Vascularization of the Hypothalamo-Hypophysial Complex in Japanese Elasmobranchs: A Scanning Electron Microscope Study of Blood Vascular Casts

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Summary. To study in detail vascularization in the hypothalamo-hypophysial complex in some Japanese elasmobranchs, injection casts of blood vessels were observed by scanning electron microscopy. Materials used were a gummy shark (Mustelus manazo), a cloudy dogfish (Scyliorhinus torazame) and a skate (Raja porosa).

The vascular supply to the hypothalamo-hypophysial region of the elasmobranchs is carried out by the inferior hypothalamic arteries and their branches that originate from the internal carotid artery. In the gummy shark, a pair of inferior hypothalamic arteries send several branches to the median eminence running along the anterolateral sides of the distal adenohypophysis. These branches form the capillary plexus, displaying anastomosis and convolution at the ventral surface of the median eminence. The plexus assumes capillary glomeruli at the lateral region of the posterior median eminence. Numerous branches derived from the plexus are directed backward through the pars distalis, join with the capillary net work (which develop in the neuro-intermediate lobe) and are finally gathered together to form a thick hypophysial vein. The ventral lobe receives several arterial branches from the internal carotid arteries and carotid artery to form a unique vascular bed. Therefore, it is assumed that in elasmobranchs the hypothalamic control of the ventral lobe is weaker than that of adenohypophysial components.

After MELLINGER (1964) and MEURLING (1967) made their detailed study of the vascular supply of the hypothalamo-hypophysial region of the European elasmobranchs by injection of India ink, no further work dealing with the vascularization of these fishes by application of another method has been made. Investigation of the intricate microcirculation and the three-dimensional distribution of vessels remains difficult in either transparent specimens or thick paraffin sections.

However, a new method for studying the three-dimensional vascularization was devised by MURAKAMI (1971, 1975) who combined methylmethacrylate plastic casting and scanning electron microscopy (SEM). This technique of making microdissections of the casts has been applied to the study of the hypothalamo-hypophysial system in various vertebrates, such as in mammals by MURAKAMI (1975), PAGE et al. (1976), PAGE and BERGLAND (1977) and OHTANI (1981), and in the toad by LAMETSCHWANDTNER and SIMONSBERGER (1975), LAMETSCHWANDTNER et al. (1976, 1977a, b, c) and ALBRECHT et al. (1980).

In our study of the comparative anatomy of fish brain-ventricular-hypophysial system, we felt the necessity of learning the details of the vascularization of this system. With the present study we aimed to examine the vascular supply of Japanese
elasmobranchs by applying this technique.

MATERIALS AND METHODS

The elasmobranchs used in this study were collected during a period from May to September, 1985 from a set net and gill net installed off the coast of the Sado Marine Biological Station, Niigata University, located on the northwestern coast of Sado Island in the Japan Sea. They consisted of two species of sharks (Mustelus manazo and Scyliorhinus torazame) and one species of ray (Raja porosa). Fish of both sexes and various sizes were included.

Immediately after capture, the fish were anesthetized with 0.1% aminobenzoate-methansulfonate (MS-222), dissected to expose the heart region, the vascular system perfused with physiological saline for elasmobranchs with 5% sucrose and 4% sodium citrate, then fixed in 4% glutaraldehyde. Next, a commercially available methylmethacylate medium (Mercox; Japan Vilene Hospital) was injected and polymerized by placing the specimens in an oven at 60°C for several hours.

After hardening, each specimen was decalcified with 5% formic acid, corroded with 20% pottasium hydroxide (KOH) or sodium hydroxide (NaOH), and then rinsed with water. In order to obtain the vascular casts, the whole organs and tissues were then corroded by repeated immersion in 20% KOH or NaOH. After drying in the air, the casts were trimmed and dissected with a pair of sharp forceps under a dissecting microscope.

The samples, stuck on the stubs, were sputter-coated with gold, and examined in a Hitachi S-500 scanning electron microscope (SEM) with an acceleration voltage of 15 kV.

RESULTS

The hypothalamo-hypophysial complex in Chondrichthyes, excepting the ventral lobe, is mainly supplied by a pair of inferior hypothalamic arteries derived from the internal carotid arteries (Fig. 1, 2). However, the inferior hypothalamic artery has two origins. In the case of the cloudy dogfish (Scyliorhinus torazame), it is derived directly from a pair of communal cerebral arteries that are combinations of the internal carotid arteries and carotid arteries. In the case of the cloudy dogfish (Mustelus manazo) and skate (Raja porosa), it is the branches of a pair of posterior cerebral arteries derived from the communal cerebral arteries (Fig. 3a). In the cloudy dogfish the right and left branches of the internal carotid arteries cross and join together on the ventral side of the ventral lobe, whereas in the cloudy dogfish and skate, these branches cross each other (Fig. 3b).

