Vascular architecture of the carotid labyrinth in larval and adult *Xenopus laevis* – Histomorphology and scanning electron microscopy of vascular corrosion casts

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Summary. In *Xenopus laevis* Daudin the anlage of the carotid labyrinth, a baro- and chemoreceptor, appears in early premetamorphosis (NF stage 53) as a swelling of the third aortic arch at the point where the common carotid artery gives off the external carotid artery. A conspicuous accumulation of melanocytes preceeds the formation of the labyrinth maze which starts to grow at the end of metamorphic climax (NF stage 64) and continues so through juvenile age. In adult animals the carotid labyrinth displays a spherical to ovoid shape, has an average length of 1.4 mm, a width of about 0.8 mm and a height of about 0.4 mm. The maze vessels form by sprouting angiogenesis from the common carotid artery and the internal carotid artery. Scanning electron microscopy of vascular corrosion casts reveals that size, overall shape, surface morphology and vascular patterns of the carotid labyrinth vary greatly between individuals,

whereby intussusceptive microvascular growth (= non-sprouting angiogenesis) and its facets model and contribute to the maturation of the labyrinth's final vascular patterns.

Key words: *Xenopus*, carotid labyrinth, vascular anatomy, vascular casts, histomorphology

Introduction

The amphibian carotid labyrinth locates at the bifurcation of the common carotid artery into external and internal carotid arteries. It is a baro- and chemosensory arterial organ (Ishii *et al.*, 1966) homologous to the mammalian carotid bodies. It consists of a maze-like vascular structure and an intervacular stroma composed of clusters of glomus cells interposed between connective tissue cells, smooth muscle cells, and nerve fibers. At the ultrastructural level glomus cells contain a large oval nucleus and dense cored vesicles filled with catecholamines (Ishii and Kusakabe, 1982). Upon stimulation of the glossopharyngeal nerve catecholamines are discharged at the glomus cell-smooth muscle cell (g-s) connections leading to smooth muscle contraction (Ishii and Kusakabe 1982). This in turn adjusts vascular tone and blood flow into the internal carotid artery (Ishii and Kusakabe, 1982; Kusakabe *et al.*, 1987). Denervation experiments designed to further clarify the role of the carotid labyrinth on breathing in unrestrained adult *Xenopus laevis* have shown that the

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carotid labyrinth does not play a major role in regulating breathing under hypoxic or hypercapnic conditions (Jones and Chu, 1988).

Studies on the vascular architecture of the developing and adult amphibian carotid labyrinth by scanning electron microscopy (SEM) of vascular corrosion casts (VCCs) are done primarily in Ranidae, Hylidae, Bufonidae, Pipidae, Salamandridae, Hynobiidae and Ambystomatidae, whereby the bullfrog, *Rana catesbeiana* is studied most thoroughly (Toews *et al.*, 1982; Kusakabe, 1990, 1991, 2002).

The vascular architecture of the carotid labyrinth in adult *Xenopus laevis* has been investigated by SEM of vascular corrosion casts in two studies only (Toews *et al.*, 1982; Kusakabe, 1990). In larval *Xenopus* corresponding studies and light microscopical analyses of stained serial tissue sections are still lacking. We therefore analyzed carotid labyrinths from larval stages 48–66 (NF stages; Nieuwkopp and Faber, 1994) and of adult *Xenopus laevis* by light microscopy of paraffin-embedded stained serial tissue sections and investigated vascular corrosion casts by scanning electron microscopy (SEM) (Murakami, 1971; Lametschwandtner *et al.*, 1990; Aharinejad and Lametschwandtner, 1992; Motta *et al.*, 1992) and 3D morphometry (Minnich *et al.*, 1999) to gain insight into spatio-temporal aspects of the developing and adult carotid labyrinth in a permanently aquatic living anuran.

