Journal of Fish Biology (1995) **47**, 769–774

Endocardial pores in the sinus venosus and atrium of the dogfish

R. Muñoz-Chápuli, A. Gallego, D. Macías, C. Ramos and A. V. De Andrés

Department of Animal Biology, Faculty of Science, University of Málaga, Spain

(*Received 14 June 1994, Accepted 3 January 1995*)

A scanning electron microscopy study showed the presence of large pores in the endocardium of the sinus venosus of the dogfish *Scyliorhinus canicula*. The pores were always found on large bundles which protruded into the cardiac lumen. The bundles were mainly constituted of granule-containing nerve fibres. The average diameter of the pores was 3·2 μm (range = 1·5–5·0 μm), and their density, was about 822 pores mm^{-2}. Endocardial pores were absent in other areas of the sinus venosus, but they were observed on the little bundles of granulated nerve fibres which were scattered throughout the atrium. The existence of large endocardial pores associated with bundles of granulated nerve fibres supports the hypothesis for the neuroendocrine nature of the elasmobranch sinus venosus wall.

Key words: heart; sinus venosus; endocardium; endocardial pores; endothelial cells; neuroendocrine system; *Scyliorhinus canicula*; dogfish.

INTRODUCTION

The cardiac sinus venosus of the dogfish *Scyliorhinus canicula* (L.) is characterized by the presence of large subendocardial bundles of nerve fibres which contain membrane-bound, dense core granules of about 150–200 nm in diameter. This fact was related to a 'neurosecretory system of unknown function' (Saetersdal *et al.*, 1975; Yamauchi, 1980; Santer, 1985).

Since the granulated fibres are found in the subendocardial space, the neurosecretion must pass the endocardium in order to reach the blood stream. The first report on this matter by Saetersdal *et al.* (1975), in the shark *Galeus melastomus* Rafinesque, described how the endocardium lining the granulated fibres was attenuated and often fenestrated, with fenestrae 30–50 nm wide, bridged by diaphragms of about 4–5 nm thickness. Large endothelial pores, 0·4–1·6 μm in diameter, through which granulated fibres protruded in the cardiac lumen, were also observed by these authors. However, their transmission electron microphotographs showed only two images of what was described as disintegrated granule-containing fibres protruding through endothelial pores. The reference to the disintegration of the fibres and also to the presence of 'disrupted cellular organelles' on the fibres of the luminal side of the cardiac wall, could suggest some kind of artifact. On the other hand, Helle *et al.* (1983) state that only fenestrations (less than 0·1 μm in diameter), not the pores reported by Saetersdal *et al.* (1975) are apparent in the endocardium of the shark *Scyliorhinus stellaris* (L). Furthermore, the association between these

0022–1112/95/110769+06 $12.00/0

fenestrations and the granulated fibres seems uncertain since these authors report the presence of fenestrations not only in the sinus venosus, but also in the ventricular endocardium, where granulated fibres are not present.

No other information about the presence, number, size, density or distribution of endocardial discontinuities in the sinus venosus of the sharks has been provided. Studies on the ultrastructure of the sinus venosus of chondrichthyan fishes have not confirmed the presence of fenestrae or pores (Kisch & Philpott, 1963; Berge, 1979; Leknes & Saetersdal, 1980; Yamauchi, 1980; Santer, 1985). If the pores are sparsely distributed along the sinus venosus, it is not surprising that they cannot easily be observed in ultrathin sections. Thus, it is evident that only SEM studies can provide detailed information on the presence and distribution of endocardial pores. On the other hand, the confirmation of the presence of pores would support the hypothesis of a neuroendocrine function of the subendocardial fibres, and it would probably constitute the only case known of endocardial pores among adult vertebrates.

The aim of this study is to verify the presence of the endocardial pores in the dogfish and to assess their size, density and distribution by means of scanning electron microscopy.

