Blood Vascular Organization of the Rat Carotid Body: A Scanning Electron Microscopic Study of Corrosion Casts

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Received April 25, 1986

Summary. The blood vascular bed of the rat carotid body was reproduced with methacrylate and observed under the scanning electron microscope. The carotid body received the proper carotid body artery from the common carotid body artery, which arose from the external carotid or occipital artery and gave off subsidiary branches to the tissues near the carotid body. The proper carotid body artery divided in the carotid body, ultimately breaking up into thick (main) or thin (subsidiary) arterial terminals to form the vascular plexus of the carotid body. This plexus contained both thick and thin capillaries. The thick capillaries arose from the thick and thin arterial terminals and formed the basic capillary network of the carotid body. The thin capillaries were only subsidiary, intercalated among the thick capillaries. A few accessory twigs of the proper carotid body artery passed through the carotid body and supplied the adipose and other tissues around the carotid body. Many venules arose from the thick capillaries of the carotid body and were collected into rostral and caudal efferent veins. These efferent veins received the veins from the tissues adjacent to the carotid body, and drained into the internal jugular vein. No arterio-venous anastomosis was found in, on or around the carotid body. The common carotid body artery and its subsidiary branches showed, at their origins, marked constrictions indicative of the arterial cushions, though the proper carotid body artery and its accessory twigs were not provided with such clear constrictions. These findings suggest that the inflow of blood into the common carotid body artery may be regulated by its constriction, especially of its arterial cushion, and that the subsidiary branches of the common carotid body artery and the accessory twigs of the proper carotid body artery may act as bypass-routes to eliminate the excessive inflow of blood into the carotid body. It is considered that the thin arterial terminals and thin capillaries may act as buffer channels to homogenize the blood flow within the carotid body.

The carotid body is regarded as an arterial chemoreceptor sensitive to the partial pressure of oxygen and carbon dioxide (Biscoe, 1971). Many authors have described the occurrence of arterio-venous anastomoses in or around the carotid body, suggesting their regulation of the blood flow into or within the carotid body (de Castro, 1951, 1962; Serafini Fracassini and Volpin, 1966; Schäfer et al., 1973; Acker and Lübbers, 1977; Acker, 1980; Acker and O'Regan, 1981; McDonald and Larue, 1983; McDonald and Haskell, 1984; Habeck et al., 1984). However, the existence of such arterio-venous anastomoses has been refused by some authors (Niedorf, 1970; Seidl, 1976).
The vascular architecture of the carotid body has been primarily studied by light microscopy of serial sections (Serafini-Fracassini and Volpin, 1966; Niedorf, 1970; Seidl, 1976; McDonald and Larue, 1983; McDonald and Haskell, 1984; Habeck et al., 1984), India ink- or gelatine-injected specimens (Muratori, 1943; de Castro, 1951, 1962) or latex-perfused samples (Chungcharoen et al., 1952). Recently, some authors have employed a vascular corrosion casting/scanning electron microscope technique (Mura- kami, 1971), and examined the microvasculature of the body in the fish (Olson et al., 1981), the frog (Kobayashi and Murakami, 1975; Weigelt and Acker, 1977), the water lizard and the salamander (Noguchi and Kobayashi, 1977), the cat (Keller et al., 1972; Schäfer et al., 1973; Weigelt and Acker, 1977), the rabbit (Weigelt and Acker, 1977), and the rat (McDonald and Larue, 1983). However, the microvascular architecture of the carotid body has not been fully elucidated because of the limited resolution and shallow focus inherent to light microscopy as well as the omission of microdissection in scanning electron microscopy. Therefore a convincing arterio-venous anastomosis has yet to be demonstrated in or around the carotid body.