Materials and Methods

Animals

Fiftytwo tadpoles (NF stages 48–66; total lengths: 15–47 mm), 6 juveniles (snout-vent lengths: 12–16 mm) and 10 adult animals (four males, 34–43 grams, body lengths: 7.5–8.0 cm; six females; 33–100 grams, body lengths: 6.3–10.0 cm) of the African Clawed Toad, *Xenopus laevis* Daudin were studied. Tadpole staging (NF stages) was according to Nieuwkoop and Faber (1994). Tadpoles were kept in plastic containers filled with tap water (20°C) under artificial light (12 h bright:12 h dark). Containers housing adults were equipped with aquarium filters, those of the tadpoles with airlift pumps. Adults were fed twice a week with either dried *Gammarus pulex* or grinded beef heart. Tadpoles were fed with nettle powder every other day.

Histomorphology

Tadpoles

Nineteen tadpoles (NF stages 48–66; body lengths: 15–47 mm) were killed by immersion in an overdose of an aqueous solution (0.03%) of tricaine methanesulfonate (MS 222: 3-aminobenzoic acid ethyl ester, Sigma Chemicals, St. Louis). Animals were then pinned in supine position on a wax plate. The heart with bulbus cordis and truncus arteriosus was exposed and a ligature was placed around the bulbus cordis. Next, the ventricle was cut open and a fine glass cannula was – using a micromanipulator – inserted through the opened ventricle into the truncus arteriosus. Subsequently, the cannula was tied in place with a ligature from 11-0 (metric) suture material (Ethicon, Basle, Switzerland) to ensure its stability during consecutive rinsing and fixing processes. Finally, the sinus venosus was cut open to allow efflux of blood and rinsing with amphibian ringer solution (Adam and Czihak, 1964) was started using an infusion pump (Habel, Vienna) with a flow rate of 3–7 ml/h. When clear reflux drained from the opened sinus venosus fixation with Bouins solution (Adam and Czihak, 1964) was started using the same flow rate. Perfusion-fixed tadpoles were dehydrated in an ascending series of ethanol and embedded in paraplast. Longitudinal and transversal sections (7 µm) were stained with Goldner (Romeis, 1968), or AZAN or were stained with Brooke's or Cleveland-Woolfe (Adam and Czihak, 1964) and examined with a light microscope (Olympus BX 51). Images were taken with a digital camera (Color View III; Soft Imaging Systems; FRG). Brightness and contrast of recorded images were adjusted according to needs.

Adults

One male (body weight: 77 grams, body length: 8.0 cm) and one female animal (body weight: 78 grams, body length: 10.0 cm) were killed by immersion into an aqueous solution of MS 222 (0.5%). After recording weight animals were pinned in supine position on a wax plate. The heart with bulbus cordis and truncus arteriosus was exposed by thoracotomy and a ligature was placed around the bulbus cordis. Next, the ventricle was cut open and a blunt grinded vein flow G19 (Braun, Melsungen, FRG) was – using a micromanipulator –

inserted through the opened ventricle into the truncus arteriosus. Subsequently, the blunted needle was tied in place with a ligature from thread to ensure its stability during consecutive rinsing and fixing processes. Finally, the sinus venosus was cut open to allow an efflux of blood and rinsing with amphibian ringer solution (Adam and Czihak, 1964) was started using an infusion pump (Habel, Vienna) with a flow rate of 40 ml/h. When clear reflux drained from the opened sinus venosus fixation with 10 ml Bouins solution (Adam and Czihak, 1964) was started using the same flow rate. After fixation right and left common carotid arteries with carotid labyrinths and proximal portions of internal carotids, external carotids, and muscular arteries were excised and fixed by submersion in fresh fixative. Further steps in specimen processing were as described under "Tadpoles" with the exception that 7 μ m thick tissue sections were stained according to Goldner (Adam and Czihak, 1964) only. Tissue sections were analyzed with an Olympus X51 microscope. Images were recorded by a Color View III digital camera (Soft Imaging Systems, FRG). If necessary brightness and contrast of images were adjusted using Photoshop 7.0 (Adobe Inc., Redwood, CA).

