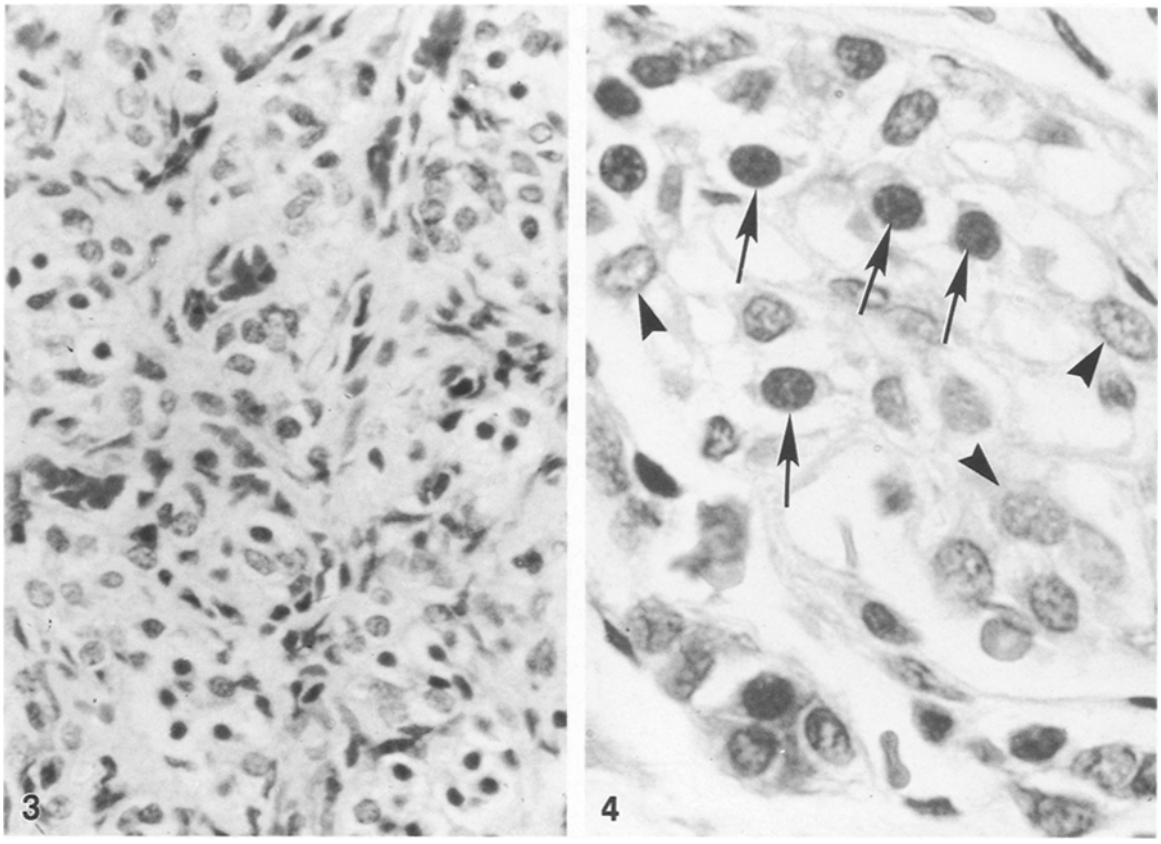


Fig. 3, 4. Low and high power views of carotid body tissue fixed 4 h after death of the animal. Some type I cell nuclei have densely staining chromatin patterns (*arrows*) and that these cells show a markedly eosinophilic cytoplasm (*arrows* in Fig. 4). Other cells (*arrowheads* in Fig. 4) have a similar chromatin pattern to that seen in Figs. 1, 2 but increased vacuolisation of the cytoplasm

