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On the Vascular Architecture of the Carotid Labyrinth in *Cynops pyrrhogaster* and *Onychodactylus japonicus**,**

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Summary. The fine vascular arrangement of the carotid labyrinth in two species of urodeles, *Cynops pyrrhogaster* and *Onychodactylus japonicus* was investigated. India ink-gelatin injected preparations were examined under the dissecting microscope. Resin-casts of the blood vessels prepared using the method of MURAKAMI (1971) were observed in the scanning electron microscope.

In both species, the common carotid artery terminated in the central chamber from which arose two retia mirabilia, i.e. an external carotid rete and an internal carotid rete. The external carotid artery arose recurrently from the external carotid rete, whereas the internal carotid artery received vessels from the internal carotid rete which apparently occupied more than two thirds of the carotid labyrinth.

The present observations indicate that the previous descriptions of the urodele carotid labyrinth should be corrected: a vascular ring exists around the common carotid artery and the latter shows a corresponding narrowing at the entrance to the carotid labyrinth; the vascular ring forms the most proximal part of the external carotid rete; the central chamber occupies only a part of the proximal end of the carotid labyrinth; the internal carotid rete and the external carotid rete constitute a continuous rete mirabile; and a distinct furrow exists in the boundary between these two portions of the rete mirabile. Individual variations were frequently encountered, but were confined to the external carotid artery and its tributaries.

The complicated and elaborate vascular architecture of the amphibian carotid labyrinth suggests that this organ should exert blood-flow regulating and probably other unknown functions as an effector organ in addition to its accepted activity as a chemo- and baro-receptor.

In urodeles and anurans, a specialized swelling occurs at the bifurcation of each common carotid artery into the internal and external carotid arteries. This swelling has been called the carotid labyrinth, the carotid gland or the carotid body (see CARMAN, 1955; ADAMS, 1958). Although the relationship of the carotid labyrinth to the carotid arteries in amphibians closely resembles that of the carotid body to the carotid sinus in mammals, the pattern of vascular supply to the organ in the two phyla is very different. Hence, it has long been debated whether or not the carotid labyrinth of amphibians is homologous with the carotid body of mammals.

On the other hand, ultrastructure research works and histochemical studies on the amphibian carotid labyrinth recently have revealed (1) that a characteristic assemblage of granulated cells, non-granulated cells and nerve terminals which is

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identical in ultrastructure and histochemistry to that in the mammalian carotid body, is present in all amphibian species examined so far; (2) that free nerve fibers are present in the carotid labyrinth which are indistinguishable in ultrastructure from the supposed baro-receptor terminals of the mammalian carotid sinus; (3) that there are adrenergic innervations within and near the carotid labyrinth; and (4), that the region of the rete mirabile contains a sufficient number of smooth muscle cells which could actively control the size of the fine blood vessels here (see KOBAYASHI, 1971a,b). Furthermore, chemo- as well as baro-receptor functions of the carotid labyrinth have been confirmed in the toad by means of electroneurographical methods (ISHII, HONDA and ISHII, 1966; ISHII and ISHII, 1976). Therefore, it seems reasonable to state that the carotid labyrinth is the amphibian equivalent of the carotid body/carotid sinus of mammals.

The vascular architecture in the carotid labyrinth of amphibia has been studied using serial sections and reconstruction techniques (ISHIDA, 1954; CARMAN, 1955, 1967 a, b). However, this methodology is too laborious to examine the large number of specimens necessary to reach a definitive statement of structures. KOBAYASHI and MURAKAMI (1975) introduced the application of resin-casting associated with scanning electron microscopy to this field, using the carotid body of *Rana catesbeiana* for the investigation of the vascular architecture in the carotid labyrinth. This technique allowed three-dimensional and unequivocal visualization and photographic recording of the finest blood vessels distributed in the labyrinth.

In the present study, we used not only the scanning electron microscopy of the resin vascular casts but also light microscopy of India ink-gelatin injected materials. We noticed that the latter method was quite useful because the vascular architecture of the carotid labyrinth of small amphibians could easily be observed as a whole under the dissecting microscope. In the India ink injection method, however the relation of delicate vessels such as connections and cross overs may be erroneously judged because of the low resolution and small focal depth in light microscopy. It was expected that the two methods used in this study would complement each other in the elucidation of the vascular architecture in the carotid labyrinth.