Vascular corrosion casting

Tadpoles

Thirtythree tadpoles (NF stages 48–66; snout-vent lengths: 15–47 mm) were used. For preparatory steps up to rinsing with Amphibian Ringer solution see Histomorphology. When clear reflux escaped from the opened venous sinus 5 ml of Mercor CL-2B (Dainippon Ink and Chemicals, Tokyo, Japan; Ladd Burlington, Vermont, USA) diluted with monomeric methylmethacrylate (4+1, v+v, 10 ml monomeric methylacrylate contained 0.85 g initiator paste MA) was injected with the infusor (see Histomorphology) at a flow rate of 3–7 ml/h. When the effluent resin became viscous or the whole amount of resin had been perfused the injection was stopped and the animals were left for about 30 minutes at room temperature to allow polymerization of the injected resin. Tadpoles were then put into a water-bath (60°C; 12–24 hours) to temper the injected resin. Next, specimens were macerated in potassium hydroxide (7.5%; 40°C; 2–24 h), rinsed three times in distilled water, submerged in formic acid

(5%; 20°C; 5–15 minutes) to dissolve any residual organic matter adhering to the cast surfaces, rinsed another three times in distilled water and finally frozen in the latter. Ice-embedded casts were freeze-dried in a Lyovac GT2 (Leybold-Heraeus, Cologne, FRG). Dry casts of whole tadpoles were mounted onto specimen stubs using the "conductive bridge-method" (Lametschwandtner *et al.*, 1980), either evaporated with carbon and gold and/or sputter-coated with gold, and examined in the scanning electron microscope XL-30 (FEI, Eindhoven, The Netherlands) at an accelerating voltage of 10 kV. If necessary, individual common carotid arteries with the carotid labyrinths and abutting proximal portions of internal and external carotid arteries as well as muscular branches were excised under binocular control.

Juveniles and Adults

Preparatory steps were the same as described under "Histomorphology" with the exception that depending on the size of the animal for rinsing and resin injections either veinflow cannulae sized G19 or G23 (Braun, Melsungen, FRG) were used to better match the inner diameters of the arterial trunks and to enable better flow patterns in rinsing and casting. In detail, flow rate for rinsing was set to 51 ml/h, flow rate for resin injection was set to 41 ml/h. All further steps in cast processing, drying, mounting and analyses were as described for tadpoles.

In some cases vascular casts of right and left carotid labyrinths were imaged *in situ* before they were excised using microscissors. Individual dry carotid labyrinths were either mounted *in toto* onto specimen stubs for detailed SEM analyses, or were cut longitudinally or transversely by a razor blade before mounting. Some carotid labyrinths were submerged into distilled water, frozen and sectioned while embedded in ice using a mini-wheel saw placed in the cryo-chamber of a cryo-microtome (Lametschwandtner and Lametschwandtner, 1992). Transverse or longitudinally sectioned casts were cleaned, air-dried, mounted and finally analyzed in the SEM.

3D Morphometry

For 3D morphometry stereopaired images were taken in the

SEM with a working distance of 10 mm and a tilt angle of 6° between corresponding images. Images were then imported into the 3D-Morphometry Software M³ (ComServ OG, Ebenau, Austria) and diameters and lengths of individual carotid labyrinths were measured.

Results

Towards the end of premetamorphosis (NF stage 53) the initial segment of the internal carotid (artery) just after the origin of the external carotid (artery) increases 10–20 µm in diameter and melanocytes aggregate at this site (Fig. 1). During the following prometamorphosis (NF stages 54–57) the segment further enlarges indicating ongoing carotid labyrinth formation (Fig. 2). At early metamorphic climax (NF stages 58–60) the number of melanocytes still increases in the outbulging section of the internal carotid and melanocytes form a maze-like pattern with an average length of 250 µm. At this stage the wall of the common carotid for the first time displays a distinct intima, media and adventitia (Fig. 2). Its luminal diameter averages 24 µm, that of the external carotid 17 µm. During mid metamorphic climax (NF stages 61–63) the number of melanocytes decreases. At this time (NF stage 62) the developing carotid labyrinth is approximately 150 µm long, 65 µm wide and 105 µm high. In the following NF stages 64–66 it's size increases to approximately 350 µm in width and 105 µm in heights. Melanocytes are still present. The interior of the labyrinth shows a course vascular maze only (Fig. 3).