MATERIALS AND METHODS

Thirty male Wistar rats weighing 200–400 g were used. Vascular corrosion casts were prepared by the original or a modified method of Murakami's (1971, 1975). Under inhalation of anesthesia with diethyl ether, the thoracic cavity was opened and the ascending aorta was cannulated. After ligating the descending aorta, the animals were perfused with physiological saline through the cannulated aorta until the effluent from the superior vena cava was clear. After this perfusion, about 20 ml of laboratory-prepared low viscosity methacrylate or commercially available Mercox resin (Japan Vilene Hospital, Tokyo) was injected into the vascular bed through the cannulated aorta (Murakami, 1971, 1975).

The carotid body and its surrounding tissues were then isolated, placed in a hot water bath (60°C) for 2–3 hrs, corroded in a hot 10–20% NaOH solution (60°C) overnight, washed in running water, and air-dried. The casts thus prepared were trimmed with needles and forceps under a light microscope (SMZ-10, Nikon, Japan), mounted on metal stubs with silver paste (Dotite Type 550, Fujikura Kasei, Sano, Japan). They were further microdissected under the light microscope with sharpened needles and forceps in order to expose the desired vessels (Fujita and Murakami, 1973). The micro-dissected specimens were coated with gold in an ion coater (IB-3, Eiko), and observed with a scanning electron microscope (JSM-U3, JEOL, or HHS-2R, Hitachi) using an accelerating voltage of 5 kV. This series of microdissection and scanning electron microscopy has yet to be demonstrated in or around the carotid body.
microscopy was repeated until the vessels of interest were thoroughly observed. The microdissection included the freeze-cutting of specimens with razor blades (Kobayashi and Murakami, 1975).

Fig. 1. Legend on the opposite page.
RESULTS

Injection of 20 ml of laboratory-prepared low viscosity methacrylate or commercially available Mercox resin through the ascending aorta after thorough perfusion with a saline solution resulted in the preparation of appropriate blood vascular casts of the carotid body and other cervical organs in adult rats (Fig. 1, 2). Few undesired discontinuities of the vessels were noted in the cast samples, though some leakage of the injected resin was observed (Fig. 2). Light microscopic trimming and repeated microdissection of the fully injected casts followed by scanning electron microscopy allowed clear visualization and also detailed analysis of the capillary plexus of the carotid body as well as its afferent and efferent vessels (Fig. 1–4). The results obtained by these casting/dissection/scanning techniques are described below and schematically diagrammed in Figure 5.

Carotid body artery

The carotid body received an artery (the proper carotid body artery) from a common trunk (the common carotid body artery), which arose from the external carotid or occipital artery and gave off one to three (usually two) subsidiary branches to the sympathetic cervical ganglion, vagus nerve, carotid sinus and other tissues near the carotid body (Fig. 1). Regardless of its origin, the common carotid body artery always showed a conspicuous constriction at its origin (Fig. 1). The subsidiary branches to the sympathetic cervical ganglion and other tissues also showed such marked constrictions at their origins. In contrast, the proper carotid body artery showed no constriction at its origin from the common carotid body artery (Fig. 1).

Microvasculature of the carotid body

Either shortly before or immediately after entering the carotid body, the proper carotid body artery divided into several primary branches (Fig. 2, 3). Some of these primary branches with shorter and thicker trunks divided four or five times in the carotid body and ran, as the secondary branches, deep into the carotid body where they broke up into arterial terminals of two types: thick ones (averaging 20 μm in diameter) and thin ones (averaging 10 μm in diameter). These then continued into the deeper capillaries (Fig. 4). Other primary branches with longer and thinner trunks ran straight for short distances toward the superficial layer of the carotid body, where they broke up rather abruptly into thick and thin arterial terminals continuous with the superficial capillaries in the carotid body (Fig. 3). The occurrence of the thin arterial terminals was at best occasional. About one-fifth of the arterial terminals were thin ones, running through the superficial and deep layers of the carotid body.