MATERIALS AND METHODS

Nine adult dogfish, measuring about 400 and 500 mm in total length, were anaesthetized in sea water with 0·08% tricaine methanesulphonate (MS-222, Sigma, U.K.). The pericardial cavity was opened and the hearts were perfused *in situ* through one of the ducti Cuvieri with elasmobranch buffer (16·38 g l^{-1} NaCl, 0·89 g l^{-1} KCl, 1·11 g l^{-1} $CaCl_2$, 0·38 g l^{-1} $NaHCO_3$, 0·06 g l^{-1} NaH_2PO_4, 21·6 g l^{-1} urea, pH=7·2). Then, the hearts were perfused with 1% glutaraldehyde and 1% paraformaldehyde in elasmobranch buffer. The hearts were removed and immersed in fixative for 4 h. After two washes with elasmobranch buffer (1 h), the hearts were post-fixed in 1% OsO_4 in distilled water for 2 h. Dissection was then made to isolate and to expose the sinus venosus. The sinus venosus was then washed in distilled water and dehydrated in a series of alcohol at 4° C. The specimens were dried by the critical point method, sputter-coated with a 30 nm gold layer and observed in a Jeol JSM 35 scanning electron microscope.

The hearts of two dogfish were immersion-fixed overnight in 1% glutaraldehyde and 2% paraformaldehyde in elasmobranch buffer, washed, post-fixed in 1% OsO_4 for 2 h at 4° C, washed, dehydrated and embedded in Araldite 502. Semithin and ultrathin sections were obtained in an Ultracut E Reichert-Jung ultramicrotome. Semithin sections were stained with toluidine blue, while ultrathin sections were stained with lead citrate and uranyl acetate, observed and photographed in a Philips 300 transmission electron microscope.

RESULTS

Three layers can be distinguished in the wall of the dogfish sinus venosus, a subepicardial collagen-rich layer, a muscular layer, and a subendocardial neural layer (Fig. 1), constituted of intramural ganglion cells and bundles of nerve fibres. Ultrathin sections show that the fibres are constituted of granule-containing nerve fibres, and reveal the presence of endocardial discontinuities (Fig. 2). We have observed that some nerve fibres, in the proximity of the endocardial pores, show abundant clear vesicles, smaller than the dense core, membrane-bound granules.

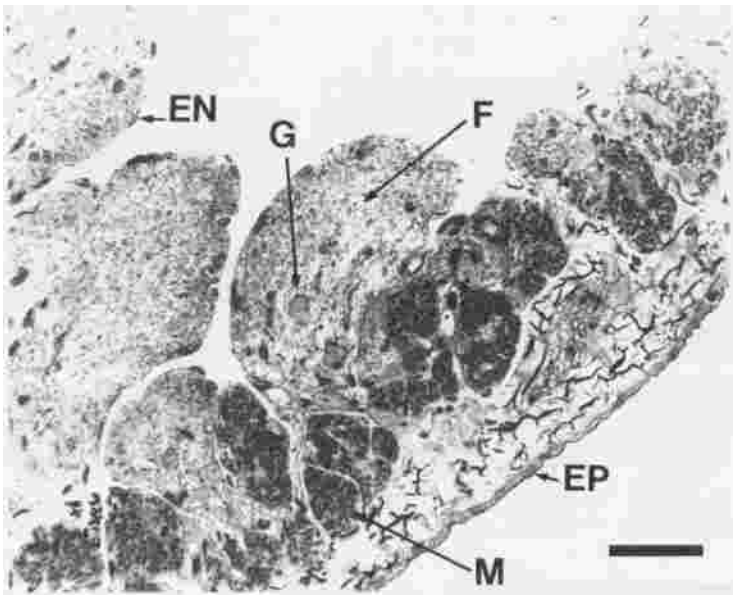

FIG. 1. Semi-thin section of the sinus venosus showing its organization. Below the epicardium (EP) there is a layer of thick collagen fibres and a muscular layer (M). The innermost layer is constituted of large bundles of unmyelinated granule-containing fibres (F) lined by the endocardium (EN). Ganglion cells (G) can also be seen. Bar=40 μm.