Juveniles and adults

During postmetamorphosis the vascular maze continues to grow and its density increases (Fig. 4). In adult *Xenopus* the carotid labyrinth has a spherical to ovoid-like shape. It has an average length of 1400 µm, a width of about 800 µm and a height of about 400 µm. In sagittally sectioned carotid labyrinths the proximally incoming common carotid, the origin of the external carotid, and the distally departing internal carotid and its root vessels can be seen (Figs. 5 and 6). Inside the proximal maze the lumen of the incoming common carotid decreases (neck region) and splits up into several large branches (Figs. 5 and 6). At the periphery of

the proximal vascular maze these branches run close beneath the surface and are covered by one or two layers of maze-vessels only. Central branches are also present (Figs. 5 and 6). Within a short distance they form capillary-sized vessels which towards the distal maze region gradually merge to form the root vessels of the internal carotid (Figs. 5 and 6).

Vascular anatomy of the carotid labyrinth as revealed by scanning electron microscopy of vascular corrosion casts

Tadpoles

Up to NF stage 50 the lumen of the initial segment of the internal carotid – the section where the carotid labyrinth will develop – shows no enlargement. A muscular branch artery branches off the common carotid. It either arises short before the external carotid (Fig. 7) or originates directly from the external carotid (Fig. 8). At NF stage 62 the initial segment of the internal carotid for the first time shows slight ventral outbulgings and small flaccid vascular structures (Fig. 9). These structures increase in size and complexity in the next NF stage 63 and for the first time form a single-layered vascular maze covering about 2/3 of the circumference of the proximal internal carotid (Fig. 10). At NF stage 64 the vascular maze further increases in size and covers the initial portion of the internal carotid and a part of the proximal portion of the external carotid (Fig. 11). Gradually tapering blind ending vessels and holes within cast maze vessels indicate ongoing sprouting and non-sprouting (intussusceptive microvascular growth, IMG) angiogenesis. In NF stages 65 and 66 the external morphology of the carotid labyrinth remains the same (Fig. 12). Maze vessels still do not arise from all over the circumference of the internal carotid.

Juvenils

In postmetamorphic juveniles the vascular maze of the carotid labyrinth is slightly larger whereby great interindividual differences exist with respect to the maturation of the vascular maze (Figs. 13 and 14). Some portions of the ventral circumference of the labyrinth still display a few maze vessels only (Figs. 13–15). The incoming common carotid splits up into few branches which merge within a short distance and

Figs. 1–6. Histomorphology of the larval, juvenile and adult carotid labyrinth in *Xenopus laevis*. Paraplast embedding. Goldner staining. **(1)** Tadpole at prometamorphosis (NF stage 48). Transverse section (7 μ m) at the level of the bifurcation of the common carotid (artery) into external and internal carotid (artery) (arrows). bc branchial cavity, cv cerebral ventricle, vv ventral velum, th thymus. **Inset:** Bifurcation of the common carotid into external (ec) and internal carotid (ic) at the end of premetamorphosis (NF stage 56). Note melanocytes (arrows) at the site of the future carotid labyrinth. **(2)** Histomorphology of internal (ic) and external carotid (ec) at the begin of metamorphic climax (stage 59). Note that the internal carotid is sectioned longitudinally while the external carotid is sectioned transversely. The internal carotid for the first time presents a clearly visible tunica media made up of a layer of vascular smooth muscle cells (arrowheads) and a tunica externa from faint connective tissue (double arrows). Arrows point at melanocytes. **(3)** Histomorphology of common carotid (cc) and growing carotid labyrinth (cl) at the end of metamorphic climax (NF stage 66). Longitudinal section (7 μ m) through carotid labyrinth. ec external carotid. **(4)** Histomorphology of transversely sectioned (7 μ m) carotid labyrinth (cl) in juvenile *Xenopus*. The vascular maze (asterisks) still consists of a few wide vessels only. ao aorta, fc fat cells, nf nerve fiber bundle. **(5)** Carotid labyrinth in adult *Xenopus*. Longitudinal section (7 μ m). Note the large branches of the common carotid (arrows) which give rise to the sponge-like vascular maze (ma). cc common carotid, ic internal carotid. **(6)** Same as Fig. 5, but different section plane. The root vessels (arrowheads) of the internal carotid (ic) emanate from deep inside the carotid labyrinth. Note the narrowing (neck region) of the common carotid (arrows). cc common carotid.