Materials and Methods

Cynops pyrrhogaster and *Onychodactylus japonicus*, collected in March from Niigata Prefecture and in June from Fukushima Prefecture respectively, were kept in the laboratory at room temperature with occasional feeding. For observation under the dissecting microscope the animals were anesthetized with urethane and held on a board. The thorax was opened and a fine needle connected with a syringe was inserted into the ventricle of the heart. About 0.5 ml of an India ink-gelatin mixture was injected into the heart through the needle. The mixture was prepared by adding 10 g of gelatin to 100 ml of Tusche nach Burri (Merck). The India ink-gelatin injected animals were then dissected carefully under the dissecting microscope and sketches and photographs of the blood vessels in and around the carotid labyrinth were made.

The resin casts of the carotid labyrinth were prepared by the method devised by MURAKAMI (1971) (see KOBAYASHI and MURAKAMI, 1975). A mixture of methylmethacrylate (200 ml) and 2, 4-dichlorobenzoyl peroxide (4 g) was heated to 60–65°C with agitation. The temperature of the mixture rose further by polymerization and

when it reached 85–90°C, it was rapidly cooled with running water. When this half-polymerized resin became as viscous as 50% glycerin, it was added with benzoyl peroxide (1.5–2.0%, w/w) and N-N-dimethylaniline (2.0–3.0%, v/v). This mixture then was immediately injected into the heart in the same way as the India ink-gelatin mixture.

After the heart and blood vessels were filled with resin, the animals were bathed in hot water (40–50°C) for 30 min to 1 hr to facilitate further polymerization of the resin.

The neck portion of the animals containing the carotid body was removed and kept in a 10% solution of potassium hydroxide overnight in order to macerate the tissue. The resin casts of the carotid labyrinths thus prepared were then washed repeatedly with distilled water and air dried at room temperature.

The casts were mounted on a metal stub with an electricity-conducting paint, coated in a vacuum evaporator with a thin film of carbon followed by gold, and photographed in a scanning electron microscope.

Results

1. *Cynops pyrrhogaster*.

The carotid labyrinth of the adult *Cynops pyrrhogaster* was oval in shape and measured approximately 0.75 mm in length and 0.54 mm in width. It is comprised of a complicated network of minute blood vessels called the rete mirabile. The distal pole of this organ was continuous with the internal carotid artery and the proximal pole extended to the common carotid artery. The external carotid artery arose recurrently from the cranial side of the proximal pole of the organ.

The common carotid artery, about 0.8 mm in diameter, entered the labyrinth at its proximal end. It tapered distally and formed a coniform space called the central chamber.

The central chamber thus occupied an axial position, though only a small proximal extent, in the carotid labyrinth. Many small vessels of relatively uniform caliber originated from all regions of the central chamber. The vessels which came from the proximal portion, after forming a typical rete mirabile, were gathered into a vascular ring which, at the proximal margin of the labyrinth, embraced the central chamber. On its cranial side, this ring poured into the external carotid artery with an approximate diameter of 0.4 mm. On the other hand, the small vessels which originated from the distal portion of the central chamber formed several routes which were oriented essentially in a longitudinal direction and anastomosed by side branches of thinner caliber. These routes, caudally became confluent to the internal carotid artery, about 0.6 mm thick.

The common carotid artery, at its entrance into the carotid labyrinth, was more or less markedly constricted by being pressed by the vascular ring surrounding it. After the ring and rete mirabile was removed by microdissection, a furrow on the common carotid artery was revealed by scanning electron microscopy.

As described above, the central chamber was ensheathed in the rete mirabile, the proximal portion of the latter was called the external carotid rete, while the distal portion, the internal carotid rete. The former occupied the proximal one third, whereas the latter, the distal two thirds of the carotid labyrinth. Although some

Fig. 1. Examples of the sketches of the India ink-gelatin injected carotid labyrinth of *Cynops pyrrhogaster*. Notice that the vascular architecture considerably different among animals. 1-12 animal number. R and L: right and left organ; V and D: ventral and dorsal view.

blood vessels bridged the internal carotid rete and external carotid rete, a definite furrow encircled the carotid labyrinth at the junction of the two portions of the rete mirabile. The boundary between the two carotid retia was thus more or less distinct.

Individual variations: The detailed architecture of the carotid labyrinth varied considerably among individuals. Figure 1 illustrates examples of the carotid labyrinth of the *Cynops pyrrhogaster*. The sketches were made based on the observations of the India ink-injected materials under the dissecting microscope.