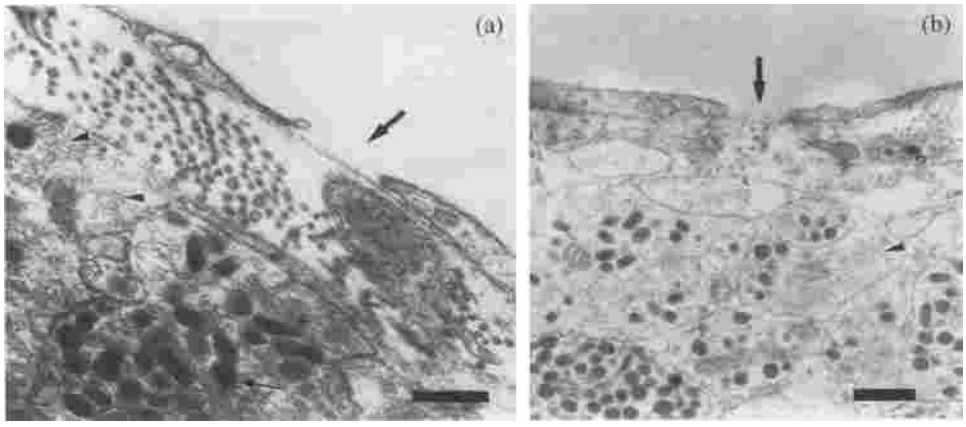

FIG. 2. Transmission electron microphotographs at the level of an endocardial pore (large arrows). Note the presence of basal lamina, the wide collagen-rich subendocardial space, and the abundance of fibres containing membrane-bound electron dense vesicles [small arrow in (a)] and other clear vesicles (arrowheads). Scale bars (a) 400 nm; (b) 1 μm.

Most of the luminal surface of the dogfish sinus venosus is covered by large bundles of nerve fibres, bulging in the cardiac cavity [Fig. 3(a)]. The caudal-most bundles are elongated, about 1–2 mm long and 100–135 μm wide. The cephalic ones are semi-spherical, between 80–300 μm in diameter.

Endocardial pores, can be seen in the endocardium lining the bundles [Fig. 3(b) (d)], but they are absent in other parts of the sinus venosus, such as in the spaces between the bundles, the openings of the ducti Cuvieri and the