Figs. 7–12. Third aortic arches and vascular anatomy of the carotid labyrinth in larval *Xenopus laevis*. Vascular corrosion casts. Scanning electron microscopy. **(7)** Third aortic arch at prometamorphosis (NF stage 51). Ventral aspect. Note that the muscular branch (mb) arises from the common carotid (cc) slightly before the external carotid (ec). The internal carotid (ic) has the same diameter as the common carotid. ba branchial artery (becomes later the aorta), pa pulmonary artery. **(8)** Right third aortic arch at prometamorphosis (NF stage 51). Ventral aspect. Note that the muscular branch (mb) arises from the external carotid (ec). cc common carotid, ic internal carotid. Note the characteristic elongated endothelial cell nuclei imprints of arteries orientated parallel to the long axis of the arteries (arrowheads). **(9)** Left third aortic arch at metamorphic climax (NF stage 62). Ventral aspect. Note the two slight outbulgings at the ventral circumference of the internal carotid (asterisks) and two flaccid vascular structures (arrows) overlaying the internal carotid (ic). cc common carotid, ec external carotid (broken away), mb muscular branch. **(10)** Vascular architecture of a left carotid labyrinth at metamorphic climax (NF stage 63). Ventro-caudal aspect. Differently shaped and sized maze vessels overlay the internal carotid (ic). Note the immature looking flaccid maze vessels (asterisks). cc common carotid, ec external carotid. **(11)** Same as **(10)** but NF stage 64. Ventro-cranial aspect. The vascular maze (ma) consists of few differently sized interconnecting vessels. Note tapering blind ending vascular profiles (vascular sprouts) directed toward the common carotid (small arrows) and the internal carotid (large arrows). cc common carotid, ec external carotid, ic internal carotid, ma maze. **(12)** Same as **(10)** but NF stage 66. Ventro-cranial aspect. Maze vessels are wide and few. The muscular branch (mb) arises from the proximal maze, the external carotid (ec) originates from the common carotid (cc). Note the internal carotid (ic) arising from the mid-caudal circumference of the carotid labyrinth. ao aorta, ma maze.

Figs. 13–16. Vascular architecture of the carotid labyrinth in juvenile *Xenopus laevis*. **(13)** Left carotid labyrinth in a 14 mm large (body size) and 314 mg (body weight) weighing juvenile. Cranial aspect. Wide and narrow maze vessels are present. Note vascular sprout (small arrow) and the connection of the external carotid (ec) with the maze (arrowhead). A single vessel originates from the distal maze (large arrow). cc common carotid, ic internal carotid. **(14)** Same as **(13)** but in a 14 mm large and 330 mg weighing juvenile. Dorsal aspect. Note that the transition from the common carotid artery (cc) to the internal carotid artery (ic) at the dorsal side is via few wide vessels (asterisks). ec external carotid. **(15)** Internal vascular architecture of a right carotid labyrinth in a 14 mm large and 329 mg weighing juvenile. Longitudinal section. Note that common carotid (cc) and internal carotid (ic) are wide and overlap some distance (arrowheads). Maze vessels are few (compare with **14**). **(16)** Endothelial cell nuclear imprint patterns of maze vessels of the carotid labyrinth in a juvenile (box in **15**). Note oval to round imprints (arrowheads) characteristic of venous vessels and elongated imprints (arrows) characteristic for arteries lying in close vicinity. Deepened square at the center of the figure (asterisk) results from a too long observation of the cast surface at too high a magnification.

form the root vessels of the leaving external carotid (Figs. 14 and 15). A longitudinal section through a vascular corrosion cast shows that the incoming common carotid still gives off few maze vessels (Fig. 15). For the first time an individual vessel is seen to branch off the distal maze (Figs. 13 and 14, arrow). Interestingly, cast peripheral maze vessels display differently shaped and orientated imprints of endothelial cell nuclei (Fig. 16). Few imprints are elongated characteristic for arterial vessels (Fig. 16, arrows), while most of them are ovoid to round characteristic for venous vessels (Fig. 16, arrowheads).