As one progresses to the posterior portion, a pair of inferior hypothalamic arteries in the cloudy dogfish give off several branches to the anterior median eminence, running ventrolaterally along the inferior lobe, and then invading the neuro-intermediate lobe and saccus vasculosus crossing the anterior ends of the proximal pars distalis of the neuro-intermediate lobe (Fig. 1, 2). On the other hand, in the gummy shark, the inferior hypothalamic arteries extend down on either side of the pars distalis after giving off several branches to the median eminence, and reach the neuro-intermediate lobe and saccus vasculosus (Fig. 4a). In the cloudy dogfish and
skate, each branch on either side of the inferior hypothalamic arteries joins each other on the median line and then empties into the median eminence just before the cephalic end of the pars distalis. In the gummy shark, the neuro-intermediate lobe receives vessels coming directly from the inferior hypothalamic arteries to the neuro-intermediate lobe, or else indirectly through the vascular net (rete mirabile) of the median eminence (Fig. 4a, b).

The anterior branches of the inferior hypothalamic arteries in the gummy shark invade deeply into the median eminence, and the posterior branches flow into the pars distalis after turning to the lateral side of the posterior median eminence (Fig. 4a,b, 5). Furthermore, in the lateral area of the posterior median eminence these branches form the capillary glomeruloids and then are directed toward the proximal pars distalis (Fig. 5). There is capillary communication between the pars distalis and neuro-intermediate lobe, and also between the neuro-intermediate lobe and saccus vasculosus. In the case of the skate, the capillary communication is discerned between the neuro-intermediate lobe and ventral lobe (Fig. 7), and also between the inferior lobe and saccus vasculosus.

Numerous vessels invading the pars distalis run backward without noticeable branches and flow into the hypophysial vein, namely, the hypophysial sinus or venus sinus, on the ventral side of the proximal pars distalis. The vascular supply to the ventral lobe in the gummy shark is carried out mainly by a branch of the carotid artery (Fig. 2), but direct irrigation by several arterioles derived from the internal carotid arteries to the ventral lobe is often encountered (Fig. 2). Delicate arteries are distributed within the

Fig. 1. Vascular cast of the hypophysis in the gummy shark (Mustelus manazo) (ventral view). The hypophysial region except for the ventral lobe is supplied by a pair of inferior hypophysial arteries (IHA; see also Fig. 2). The arteries, after supplying the hypophysis, flow into a comparatively thick vein (arrow) located in the median portion of the hypophysis. RPD rostral pars distalis, PPD proximal pars distalis, NIL neuro-intermediate lobe. ×30
ventral lobe, whereas a network consisting of thick veins covers the surface of the ventral lobe (Fig. 2). In the skate, on the other hand, the thickness of the vessels of the ventral lobe is comparatively uniform, and the structural pattern of the network is regular (Fig. 7). In addition, there is an arterial convolution at the anterior end of

Fig. 2. Vascular cast of the gummy shark hypophysial region with the saccus vasculosus (SV) being exposed by microdissection (ventral view). A part of the ventral lobe (VL) remains in this specimen. HV hypophysial vein, ICA internal carotid artery, PPD proximal pars distalis. The arrowheads indicate the vascular routes from the carotid artery branches to the ventral lobe. The arrows, a branch of a hypophysial vein. ×120
the ventral lobe of the skate. In spite of these differences in the vascular distribution among the three species examined, vessels of the network in the ventral lobe are alike in flowing into the hypophysial vein at several points (Fig. 2).

The hypophysial vein of the gummy shark and cloudy dogfish runs along the anterior ventral surface of the ventral lobe, crosses with a foregoing internal carotid artery, and directs itself backward in parallel with the carotid artery along the ventral side of the internal carotid artery (Fig. 2). On the other hand, the hypophysial vein of the skate runs parallel to the posterior margin of the ventral lobe, and occupies the dorsal side of the internal carotid arteries (Fig. 7).

**DISCUSSION**

By using scanning electron microscopy to observe the injection replica, we have demonstrated in some elasmobranchs the existence of different vascular supply systems from the median eminence to the distal adenohypophysis; a possible central control over the hypophysial activity was also suggested. Similar findings have already been reported for several primitive fishes, such as polypteriform fishes, bowfin, chondrosteans, and coelacanths (LAGIOS, 1968, 1970, 1972, 1975; HANSEN, 1971, 1973), elasmobranchs (MELLINGER, 1964; FOLLÉNIUS, 1965; MEURLING, 1967) and chimaeroid (SATHYANESAN, 1965; JASINSKI and GORBMAN, 1966). The structure so described for the primitive fishes apparently corresponds to the hypophyseal-portal system of the terrestrial tetrapods, such as toads (LAMETSCHWANDTNER et al., 1975, 1977a,b,c) and mammals (MURAKAMI, 1975; PAGE et al., 1976; PAGE and BERGLAND, 1977; OHTANI et al., 1983; etc.). Therefore, the vascularization of the hypothalamo-hypophysial system in elasmobranchs markedly differs from that in teleosts with respect to the lack of the portal vessel system in the latter. In some teleosts, it has been noted that the arteries to the adenohypophysis and hypothalamus are conveyed in different ways (FOLLÉNIUS,
It has been confirmed that in elasmobranchs the vessels, after irrigating the hypophysis, gather into a thick vein to drain into a hypophysial vein. Furthermore, our findings on the microcirculation have revealed that the ventral lobe receives a weaker hypothalamic control than other adenohypophysial components.