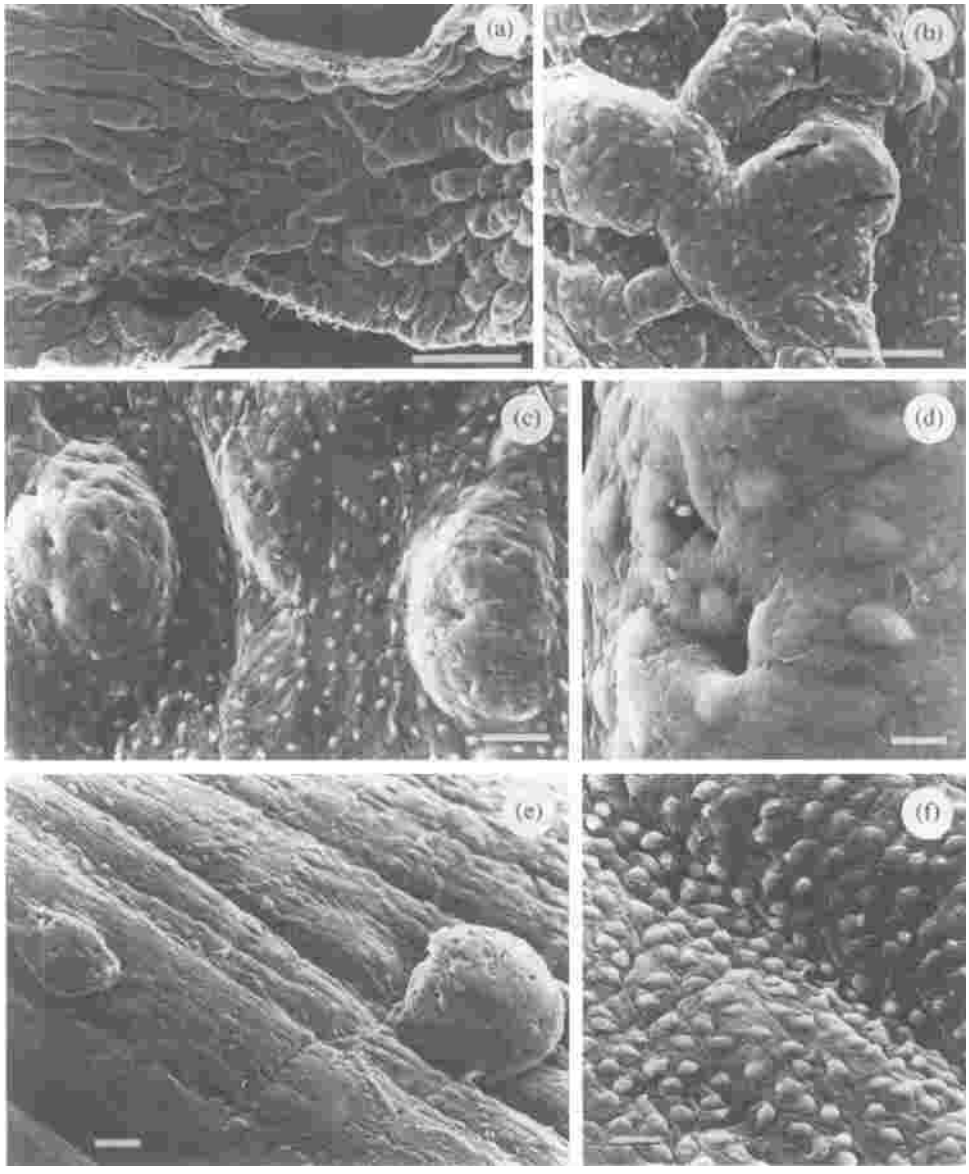

FIG. 3. (a) Low magnification scanning microphotograph of the dogfish sinus venosus seen from its luminal side. Large cords protruding in the lumen are present in the caudal region of the sinus (left), while more rounded bundles are present in the cephalic areas, closer to the sinoatrial valve (right). Scale bar, 0·5 mm. (b) Cords and bundles observed at higher magnification. Some large pores are already visible (arrows). Scale bar, 100 μm. (c) Two isolated bundles showing pores on their surface. Note the absence of pores in the endocardium which does not cover the bundles. Scale bar, 50 μm. (d) High magnification view of two endocardial pores. Scale bar, 10 μm. (e) Two small bundles on an atrial trabecula, near the atrioventricular canal. Note the endocardial pores on both bundles. Scale bar, 40 μm. (f) A view of the dorsal surface of the sinoatrial valve, in its sinusal face, which is devoid of nerve bundles. Endocardial pores are absent. Scale bar, 20 μm.

suprahepatic veins [Fig. 3(c)]. The endocardium of the sinoatrial valve is also devoid of pores [Fig. 3(f)], except for its sinusal and ventral face, where some nerve bundles are present.

The size of the pores was measured in high-magnification photographs. The mean diameter was 3·2 μm (s=1·14, n=14) with a range of 1·5–5·0 μm. The pore density was also calculated on photographs of several hearts. On a total surface of 157 000 μm^2 of endocardium covering the bundles, 129 pores were counted. This gives a density of 822 pores mm^{-2}, i.e. 1 pore per 1217 μm^2. These values are not accurate estimations, since the shrinkage of the tissue after dehydration was not considered and the density was not measured on a flat surface.

Some bundles were also observed on the atrium wall [Fig. 3(e)]. They are smaller than those of the sinus venosus (about 50–150 μm in diameter). The endocardium covering these bundles also shows endocardial pores. However, pores were not observed on the atrial trabeculated myocardium.

DISCUSSION

The large size of the endocardial pores of the dogfish discounts them from being considered as transendothelial channels or fenestrae. Transendothelial channels created by chains of fused vesicles have been described in the capillary endothelium of the shark brain (Hashimoto, 1972). However, strictures with dimensions down to 20 nm are found where a vesicle opens to the surface, and these strictures are often provided with a diaphragm (Bundgaard, 1980). Fenestrae occur in very thin endothelia associated with transporting epithelia. This is the case of the intestine, kidney tubules or choroid plexus. However, fenestrae are 40–60 nm in diameter (Bundgaard, 1980), that is, about two orders of magnitude smaller than the endocardial pores reported here. Probably, the small 'endothelial perforations' reported by Midttun *et al.* (1986) in the ventricular and atrial endocardium of the pike *Esox lucius* L. are true fenestrae, although the authors state that some of the smaller ones could be plasmalemmal vesicles. Another SEM study on the endocardial cells of a teleost, *Pollachius virens* L., including those of the sinus venosus, has not shown pores of fenestrations (Leknes, 1985).

