Electron-Dense Precipitates in Glomus Cells of Rat Carotid Body after Fixation in Glutaraldehyde and Pyroantimonate-Osmium Tetroxide Mixture as Possible Indicators of Calcium Localization*

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Summary. An attempt was made to study the subcellular localization of calcium in carotid body glomus cells of adult rats using fixation with glutaraldehyde followed by treatment with a mixture of pyroantimonate and osmium tetroxide. Precipitates were seen as electron-dense particles (EDP) in the glomus cells, mostly within membrane-bound organelles, such as dense-cored vesicles, mitochondria, small clear vesicles, multivesicular bodies, and especially in lysosomes. However, EDP were also seen in the nuclei and in the free cytoplasm of the glomus cells and even outside them.

Preincubation of carotid bodies in media containing calcium and either high potassium or calcium-ionophore A 23187 resulted in a marked increase in the general precipitation pattern, there being an increased amount of EDP both in the glomus cell nuclei and in the cytoplasm. Dense-cored vesicles more often showed precipitates than those in the controls. Some dense-cored vesicles contained multiple precipitates, typically located in the electron-lucent area between core and vesicle membrane.

Extensive diffusion of ions probably occurred during fixation before precipitation, making the localization of calcium and other precipitating cations unreliable. However, it is possible that precipitates, which were regularly seen in the dense-cored vesicles, may reflect the content of bound calcium. The possible significance of calcium in glomus cell function is discussed, and the need for more adequate methods is emphasized.

Key words: Carotid body – Calcium – Granular vesicles – Exocytosis

It has been suggested that calcium plays an important role in the function of the glomus cells of the carotid body by triggering the secretion of the amine-containing granules (Krammer 1978; Hansen and Smith 1979). As in the adrenal medulla

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Nagasawa 1977), amines are probably secreted by exocytosis from the glomus cells (cf. McDonald and Mitchell 1975). Exocytotic profiles have indeed been observed on glomus cells following incubation of carotid bodies in media containing calcium and a high concentration of potassium, while such profiles were only rarely seen in untreated carotid bodies (Grönlad et al. 1980a), possibly due to the rapidity of the secretory process (Smith and Winkler 1972).

The insulin-storing granules in pancreatic B-cells (Hellman et al. 1979) and the amine granules in adrenal medullary cells (Serck-Hansen and Christiansen 1973; Kostron et al. 1977) have been shown to actively take up calcium during secretory stimulation and in the B-cells the insulin granules probably have an important role as intracellular sites of calcium buffering during the secretion (Kohnert et al. 1979).

In view of the morphological and functional similarities between adrenal medullary cells and the amine-storing glomus cells of the carotid body (Fujita 1977; Kanno 1977), it seems possible that glomus cell granules might have an active mechanism of calcium uptake similar to that found in adrenal medullary granules. Therefore, direct ultracytochemical demonstration of calcium in dense-cored vesicles would be of obvious interest.

Using a method proposed by Oschman and Wall (1972) for subcellular localization of calcium-binding sites in tissues, Hess (1977) and Hansen and Smith (1979) observed the appearance of electron-dense particles (EDP) in the dense-cored vesicles of the glomus cells. In these two studies on the carotid body of the rat (Hess 1977) and the carotid and aortic bodies of the rabbit (Hansen and Smith 1979), 50 mM of CaCl₂ was added to the glutaraldehyde fixative, to the buffer and to the osmium tetroxide used for post-fixation. No EDP were seen when calcium was omitted from the fixative solutions (Hess 1977; Hansen and Smith 1979). It was suggested that the EDP represent granular binding sites for calcium, which possibly is bound during exocytotic extrusion of granules from glomus cells (Hansen and Smith 1979). Similar EDP have been observed in the dense-cored vesicles of the glomus cells following fixation first in glutaraldehyde and then in a mixture of potassium pyroantimonate and osmium tetroxide (Grönlad et al. 1980b), a method proposed for localizing calcium electron histochemically (Simson and Spicer 1975). Preincubation of carotid bodies in media containing calcium and the calcium-transporting ionophore A23187, which can be expected to translocate calcium across the cell membrane in the direction of the concentration gradient (Pfieffer et al. 1978), results in the appearance of EDP in some of the glomus cell granules with the pyroantimonate method (Grönlad et al. 1980b). Thus, two different methods suggest localization of exogenous calcium in the granular vesicles of the glomus cells.

In the present study, carotid bodies were preincubated either with a high concentration of potassium or the ionophore A23187 and calcium, to further examine the distribution of pyroantimonate-precipitated calcium in the glomus cells.

**Materials and Methods**

**Incubation of the Carotid Bodies**

Untreated adult male Sprague-Dawley rats were used. The animals were killed by a sharp blow on the back of the head and by cutting the spinal column. The carotid bodies were then quickly dissected with the aid of a binocular dissecting microscope and immediately immersed in the incubation medium.