We have elucidated that the vascular supply of the hypothalamo-hypophysial region in Japanese elasmobranchs, except for the ventral lobe, is achieved by the inferior hypothalamic arteries derived from the internal carotid arteries. However, two different routes and derivatives of inferior hypothalamic arteries were noted. The unification of the right and left carotid arteries found in the cloudy dogfish is rather peculiar among the three species examined, but, nevertheless, it is identical with 1965; HONMA and TAMURA, 1967; HILL and HENDERSON, 1968; JASINSKI, 1969; CHIBA, 1984).

Fig. 4. Vascular cast of the hypothalamo-hypophysial region of the gummy shark (left lateral view). a. A left branch (small arrow) of the inferior hypophysial artery (IHA) sends its branches to the median eminence (ME), and reaches the neuro-intermediate lobe (NIL) and saccus vasculosus (SV) at the level of the caudal part of the rostral (RPD) and proximal pars distalis (PPD). ×21. b. An enlarged view of the region indicated by a thick arrow in a. The arrows show the route of the inferior hypophysial artery (IHA) to the anterior median eminence (AME). ×75.
some European species (Scyliorhinus caniculus and S. stellaris) reported by MEURLING (1967). Therefore, this peculiar feature could be unique characteristic for the dogfish of the genus Scyliorhinus. It remains to be proved whether this peculiar character is a synapomorph of the dogfish clade.

The structural pattern in which several branches of the inferior hypothalamic
arteries invade deeply into the median eminence, while the remainders empty into the distal adenohypophysis after showing a convolution on both sides of the median eminence is perhaps homologous to the organization of the hypophysial-portal system in land vertebrates. A similar circulation has been described in European elasmobranchs (MELLINGER, 1964; FOLLÉNIUS, 1965; MEURLING, 1967) and American holocephalans (SATHYANESAN, 1965; JASINSKI and GORBMAN, 1966). Thus, based on the structural pattern of the hypothalamic portal system, it seems that fishes of the phylum Chondrichthyes represent the stem of the vertebrate phylogenetic tree from which the chondrosteans (HANSEN, 1971), holosteans (LAGIOS, 1968, 1970; HANSEN, 1971), brachiopterids (HANSEN, 1971), sarcopterygians (LAGIOS, 1972, 1975) and amphibians (LAMETSCHWANDTNER et al., 1975, 1977a, b, c) branch off on one side and the teleosts on the other side. The hypothalamic-hypophysial system of teleosts is highly specialized. This specialization is believed to be result from the process of intensive adaptation to the hydrosphere after having diverged from the ancestral fish. However, it remains unclear whether or not the hypophysial portal system might have been lost secondarily in the teleosts. It is assumed that the hypophysial portal system of elasmobranchs, with a remarkable convolution and capillary glomeruloids on the lateral sides of the median eminence (MELLINGER, 1964; MEURLING, 1967; the present work), is more primitive than that of the holocephalans (LAGIOS, 1970; HANSEN, 1971), coelacanth (LAGIOS, 1972), and land vertebrates (MURAKAMI, 1975; LAMETSCHWANDTNER et al., 1975, 1977a, b, c; PAGE et al., 1976; PAGE and BERGLAND, 1977; OHTANI, 1981; OHTANI et al., 1983). For example, in the holocephalan and coelacanth, there are well-developed capillary glomeruloids in the entire ventral surface of the median eminence after its forming the convolution, and in the land vertebrates, the portal vessels make a primary capillary plexus in the same place. The hypophysial vessels consist of a secondary capillary network that irrigates the distal lobe, and the neuro-intermediate lobe flows into a vein and ultimately drains into the hypophysial vein along the median line.

In addition, as described in previous (FOLLÉNIUS, 1965; MEURLING, 1967) and the present study, the capillaries which irrigate the inferior lobe and intermediate lobe in the gummy shark are gathered en masse into a common thick vein. This vein, derived from both interior and the neuro-intermediate lobe, may possibly correspond to the dorsal hypophysial vein as described in the American ratfish by JASINSKI and GORBMAN (1966).

Previous findings that the vascular supply to the chondrostean ventral lobe was accomplished mainly by the branches of the carotid artery and partly by the internal carotid arteries are yet to be confirmed. The branches of the carotid artery noted here correspond to the so-called ventral lobar arteries. The vascular floor of the ventral lobe is a peculiar structure; its capillaries in the form of a network flow into the hypophysial vein from several points. It is therefore thought that the ventral lobe is not strongly controlled by the hypothalamus. The disposition of the hypophysial vein seems to differ among species.

It is concluded that the present study on the hypothalano-hypophysial microcirculation in three species of Japanese elasmobranchs by using this modern technique has substantiated, in essential points, the previous findings obtained by MELLINGER (1964), FOLLÉNIUS (1965), and MEURLING (1967) using classical methods.
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