Most of the primary branches, including those running deep, of the proper carotid body artery showed poorly-developed constrictions at their origins, and terminated within the carotid body (Fig. 3). However, one or two primary branches (accessory twigs) of the proper carotid body artery ran through the carotid body to supply the tissues near the carotid body, including the adipose tissue (Fig. 2, 3). These accessory twigs were sometimes well-developed, but showed no marked constrictions at their origins (Fig. 2).

Both deep and superficial capillaries in the carotid body anastomosed (or joined) with each other and formed a conglomerated capillary plexus commonly supplying the whole carotid body (Fig. 3). Two types of capillaries were observed in this plexus:
A closer scanning electron micrograph of the methacrylate cast of the blood vascular bed of the rat left carotid body. Note that the rostral and caudal efferent veins (VR and VC) of the carotid body anastomose with each other (thick arrows) on the carotid body (CB). Right inset shows a much closer view of these anastomoses. The left inset shows a dissected form of this cast sample (dorsal view), where one (double arrowheads) of the primary branches (As) of the proper carotid body artery (CBA_p) passes through the carotid body (CB). V veins which receive the rostral and caudal efferent veins and continue into the internal jugular vein, ve venules of the carotid body, ve venous twigs from the tissues around the carotid body emptying into the rostral efferent veins of the carotid body; thin arrow: a broken venous twig which arises from the adjacent tissues and continues into the rostral efferent vein of the carotid body; asterisks: leakage of resin. ×140, left inset: ×70, right inset: ×200
thick ones (averaging 15 μm in diameter), and thin ones (averaging 6 μm) (Fig. 3, 4). These two types of capillaries could be clearly discriminated from each other in the fully injected or expanded casts. The majority of the capillaries were the former thick type, which was spread throughout the capillary plexus of the carotid body (Fig. 3, 4).
These thick capillaries arose directly from the thick and thin arterial terminals (see above), ran in a winding manner with repeated anastomosing, and formed a basic or skeletal capillary network of the carotid body capillary plexus (Fig. 4). The thin capillaries were only subsidiary and intervened, as bridging vessels, between the thick capillaries (Fig. 4). The thin capillaries had neither a direct origin from the arterial terminals nor a direct drainage into the venules (Fig. 3, 4).

**Venules and veins of the carotid body**

Many venules arose, in both the deep and superficial layers of the body, from the basic capillary network consisting of thick capillaries (Fig. 3, 4). They drained into two groups of efferent veins: one or two rostral efferent veins located at the rostral portion of the body, and one or two caudal efferent veins at the caudal portion (Fig. 1). Around the carotid body, the rostral and caudal efferent veins received some additional veins from the sympathetic cervical ganglion and other tissues near the carotid body, finally draining into the internal jugular vein (Fig. 1). The rostral and caudal efferent veins occasionally formed a few anastomoses on the surface of the carotid body (Fig. 2).

**Arterio-venous anastomosis**

No direct connection between an artery and a vein, or the arterio-venous anastomosis could be found either in the interior or on the surface of the carotid body. All the channels intervening between the arterial and venous sides in the carotid body fell into the categories of the thick and thin capillaries described above. In addition, no arterio-venous anastomosis was observed around the body.

**DISCUSSION**

The present scanning electron microscope observations combined with microdissection of casts prepared by thorough injection of resin show that, in the rat, no arterio-venous anastomosis exists in, on or around the organ. This finding coincides with those by NIEDOLF (1970) and SEIDL (1976) who observed serial sections of perfusion-fixed cat carotid bodies with a light microscope. Our present scanning observations of vascular casts could not support the argument for such a short-cut circulation between the arterial and venous twigs in the carotid body as contended by DE CASTRO and many other light microscopists who observed the gelatine-injected cat samples (DE CASTRO, 1951, 1962) or serial sections of the perfusion-fixed dog, cat and rat specimens (SERAFINI-FRACASSINI and VOLPIN, 1966; MCDONALD and LARUE, 1983; MCDONALD and HASKELL, 1984; HABECK et al., 1984). SCHAFER and his associates (1973) described a thick arterio-venous anastomosis on the surface of the cat carotid body by their scanning electron