The narrowing of the common carotid artery, at its entrance to the central

Labels common to all figures:

cb carotid body

cc common carotid artery

ch central chamber

ec external carotid artery

ic internal carotid artery

chamber in 2RD, 2RV, 3LV, 4RV, 4LV and 10RV was more distinct than in other specimens. When a distinct constriction of the common carotid body was observed, there was always a thick vascular ring corresponding to it. This was especially conspicuous in 2RV. The narrowing was hardly recognizable in 5RV, 5RD, 6RV, 9LV and 10LV.

The size and shape of the central chamber differed between individuals of the same species. The central chamber in 1LV was large and was located in the center of the carotid labyrinth and apparently oval in shape. A structure deserving the

name of the central chamber was hardly seen in 2LV and 8LV. In these cases, the common carotid artery abruptly branched into many small vessels when it reached the carotid labyrinth.

The detailed pattern of the external carotid rete differed greatly between individuals. The most proximal vessels of the external carotid rete of 2RV was extre-

Fig. 2 and 3. Scanning electron micrographs of the resin vascular casts obtained from the left (Fig. 2) and right (Fig. 3) carotid labyrinths of the same *Cynops pyrrhogaster*. There is a distinct separation between the two retia mirabilia, one belonging to the external, one to the internal, carotid artery. This is seen more clearly in Figure 2.

Fig. 4 and 5. Scanning electron micrographs of a resin cast obtained from the right carotid labyrinth of *Cynops pyrrhogaster*. The ventral (Fig. 4) and dorsal aspects (Fig. 5) of the proximal portion of the organ are shown. Note that the margin of the rete mirabile continuously surrounds the common carotid artery like a collar. The elongated indentations on the surface of the resin cast correspond to the nuclei of endothelial cells (arrows).

Fig. 6. A stereo-pair of scanning electron micrographs of the resin cast of the carotid labyrinth of *Cynops pyrrhogaster*. The pictures were taken at a nine degree angle difference.

mely thick, forming the main tributary of the external carotid artery. The vascular ring around the common carotid artery was not seen in 6RV, where a variant large artery, in addition to the external carotid artery, arose from the external carotid rete. The initial portion of the external carotid artery formed a bulbous swelling in 1LV and 3LV.

The internal carotid rete and internal carotid artery showed much less individual variability. The meshes in the internal carotid rete were always smaller than those in the external carotid rete. Furthermore, the former was formed by a vasculature of larger caliber than that which formed the latter.

The furrow at the boundary between the external carotid rete and the internal carotid rete showed a significant individual variation. It was particularly conspicuous in 4RV and 9LV. This furrow was hardly recognizable in 1LV, 1LD and 2RV.

Scanning electron microscopy: Twenty one resin-casts of the vasculature of the carotid labyrinth, obtained from fifteen *Cynops pyrrhogaster*, were examined in the scanning electron microscope. Figures 2 to 10 represent examples of the scanning electron micrographs.

By this method it was possible to demonstrate not only the three dimensional distribution pattern of different vessels forming the carotid labyrinth, but also the nuclei of the endothelial cells as indentations on the vascular cast.

Examination of Figure 6 using a stereoscope will, clearly visualized the three-dimensional image of the vasculature. The narrowing of the common carotid artery just before entering the central chamber is clearly demonstrated in Figures 4 and 5. In Figures, 7 and 8, the origins of the external and the internal carotid rete are demonstrated. It is also unequivocally demonstrated in these micrographs that the external carotid rete and internal carotid rete are a continuous network of small blood vessels.

2. *Onychodactylus japonicus*

Onychodactylus japonicus has a primitive lung in the early stage of development

Fig. 7 and 8. The common carotid artery shows a distinct narrowing which corresponds to the proximal margin of the external carotid rete. A part of the external carotid rete was removed to expose the narrowed portion as well as the central chamber and its branches.

but loses it in the adult stage (Kudo and Yamada, 1934). Thus, the carotid labyrinth of the *Onychodactylus japonicus* is of particular interest in the *phylogenetic* investigation of the relationship between the carotid body/carotid sinus and the respiratory function. In mammalian species, this has been established.

The carotid labyrinth of *Onychodactylus japonicus* was heavily speckled with melanocytes. The micro-architecture of the blood vessels was quite similar to that the *Cynops pyrrhogaster*. Figure 11 shows sketches of the India ink injected specimens and Figure 12 is a scanning electron micrograph of a resin cast. In the latter, it is clearly demonstrated that the external carotid artery runs side by side with the common carotid artery for a considerable distance in its initial portion.