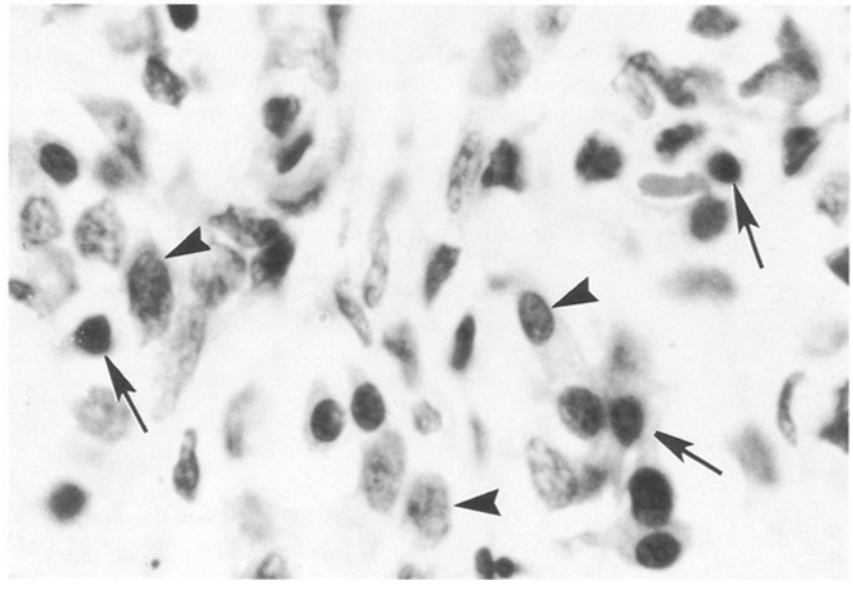


Fig. 5. High power micrograph of carotid body tissue fixed 16 h after death. Some cells lack any visible chromatin pattern in their nuclei (*arrows*) whilst others have dark nuclei as in Figs. 3, 4 (*arrowheads*)

noted in tissues were the fixation delay was of the order of 15 min (the time taken to complete the dissection). This situation is not surprising as it is well known that other rat organs, for example the liver, may undergo autolytic changes within minutes after death. The fact that previous morphological studies of rat, cat and rabbit carotid bodies failed to demonstrate dark or pyknotic cells may be explained by the fact that all of these studies used vascular perfusion of the fixative (McDonald and Mitchell 1975; Verna 1979; Pallot et al. 1986). The de-

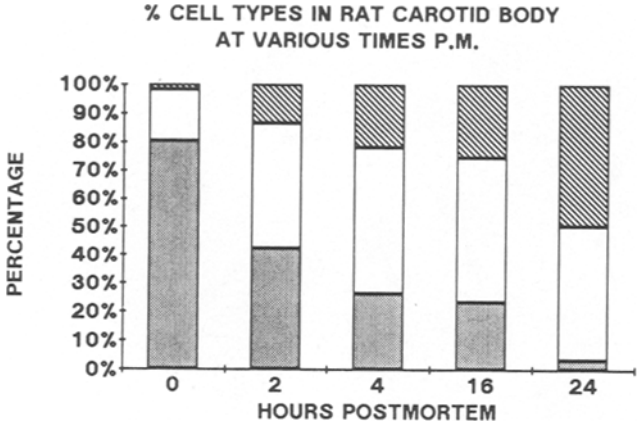


Fig. 6. Stacked bar histogram illustrating the percentage of clear, dark and pyknotic cells in the rat carotid body after various fixation delays. (▨ = clear; □ = dark; ▧ = pyknotic)

sign of these experiments was adopted in an attempt to mimic the situation that might exist in routine autopsy protocols. Thus there was a delay of 2 h before refrigeration of the carcass and permeation of the fixative rather than perfusion.

We have as yet no direct information on the precise events that lead to the appearance of dark and pyknotic cells within the carotid body. However, based upon what is known to occur elsewhere during the initiation of irreversible anoxic cell injury (Rubin and Farber 1988) and on our histopathological findings let us assume that a similar sequence of events could take place in the carotid body. According to such an assumption the clear type I cells rapidly undergo hydropic degeneration, as indicated by their vacuolated appearance, followed by an increase in the eosinophilic affinity of the cytoplasm and changes in the nuclear chromatin array. These transformations represent an intermediate step in the process of autolysis, the dark cell variant. During the following hours after death (4–24 h) the cytolytic process will continue and the nucleus becomes heavily hyperchromatic, representing the so-called pyknotic cell.

The existence of a sequential and dynamic transformation of the type I cell population as a function of the autolytic process is well illustrated in our data by the reciprocal interelationship between the decreasing number of clear cells and increasing number of pyknotic cells.

There is considerable controversy in the carotid body literature regarding the origin and nature of the dark and pyknotic cells. The possibility that these cells increased in number with increasing post-mortem delay was suggested by a number of authors (Gosses 1938; de Castro 1951), whilst others presumed them to be normal constituents of the carotid body (Smith 1924; White 1935; Smith et al. 1982). Recently it has even been suggested that the pyknotic cells in the carotid bodies of children represent "precursors" of the dark and clear cell variants (Heath et al. 1990). The confusion is compounded by the fact that to date no clear function has been ascribed to the dark and pyknotic variants.

Our studies have also revealed an apparent resistance of the type II, or sustentacular, cells to autolytic change as well as the absence of any intermediate variant between the normal and pyknotic type II cell. The existence of different patterns in response to anoxic injury in the type I and type II cells might be explained by their differing autogenetic origins and functions.