**Fig. 3.** A survey scanning view of a dissected form of the blood vascular bed of the rat left carotid body. Note that the capillary plexus of the carotid body is derived from the proper carotid body artery (CBAr) and continues into the rostral and caudal efferent veins (VR and VC). Also note that the blood vascular capillary plexus of the carotid body (CB) consists of anastomosing capillaries, which are classified into thick (main) (large arrowheads) and thin (subsidiary) (small arrowheads) ones. Arrows indicate constrictions of the primary branches (As) at their origins. The inset shows a further microdissected form of the cast sample shown in the left inset in Figure 2. Here note that a primary branch (large arrow) terminates in the superficial layer of the carotid body, and that a small primary branch (asp) of the proper carotid body artery passes through the carotid body (CB). Double arrowheads: thick primary branch penetrating the carotid body; vc venules of the carotid body, ve vein of the tissues around the carotid body. ×200, Inset: ×70
Fig. 4. Legend on the opposite page.
microscopy of cast samples. By the same method, Acker and Lübbers (1977) also recognized a similarly thick arterio-venous shunt on the surface of cat and rabbit carotid bodies. Judging from their scanning micrographs or the surface properties of their cast samples, however, their arterio-venous anastomosis seems to be a veno-venous anastomosis, which probably is identical with our veno-venous anastomosis occasionally found between the rostral and caudal efferent veins on the rat carotid body surface.

Our preliminary experiments in the present study have shown that our veno-venous anastomosis, when reproduced by insufficient injection of methacrylate resin through the ascending aorta, usually shows surface-irregularities quite similar to those in the "arterio-venous anastomosis" of the previous authors (data, not shown).

Fig. 5. Diagram showing the vascular arrangement in and around the rat carotid body (see text). The large and small arrows indicate thick and thin arterial terminals, respectively. The large and small arrowheads indicate thick and thin capillaries, respectively. The double arrows point to constrictions indicative of intra-arterial cushions, while the triple arrows, veno-venous anastomosis. For abbreviations see the legends for Figure 1-4.

Fig. 4. A closer scanning view of another micro dissected form of the left carotid body. Note that the arterial terminals in the carotid body are also classified into two types: thick (large arrows) and thin (small arrows) ones. The thick capillaries (large arrowheads) originate from both thick and thin arterial terminals. Left inset shows two typical thin arterial terminals (small arrows) continuing into thick capillaries (large arrowhead). The right inset shows some typical thin capillaries (small arrowheads) intercalated between the thick capillaries (large arrowheads). As primary branches of the proper carotid body artery, VR and VC rostral and caudal efferent veins, as secondary (or preterminal) branches of the proper carotid body artery (CBAp), ve venules of the carotid body. ×340, left inset: ×300, right inset: ×500
The present scanning electron microscope observations also show that two types of capillaries, a thin one and a thick one, occur in the carotid body, and that the thick capillaries are the basic vessels to form the vascular plexus of this body. Based on their light microscope observations of the serial sections of perfusion-fixed rat carotid bodies, McDonald and his associates described Type I capillaries (ranging from 8 to 20 μm in diameter) and Type II (averaging 6 μm in diameter), and contended that their Type II or thin capillaries arose from the arterial terminals to directly continue, without any communication with their Type I or thick capillaries, into venules. However, no thin capillaries corresponding to the Type II capillaries of McDonald and his associates could be identified in the present scanning electron microscope study of fully expanded casts prepared by injection into non-fixed vessels. Their classification as “carotid body capillaries” by McDonald and his associates seems to be inadequate.

Another preliminary experiment of ours has shown that in perfusion fixation, some vessels are fixed in dilated conditions while others remain undilated because of the uneven flow of the perfused fixative through the capillary network (data not shown). As described above, our thin capillaries reproduced by thorough injection of resin are characterized by their intervention between the thick capillaries. We regard these thin capillaries as channels in the connective tissue proper, and the thick capillaries as the vessels associated with the glomus cell clusters.