Adults

Vascular corrosion casts clearly show that the carotid labyrinth of adult *Xenopus* is spherical to ovoid with the distal end tapering in some specimens (Fig. 17). In general, a great interindividual variability is given. A close-up view at the external surface of the vascular maze shows a dense network of differently-sized vessels with locally mature capillaries connecting wide sinusoid vessels (Fig. 17, inset). After removal of superficial maze vessels the branching pattern of the incoming common carotid as well as its broad connection

Figs. 17–22. Vascular architecture of the carotid labyrinth in adult *Xenopus laevis*. **(17)** Left carotid labyrinth. Cranial aspect. Maze vessels (ma) are densely packed. The common carotid (cc) is about twice as thick as the internal carotid (ic). Note the small vessel originating from the distal maze (arrow). **Inset:** Detail from the surface of the maze. Note capillary-sized vessels (arrowheads) interconnecting wider vessels. ec external carotid, mb muscular branch. **(18)** Left carotid labyrinth. Superficial proximal maze vessels (ma) are ripped off by a sharp-tipped insect-pin to expose subsurface branches (asterisks) of the common carotid (cc) and their transitions into superficial maze vessels (arrows). Note the broad connection of the common carotid with the recurrent external carotid. **(19)** Left carotid labyrinth. Superficial vessels of the proximal maze (ma) are ripped off by a sharp-tipped insect-pin to expose the origin of the recurrent external carotid (asterisk) from the common carotid (cc). ec external carotid, ic internal carotid, mb muscular branch. **(20)** Right carotid labyrinth. Transverse section at the level of the origin of the external carotid (ec) from the common carotid (cc). Note the vessel (arrow) arising from the distal maze. ic internal carotid, ma maze vessels. **(21)** Architecture of the maze of a right carotid labyrinth. Longitudinal sections in an acute angle. Common carotid (cc) and internal carotid (ic) extend deeply into the vascular maze (ma) before the common carotid branches respectively the internal carotid forms from thick root vessels (arrows). Note how close both vessels approach each other. **(22)** Transition from common carotid (cc) into internal carotid (ic). Detail from **21** (box). Dashed line marks a transition route.

with the external carotid can be seen (Figs. 17–20). Cross sections through a cast carotid labyrinth at the level of the origin of the external carotid confirms that the external carotid arises from the proximal margin of the carotid labyrinth (Fig. 20). In many carotid labyrinths a small vessel branches off the distal maze (Fig. 20; compare with Figs. 13 and 14, arrows). Longitudinally sectioned vascular corrosion casts of the carotid labyrinth demonstrate branching patterns of common carotid, external carotid, internal carotid, and the internal maze vessel structure (Fig. 21). It becomes evident that the common carotid extends deeply into the maze and then splits up within a short distance into few thick branches which run under the surface and into the center of the labyrinth (Figs. 21 and 22; compare with Figs. 5 and 6). Within a short distance branches become thinner, merge and form the root vessels of the internal carotid (Figs. 20 and 21). These root vessels join at some distance inside the labyrinth almost at one point (Fig. 21) and form the internal carotid. Still inside the labyrinth the internal carotid enlarges abruptly and exits the labyrinth (Fig. 21). Interestingly, internal maze vessels show oval to round endothelial cell nuclear imprints characteristic for venous vessels (Fig. 21, inset). At higher magnification the architecture of the vascular maze reveals a dense network of differently-sized vessels with extremely small intervascular spaces representing the site of the intervascular stroma (Fig. 22). Smallest vessels have a (luminal) diameter of about 10 μm , whereas narrowest intervascular spaces are 1 μm (Fig. 22).

3D-morphometry measurements on vascular corrosion casts show that luminal diameters of external carotids range from 200–450 μm , those of internal carotids range from 180–460 μm , and those of common carotids range from 630–880 μm . The average ratio of common carotids diameters to those of external carotids ranges from 2.9 to 4.2, those of internal carotids to external carotids diameters ranges from 1.7 to 2.7. Average angles between common carotids and external carotids range from 31–75 degrees, those between common carotids and internal carotids range from 2–67 degrees.