Fig. 9. Proximal aspect of the carotid labyrinth in *Cynops pyrrhogaster*. The common carotid artery and part of the external carotid rete were removed to demonstrate the central chamber and the origin of the rete mirabile from it.

Discussion

With regard to the micro-architecture of the carotid labyrinth of the species of the urodeles, little progress has been made in our knowledge since the reconstruction work of ISHIDA (1954). He reported the carotid labyrinth of the *Cynops pyrrhogaster* and *Hynobius nigrescens* is comparable in the vascular arrangement to that the anurans, but the organ of the *Megalobatrachus japonicus* is less compact and less specialized than in other amphibians, forming an independent type of carotid labyrinth. ISHIDA's paper includes diagrams showing the architecture of the carotid labyrinth in these species of urodeles, which are reproduced in the monograph of ADAMS (1958).

The results of the present study in the *Cynops pyrrhogaster* and *Onychodactylus japonicus*, using light microscopy of India ink

injected materials and scanning electron microscopy of the resin vascular casts revealed several points which ISHIDA (1954) could not demonstrate in his study using serial section and reconstruction techniques in the celloidin- or paraffin-embedded

Fig. 10. Central chamber of carotid labyrinth in *Cynops pyrrhogaster*. A wide region of the rete mirabile was removed. The common carotid artery was broken at its entrance to the carotid labyrinth (see Fig. 9). The origins of several small vessels of the rete mirabile from the central chamber are seen.

Fig. 11. Examples of the India ink-gelatin injected carotid labyrinth of *Onychodactylus japonicus*. Labels: same as in Figure 1.

Fig. 12. Scanning electron micrograph of the carotid labyrinth of *Onychodactylus japonicus*. The common carotid artery supplies the carotid labyrinth from the left-bottom corner of this picture. Also seen is the internal carotid artery which is located at the top, and the external carotid artery which runs parallel to the common carotid artery but in front of it. The central area of the rete mirabile was removed to show the central chamber.

tissue. The new findings will be summarized as follows:

- 1) At the entrance of the common carotid artery into the central chamber there is distinct narrowing, which is apparently formed by the compressing effect of the vascular ring at the proximal portion of the external carotid rete.
- 2) The central chamber is smaller than described by ISHIDA (1954) and occupies only a small proximal part of the carotid labyrinth.
- 3) The external carotid rete and internal carotid rete are not isolated as illustrated by ISHIDA (1954) (see also ADAMS, 1958), but are continuous small blood vessels.
- 4) In the boundary between the external carotid rete and the internal carotid rete, a distinct furrow is present. Previous authors, using a variety of investigative

techniques, have not noticed this furrow. It is worth pointing out that KOBAYASHI and MURAKAMI (1975) who observed the cast of the carotid labyrinth in *Rana catesbeiana* by scanning electron microscopy did not recognize a corresponding furrow in this anuran species.

5) The results of the present study also showed that the vascular architecture of the carotid labyrinth varied considerably among the same species of urodeles. Variations were more frequent in the external carotid artery and its tributaries than in the internal carotid rete and internal carotid artery.

On the basis of these findings, the possible functions of the carotid labyrinth in amphibia will now be discussed. The first function of the carotid labyrinth may be as an effector. The carotid labyrinth may regulate the volume of blood entering the internal carotid artery (see CARMAN, 1955; ADAMS, 1958). The intricate structure of this organ revealed in the present study may allow any excess of blood to be dammed back into the external carotid artery, if the internal carotid rete constricts. The narrowing of the common carotid artery associated with the vascular ring at the proximal end of the external carotid rete may be a device to control the blood flow from the common carotid artery into the central chamber. It may be possible that when the blood flow passing the narrowing increases, the muscle fibers in the wall of the vascular ring constrict and the caliber of the narrowing is reduced. Furthermore, the carotid labyrinth, as an important adjunct may contribute to maintain an adequate blood flow in the external carotid artery. But it seems more reasonable to regard the function of the external carotid artery to be supplementary to the internal carotid artery, since the latter nourishes the brain which is most essential for the life of the animal. This view is also supported by the present observation that the variations in vascular architecture occurred more frequently in the external carotid rete than the internal one: if the more essential a given structure is for life, the less variation of it may be allowed.

