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Distribution, structure and projections of the frog intracardiac neurons

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ABSTRACT

Histochemistry for acetylcholinesterase was used to determine the distribution of intracardiac neurons in the frog *Rana temporaria*. Seventy-nine intracardiac neurons from 13 frogs were labelled iontophoretically by the intracellular markers Alexa Fluor 568 and Lucifer Yellow CH to determine their structure and projections. Total neuronal number per frog heart was (Mean ± SE) 1374 ± 56. Largest collections of neurons were found in the interatrial septum (46%), atrioventricular junction (25%) and venal sinus (12%). Among the intracellularly labelled neurons, we found the cells of unipolar (71%), multipolar (20%) and bipolar (9%) types. Multiple processes originated from the neuron soma, hillock and proximal axon. These processes projected onto adjacent neuron somata and cardiac muscle fibers within the interatrial septum. Average total length of the processes from proximal axon was 348 ± 50 μm. Average total length of processes from soma and hillock was less, 118 ± 27 μm and 109 ± 24 μm, respectively. The somata of 59% of neurons had bubble- or flake-shaped extensions. Most neurons from the major nerves in the interatrial septum sent their axons towards the ventricle. In contrast, most neurons from the ventral part of the interatrial septum sent their axons towards the atria. Our findings contradict to a view that the frog intracardiac ganglia contain only non-dendritic neurons of the unipolar type. We conclude that the frog intracardiac neurons are structurally complex and diverse. This diversity may account for the complicated integrative functions of the frog intrinsic cardiac ganglia.

1. Introduction

Frog is a convenient animal model to study the electrophysiology, development and structure of the intracardiac neurons (McMahan and Kuffler, 1971; Heathcote and Sargent, 1985; Clark et al., 1990; Selyanko et al., 1991). Compared to mammalian heart, the intracardiac neurons of frog lie in a position that is more accessible for researchers. Most intracardiac neurons of mammals are embedded in atrial fat pads beneath thick epicardium (Pauza et al., 2002; Batulevicius et al., 2003; Saburkina et al., 2010; Rysevaite et al., 2011a). In contrast, major clusters of the frog intracardiac neurons are easy accessible in situ by microelectrodes within intact preparations of very thin and translucent interatrial septum (McMahan and Kuffler, 1971). Living intracardiac neurons of frog may be observed by both Nomarski optics and fluorescence microscopy (McMahan and Kuffler, 1971). Finally, frog intracardiac neurons survive well in explants of atria for prolonged time at room temperature (Heathcote, 1996).

Heart of frog is innervated by the branches of vagosympathetic trunks that extend from the venal sinus into the interatrial septum and then into ventricles (Burnstock, 1969). Preganglionic parasympathetic neurons are located in the brainstem at the nucleus of the glossopharyngeal–vagal complex, and they synapse to the ganglionic nerve cells inside the frog heart (Pardini and Wurster, 1986). This nerve cell system is considered as relatively simple, because neurons in frog heart are innervated by only one or a few preganglionic axons (Baluk and Fujiwara, 1984; Gibbins and Morris, 2006). Consequently, frog intracardiac ganglia have been used as a model to study the synaptic transmission, synapse formation and effects of preganglionic denervation (Roper and Taylor, 1982; Streichert and Sargent, 1989; Horch and Sargent, 1996).

Although the locations of frog intracardiac neurons in the venal sinus, interatrial septum and atrioventricular junction have been known long ago (Burnstock, 1969; Fye, 1987), understanding of the structure of frog intracardiac ganglia is incomplete. Histochemical and electron microscopic methods did not allow the researchers to visualise the morphology of the individual intracardiac neuron including the dendritic tree and axonal projection (Baluk and Fujiwara, 1984; Heathcote and Sargent, 1987a, 1987b; Clark et al., 1994). So far, only a few studies are known to explore the structure of the frog intracardiac neurons by the intracellular injection of markers. McMahan and Kuffler (1971) injected Procion Yellow into intracardiac

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neurons of the frog *Rana pipiens*. Heathcote and Sargent (1985) filled the intracardiac neurons with horseradish peroxidase in the frog *Xenopus laevis*. These studies have shown that intracardiac neurons of adult frog are unipolar, non-dendritic and have non-branching axons (McMahan and Kuffler, 1971; Heathcote and Sargent, 1985). Up to date we found no intracellular injection study to describe the structure of the intracardiac neurons in the frog *Rana temporaria*. Furthermore, we found no data on the quantitative distribution and regional projections of the frog intracardiac neurons.

Our study was initiated for a better understanding of the structure of the frog intracardiac ganglia. The goals of this study were 1) to determine the quantitative distribution and projections of the intracardiac neurons, and 2) to review the structure of the intracardiac neurons in frog *Rana temporaria*. The presented findings would facilitate the use of frog as a model in further immunocytochemical and functional studies of the intracardiac ganglia.