The type I cells are known to originate from the neural crest (Le Douarin et al. 1972) and show similar cell markers to neuronal cells such as neuron specific enolase and neurofilament protein (Abramovici and Pallot 1990) as well as acting as a storage site of various neuropeptides (Wharton et al. 1980; Abramovici and Pallot 1990; Oomori et al. 1991) and catecholamines (Pallot 1987). Since type I cells undoubtedly respond to changes in oxygen tension it seems plausible that a sudden and dramatic drop in oxygen supply, even for a few minutes, might result in irreversible changes in the cell.

The exact origin and function of the type II cell is yet to be elucidated and it is difficult to distinguish then from the Schwann cells (McDonald 1981; Jago et al. 1984). There is some immunohistochemical evidence (they store S100 and glial fibrillary acidic protein) suggesting that they are similar to Schwann cells and oligodendroglia (Abramovici and Pallot 1990). The possibility exists that their resistance to anoxia might be related to a lower oxygen requirement than the type I cells.

The existence of vasodilatation and congestion in the longer post-mortem delay animals (4–24 h) are further post-mortem changes affecting carotid body morphology. The intensity of both the vasodilatation and the degree of congestion were related to the post-mortem delay and were further increased if the head of the animal was placed below the level of the heart suggesting that the phenomenon was entirely passive.

The data reported here would council caution in the interpretation of carotid body type I cell differential counts as it may be that the existence of substantial numbers of dark and pyknotic cells is a post-mortem artefact. This suggestion is substantiated by our recent observations of human carotid bodies fixed at various times after death where there was a positive correlation between the post-mortem delay in fixation and the number of pyknotic cells and a negative correlation between delay and the number of clear cells (Seker et al., unpublished).

References

Abramovici A, Pallot DJ (1990) An immunohistochemical approach to the study of the cat carotid body. Acta Anat (Basel) 140:70–74

Biscoe TJ (1971) The carotid body; structure and function. Physiol Rev 51:437–495

Castro F de (1951) Sur la structure de la synapse dans les chemorecepteurs leur mecanisme d'excitation et role dans la circulation sanguine locale. Acta Physiol Scand 22:14–43

Gosses J (1938) The glomus caroticum. Acta Neeol Morphol 1:38–42

Heath D, Khan Q, Smith P (1990) Histopathology of the carotid bodies in neonates and infants. Histopathology 17:511–520

Hurst G, Heath D, Smith P (1985) Histological changes associated with ageing of the human carotid body. J Pathol 147:181–187

Jago R, Smith P, Heath D (1984) Electron microscopy of carotid-bodyhyperplasia. Arch Pathol Lab Med 108:717–722

Le Douarin N, Le Lievre C, Fontaine J (1972) Recherches experimentales sur l'origin embryologique du corps carotidien chez les oiseaux. CR Acad Sci (Ser D) 275:583–586

McDonald DM (1981) Peripheral chemoreceptors: structure function relationships of the carotid body. In: Hornbein TF (ed) Regulation of breathing. Dekker, New York, p 105

McDonald DM, Mitchell RM (1975) The innervation of glomus cells, ganglion cells and blood vessels in the rat carotid body: a quantitative ultrastructural analysis. J Neurocytol 4:177–230

Oomori Y, Ishikawa K, Sato H, Matsuda M, Ono K (1991) Neuropeptide y immunoreactivity in the chief cells in the carotid body of young rats. Acta Anat (Basel) 140:120–123

Pallot DJ (1987) The mammalian carotid body. Adv Anat Embryol Cell Biol 102:99

Pallot DJ, Al-Neamy K, Blakeman N (1986) Quantitative studies of the rat carotid body Type I cells. Acta Anat (Basel) 126:187–191

Rubin E, Farber JL (1988) Pathology. Lippincott, Philadelphia, pp 4–33

Smith C (1924) The origin and development of the carotid body. Am J Anat 34:82–131

Smith P, Jago R, Smith D (1982) Anatomical variation and quantitative histology of the normal and enlarged carotid body. J Pathol 137:287–304

Verna A (1979) The ultrastructure of the carotid body in mammals. Int Rev Cytol 60:271–330

Wharton J, Polak JM, Pearse AGE, McGregor GP, Bryant MG, Bloom SR, Emson PS, Bisgard GE, Will JA (1980) Enkephalin, VIP and substance P-like immunoreactivity in the carotid body. Nature 284:269–271

White EG (1935) Die Struktur des Glomus caroticum, seine Pathologie und Physiologie und sein Nervensystem. Beitr Pathol Anat 96:177–227