The second function attributed to the carotid labyrinth may be as a receptor. The carotid labyrinth is accepted to function as a baro- and chemo-receptor as it has been electrophysiologically demonstrated in the toad (ISHII, HONDA and ISHII, 1966). In the mammalian species the baro- and chemo-receptors are separated in the carotid sinus and carotid body respectively (see CARMAN, 1955; ADAMS, 1958). The complicated vascular architecture revealed by the present study in the carotid labyrinth of *Cynops pyrrhogaster* and *Onychodactylus japonicus*, suggests that this organ in these animals necessarily might exert further activities in addition to those as a mere baro- and chemo-receptor. The carotid labyrinth in the amphibians could actually play a more vital role in the maintenance of life than the carotid body/carotid sinus do in the mammalian species.

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イモリとハコネサンショウウオの頸動脈小体における血管の微細分布について

野口良子と小林 繁

有尾両生類のイモリとハコネサンショウウオの頸動脈小体における血管の微細分布を、墨汁注入標本の実体顕微鏡観察法 および血管樹脂鑄型の走査電子顕微鏡観察法 (村上, 1971) を使って研究した。

頸動脈小体の血管の微細分布は、両種において基本的には同様であった。総頸動脈は、その直接の延長である中心血管へ注ぐ。中心血管から多数の枝が出、これらは分枝と吻合して、微小血管よりなる怪網をつくる。この怪網の近位部三分の一が外頸動脈の、遠位部三分の二が内頸動脈の形成にあずかる。

今回の私たちの研究により、有尾両生類の頸動脈小体に関する過去の記載は、次の点で改められなければならない。

1. 総頸動脈が中心血管に移行する部位に、これを襟のように取り巻く血管輪がある。
2. 総頸動脈はこの血管輪に対応してくびれを持つ。この血管輪は外頸動脈を形成する怪網のうち最も近位端の微細血管で形成される。機能上、この血管輪は効果器として内頸動脈へ流入する血流量の調節に関与するらしい。
3. 中心血管は頸動脈小体の近位端の一部を占めるにすぎない。
4. 外頸動脈を形成する怪網と内頸動脈を形成する怪網とは連続している。
5. しかし、両者の間には、明瞭な溝があって、これが境界をなしている。
6. 個体差が観察できたが、これは外頸動脈とその支流に限って見られた。

References

- Adams, W. E.: The comparative morphology of the carotid body and carotid sinus. Thomas, Springfield, 1958.
- Carman, J. B.: The carotid layrinth in *Hyla aurea*, with a note on that in *Leiopelma hochstetteri*. J. Anat. 89: 503-525 (1955).
- : The carotid labyrinth in the anuran *Breviceps mossambicus*. Trans. Roy. Soc. New Zeal., Zool., 10: 1-15 (1967a).
- : The morphology of the carotid labyrinth in *Bufo bufo* and *Leiopelma hochstetteri*. Trans. Roy. Soc. New Zeal., Zool., 10: 71-76 (1967b).
- Ishida, S.: So-called carotic body of the amphibia (Japanese text with English summary). Igaku-Kenkyu (Fukuoka) 24: 1024-1050 (1954).
- Ishii, K., K. Honda and K. Ishii: The function of the carotid labyrinth in the toad. Tohoku J. exp. Med. 88: 103-116 (1966).
- Ishii, K. and K. Ishii: The chemoreceptors of amphibians. In: (ed. by) A. S. Paintal: Morphology and mechanisms of chemoreceptors. Vallabhbhai Chest Institute, Univ. Delhi, Delhi, 1976. (p. 265-274).
- Kobayashi, S.: Comparative cytological studies of the carotid body. 1. Demonstration of monoamine-storing cells by correlated chromaffin reaction and fluorescence histochemistry. Arch. histol. jap. 33: 319-339 (1971a).

- Kobayashi, S.:** Comparative cytological studies of the carotid body. 2. Ultrastructure of the synapses on the chief cell. Arch. histol. jap. 33: 397-420 (1971b).
- Kobayashi, S. and T. Murakami:** Scanning electron microscopic observation of the fine three-dimensional distribution of the blood vessels in the frog carotid labyrinth. In: (ed. by) M. J. Purves: The peripheral arterial chemoreceptors. Cambridge Univ. Press, Cambridge, 1975. (p. 301-313).
- Kudo, T. and N. Yamada:** On the development of the lung in the lungless salamander, *Onychodactylus japonicus*. (In Japanese). Acta inst. anat. niigata. 4: 1-18 (1934).
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