Discussion

In his comparative studies on the vascular organization of carotid labyrinths of anurans and caudates Kusakabe (1990,

2002) uses scanning electron microscopy of vascular corrosion casts and reports that the carotid labyrinth in adult *Xenopus laevis* is 0.5–0.6 mm long. Toews *et al.*, (1982) show in their Fig. 1D a cast carotid labyrinth of adult *Xenopus* having a length of about 2.3 mm and a width 1.9 mm. Applying the same method the carotid labyrinth in our specimens of adult *Xenopus laevis* has an average length of 1.4 mm and an average width of 0.8 mm. Regretably, neither Kusakabe (1990) nor Toews *et al.*, (1982) report any biometrical data (male, female; body weight, snout-vent-length, total length) of their experimental animals. Carman (1955) who studied the carotid labyrinth in adult *Hyla arborea* by light microscopy of stained serial tissues sections (10 μm) in great detail describes the carotid labyrinth in 8–10 cm body-sized *Hyla arborea* as a 1 mm long and 0.6 mm wide structure, whereas Kusakabe (1990, 2002) reports the carotid labyrinth in *Rana nigromaculata* and in *Hyla arborea* as a nearly spherical structure with a diameter of 0.4–0.6 mm. In 4 cm body-sized *Leiopelma hochstetteri* Carman (1955) reports a slender carotid labyrinth with a length of about 0.8 mm whereas Toews *et al.*, (1982) report an ovoid carotid labyrinth with a diameter of 1.0–1.5 mm in a 400 g *Bufo marinus*. If Kusakabe (1990) has studied smaller specimens of *Xenopus* than we did or if the differences in the size of the carotid labyrinths are due to an under- or overfilling of the carotid labyrinth's vascular beds during resin casting, cannot be discussed further as Kusakabe (1990, 1991) does not give any data on the flow rate or injection pressure he used in his casting studies. As Toews *et al.*, (1982) infused the resin (Batson #17) with an initial pressure of 40 mm Hg into the common carotid artery of adult *Xenopus* artificial distension of the carotid labyrinth vascular bed by applying a too high injection pressure is very unlikely to explain the large size of the cast carotid labyrinth. If the large carotid labyrinth originates from a *Xenopus* much larger than those studied by us remains open.

In *Rana nigromaculata* and *Hyla japonica* Kusakabe (1990, 2002) describes a complete vascular ring at the proximal maze of the carotid labyrinth. From the cranial side of this vascular ring the external carotid artery opens. The cast carotid labyrinth specimens of adult *Xenopus* studied by Toews *et al.*, (1982) and our specimens lack a clear vascular ring. The external carotid artery opens here with a broad connection from the common carotid artery immediately

where the latter enters the labyrinth. The vascular ring is also absent in *Hyla arborea* (Carman, 1955), *Rana esculenta* (Zimmermann, 1887) and in *Bufo vulgaris* and *Rana esculenta* (Pischinger, 1934).

The observation that the common carotid artery branches into arterioles and capillaries which in turn merge to form arterioles and arteries (external carotid, internal carotid) again, makes the carotid labyrinth a true rete mirabile as described by Noguchi and Kobayashi (1977) in the caudate species *Cynops pyrrhogaster* and *Onychodactylus japonicus*. It has to be pointed out that it is difficult to follow the course of first order branches of the common carotid artery or of the root vessels of external and internal carotid arteries within the carotid labyrinth because casts often break if whole carotid labyrinths or parts of it are dissected stepwise using forceps and fine tipped insect pins to expose internal vascular patterns.

The presence of a rete mirabilis in the carotid labyrinth of anuran and caudate amphibians contrasts with the situation in the carotid bodies in mammals where one or more supplying carotid body arteries branch into arterioles which in turn capillarize and continue into postcapillary venules and veins which in turn drain into internal or external jugular veins (Taguchi *et al.*, 1992).

The observation that in NF stages 54–57 melanocytes accumulate in the area of the future carotid labyrinth to a much higher degree than in other areas and that during NF stages 58–60 they arrange in a similar pattern as maze vessels do later rises the question if these melanocytes are somehow involved in the formation of maze vessels.

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