The endocardial pores of the dogfish fit into the category of the large gaps (several hundreds of nanometres) between endothelial cells, which have been reported at the venular end of the microcirculation in response to inflammation or after treatment with histamin, snake venom, serotonin, bradykinin and prostaglandin (Bundgaard, 1980). This author states that the rarity of large endothelial pores makes it rather unlikely that they could be found systematically and identified. Thus, the sinusal endocardium of the dogfish might offer a unique opportunity for the study of transport across large endothelial pores.

Endocardial pores have also been described in the embryos of several vertebrate species (Pexieder, 1981).

Atrial bundles constituted of a few ganglion cells and many granule-containing nerve fibres similar to those of the sinus venosus have already been reported by Muñoz-Chápuli *et al.* (1994). The presence of endocardial pores on these atrial bundles makes evident an anatomical association and presumably a functional connection between ganglion cells, granule-containing fibres and endocardial pores.

We think that the presence of the endocardial pores is functionally related to the granulated fibres which accumulate in the subendocardial areas, forming

cords and bundles. We have recently demonstrated a distinct substance P-like immunoreactivity in these fibres as well as in most intramural ganglion cells (Muñoz-Chápuli *et al.*, 1994). A tachykinin-related peptide, alone or together with other substances, is thought to be released into the blood stream from these neuroendocrine cells and fibres through the endocardial pores.

The higher frequency of clear vesicles in the granulated fibres close to the pores was already noted by Helle *et al.* (1983). These vesicles might be secretory granules depleted of their content, thus supporting a role for the pores to allow diffusion from the extracellular matrix of the subendocardial space to the blood stream. Therefore, the contention that a neuroendocrine system is located in the sinus venosus of the dogfish (Muñoz-Chápuli *et al.*, 1994) receives some support from the structure of the sinusal endocardium.

We thank Juan José Canca and Gregorio Martín for expert technical assistance; Borja Fernández for valuable help; and Paul Alen for the English version. This study was supported by the research grants PB89-0577 and PB92-0413 from the DGICYT, Spanish Ministry of Education and Science.

References

Berge, P. I. (1979). The cardiac ultrastructure of the *Chimaera monstrosa* L. (Elasmobranchii: Holocephali). *Cell Tissue Research* **201,** 181–195.

Bundgaard, M. (1980). Transport pathways in capillaries – in search of pores. *Annual Review of Physiology* **42,** 325–336.

Hashimoto, P. H. (1972). Intracellular channels as a route for protein passage in the capillary endothelium of the shark brain. *American Journal of Anatomy* **134,** 41–58.

Helle, K. B., Miralto, A., Pihl, K. E. & Tota, B. (1983). Structural organization of the normal and anoxic heart of *Scyllium stellare*. *Cell Tissue Research* **231,** 399–414.

Kisch, B. & Philpott, D. E. (1963). Electron microscopy of the heart of fish. II. The heart of selachians (dogfish and torpedo). *Experimental Medicine and Surgery* **21,** 54–74.

Leknes, I. L. (1985). A scanning electron microscopic study on the heart and ventral aorta in a teleost. *Zoologische Anzeiger* **214,** 142–150.

Leknes, I. L. & Saetersdal, T. S. (1980). On the ultrastructural organization of the sinus venosus in *Chimaera monstrosa* L. (Elasmobranchii: Holocephali). *Zoomorphology* **96,** 183–189.

Midttun, B. & Okland, S. (1986). Surface morphology of endocardial endothelium of the pike *Esox lucius* L. (Teleostei: Esocidae). *Zoologische Anzeiger* **216,** 257–266.

Muñoz-Chápuli, R., de Andrés, V. & Ramos, C. (1994). Tachykinin-like immunoreactivity in the sinus venosus of the dogfish (*Scyliorhinus canicula*). *Cell Tissue Research* **278,** 171–175.

Pexieder, T. (1981). Prenatal development of the endocardium: A review. *Scanning Electron Microscopy* **1981,** 223–253.

Saetersdal, T. S., Sorensen, F. & Myklebust, R. (1975). Granule containing cells and fibres in the sinus venosus of elasmobranchs. *Cell Tissue Research* **163,** 471–490.

Santer, R. M. (1985). Morphology and innervation of the fish heart. *Advances in Anatomy, Embryology and Cell Biology* **89,** 1–102.

Yamauchi, A. (1980). Fine structure of the fish heart. In *Heart and Heart-like Organs* (Bourne, G. H., ed.), pp. 119–148. New York: Academic Press.