2. Material and methods

We used 53 adult (20–35 g in weight) *Rana temporaria* frogs. The experiments were done in accordance to local and state guidelines for the use of experimental animals. The frogs were euthanized by decapitation.

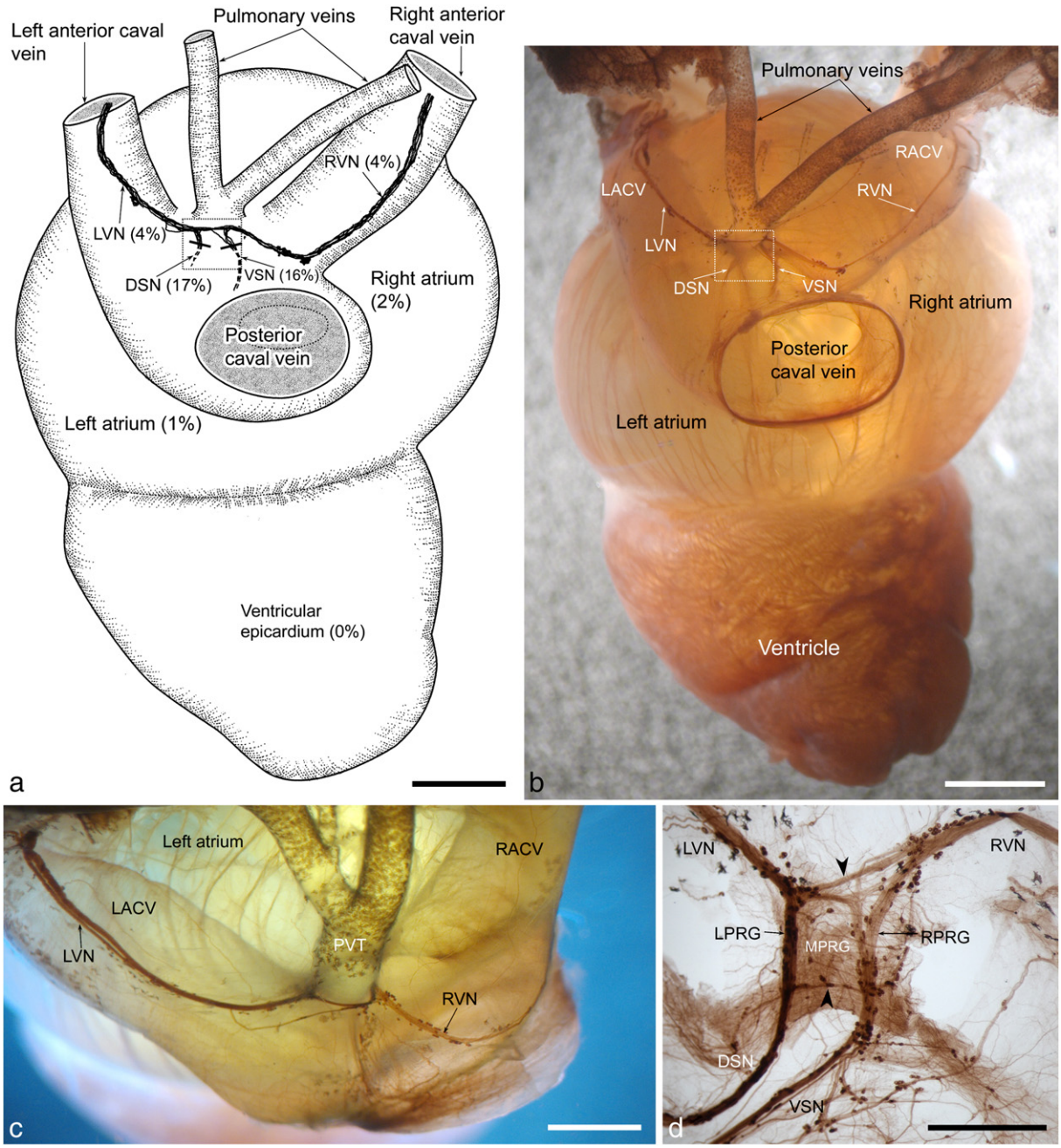

Fig. 1. Reconstruction (a) and macrographs (b–d) of the dorsal view of pressure-inflated frog heart stained for acetylcholinesterase. Macrograph in panel b was used for reconstruction, while the panels b–d represent three different hearts. Boxed area in a–b represents the Remak's ganglion, and it is enlarged in panel d. Numbers indicate the percentage of the total number of neurons in the region. Note the right (RVN) and left (LVN) venal nerves proceeding into the Remak's ganglion as well as ventral (VSN) and dorsal (DSN) septal nerves originating from the Remak's ganglion. LACV, left anterior caval vein; LPRG, left part of Remak's ganglion; MPRG, middle part of Remak's ganglion; PVT, pulmonary vein trunk; RACV, right anterior caval vein; RPRG, right part of Remak's ganglion. *Bars* 3 mm (a–b), 1.5 mm (b), 1 mm (c).