Besides their two types of capillaries, McDonald and his associates further observed another vascular system in the rat perfusion-fixed specimens. More strictly, they described two out of 14 terminal arterioles as being lined with endothelial cells with marked nuclear protrusion into the vascular lumen, and abruptly continuing into thin walled vessels averaging about 10 μm in diameter. They considered these vascular connections to be the arterio-venous anastomoses, and thin walled vessels to be small venules. However, they did not clarify further connections of their thin walled vessels. As described above, our scanning electron microscope observations of vascular casts clearly show that arterial twigs with similar diameters ranging 10–15 μm run toward the superficial layer of the carotid body, abruptly to continue into the thick or basic capillary plexus of this body. Furthermore, it should be noted in the present study that the arterial terminals are sometimes thin (10 μm in diameter), continuing into the thick or basic capillaries in the carotid body. It is presumed that these vessels were misunderstood as the arterio-venous anastomoses by McDonald and his associates.

Acker and his associates estimated the tissue oxygen contents and hydrogen pressures (or pH levels) in various areas of the cat and rabbit carotid bodies, and found out a fact that the local blood flow (or capillary blood flow) in the carotid body was kept constant, even when the blood pressure or inflow into the carotid body (or common carotid body artery) was altered through the stimulation of autonomic nerves or by clamping the external carotid artery (Acker, 1980; Acker and O'Regan, 1981). They also examined serial sections of the cat and rabbit carotid bodies under a light microscope, and also discovered that in each slice of the carotid body only 20% of the vessels contained red blood cells. Such plasma skimming or flow of blood with low-concentrated red blood cells in the carotid body was also confirmed by other authors who compared the oxygen contents of the carotid body with microelectrodes in the normal and Locke-perfused cats (Whalen and Nair, 1977). Most of these authors believed that this constant blood flow and plasma skimming in the carotid body might be caused by arterio-venous anastomoses (Acker, 1980; Acker and O'Regan, 1981). As discussed above, however, the occurrence of the arterio-venous anastomosis in and around the
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The carotid body could not be confirmed. As has been suggested by a few authors (McDonald and Larue, 1983) and clearly confirmed in the present study, it is noteworthy that the proper carotid body artery arises from the common carotid body artery together with some subsidiary branches to the tissues near the carotid body, and that a few primary branches or accessory twigs of the proper carotid body artery pass through the body. It is also noteworthy that the common carotid body artery and its subsidiary branches to other tissues have, at their origins, marked constrictions, indicative of intra-arterial cushions (McDonald and Larue, 1983). These special arterial branchings and structures seem important in regulating the blood flow into the carotid body. The inflow of blood into the common carotid body artery may presumably be regulated by the intra-arterial cushion at the origin of this vessel. The subsidiary branches of the common carotid body artery may possibly act as a bypass-route to eliminate the excessive inflow of blood into the proper carotid body artery. The primary branches or accessory twigs of the proper carotid body artery, which pass through the carotid body, can also be proposed to act as a bypass-route to control the inflow of blood into the carotid body, though they showed no marked constrictions indicative of the arterial cushions. The occurrence of the arterial cushion at the origin of the common carotid body artery has been clearly demonstrated by light and electron microscopy of sectioned samples (McDonald and Larue, 1983), though its occurrence at the origins of the subsidiary branches has not been shown previously. It should be noted further that the proper carotid body artery is provided with thick and thin arterial terminals. The thin arterial terminals may act as an additional route to convey blood into the capillary bed of the carotid body, since their occurrence is only occasional. The thin capillaries intercalated between the thick or main capillaries of the carotid body may act as a buffer route to homogenize the blood flow within the carotid body or its basic plexus consisting of thick capillaries.

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