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Immunohistochemical characteristics of neurons in nodose ganglia projecting to the different chambers of the rat heart

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ABSTRACT

Despite the contribution of nodose ganglia neurons to the innervation of the heart being the subject of several studies, specific neuronal subpopulations innervating the four different chambers of the heart have not been distinguished.

In our study, the application of Fast Blue-loaded patch to the epicardial surface of different chambers of the rat heart (the right or left atrium or the right or left ventricle) resulted in labeling of discrete populations of immunohistochemically diverse neurons. About one half (55%) of these neurons showed immunoreactivity for the 200-kDa neurofilament protein (marker of myelinated neurons), with a higher proportion of positive staining among neurons projecting to the left than to the right ventricle. Isolectin B4 immunoreactivity (characteristic for a subset of nonmyelinated non-peptidergic neurons) was more abundant among neurons projecting to the right side of the heart (right atria and right ventricles) compared to the left side (23% vs. 16%). Calretinin immunoreactivity (possible marker of mechanosensitive neurons) was significantly higher among neurons projecting to the ventricles than among those projecting to atria (36% vs. 11%). These findings reveal that chambers of the rat heart are innervated with immunohistochemically different subpopulations of neurons from the nodose ganglia.

1. Introduction

The heart is a well innervated visceral organ supplied by heterogeneous populations of neuronal projections. Afferent neurons originating from nodose and dorsal root ganglia (DRG C7-T7) are the two main sources of cardiac afferent innervation (Kuo et al., 1984; Quigg et al., 1988; Hopkins and Armour, 1989; Hoover et al., 2008). The largest number of primary heart-projecting somata is located in the nodose ganglia (Hopkins and Armour, 1989).

The contribution of nodose ganglia neurons to innervation of the heart was the focus of interest of many studies that used various retrograde and anterograde tracers (Hopkins and Armour, 1989; Quigg, 1991; Cheng et al., 1997; Hopkins et al., 1997; Corbett et al., 2005; Hoover et al., 2008).

Retrograde tracers were usually applied through a surgical opening made in one or more intercostal spaces to allow injecting into the cardiopulmonary nerves or directly into different regions of the heart. Interestingly, when two different neuronal tracers were injected into different parts of the heart simultaneously, none of the neurons in the heart associated extracardiac ganglia was double labeled (Hopkins et al., 1997).

Regardless of the fact that atria and ventricles have distinct developmental, structural and physiological properties (Forbes and van Neil, 1988; Chen and Fishman, 2000; Zhao et al., 2002), and the fact that there are even structural differences between the right and left atria (Forbes et al., 1990) and between the right and left ventricles (Kondo et al., 2006), to the best of our knowledge there are no studies comparing nodose afferent neurons projecting to different chambers of the heart.

The goal of this study was to identify neurons of nodose ganglia that project to the different parts of the rat heart (to the right atria or ventricle or to the left atria or ventricle), to compare them quantitatively and examine their morphological and immunohistochemical properties.

To achieve precise and reproducible neuronal labeling, we decided to apply the neuronal tracer Fast Blue (FB) in the form of a patch, as previously described by Corbett at al. (1999), using a modification of the abdominal approach to the rat heart (Huikeshoven et al., 2000). The FB-labeled neurons were characterized by immunohistochemical staining for isolectin B4 (IB4) and 200-kDa neurofilament protein (N52), previously described as a marker for a subset of nonmyelinated non-peptidergic and myelinated neurones, respectively (Lawson and Waddell, 1991; Wang et al., 1994). For further characterisation of labeled neurons, we also used calretinin (CALR) since it has been reported that a significant number of nodose ganglia neurons contain calcium-binding proteins (Ichikawa and Helke, 1995, 1996) and specifically CALR (Ichikawa et al., 1991). CALR was previously described (Kressel and Radespiel-Troger, 1999) as a possible marker

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of mechanosensitive afferents, but little is known about the presence and/or distribution of this protein among cardiac projecting neurons.

2. Materials and methods

2.1. Surgical procedures and tracer application

The experimental protocol was approved by the Ethics Committee of the University of Split School of Medicine, and laboratory animal guidelines (European Communities Council Directive of 24 November 1986) were followed and applied in all experiments. Female Sprague–Dawley rats (160–180 g, $n = 13$) were divided into 4 groups (one group consisting of four animals) based on the location of the patch placement on the heart surface. Rats were anaesthetized with an intramuscular injection of a mixture containing ketamine (Ketaminol, Intervet, EU, 60 mg/ml) and xylazine (Xylapan, Vetoquinol, Bern, Switzerland, 50 mg/ml). Surgical procedures were performed with the aid of a surgical microscope (Leica, M520 MC1, Switzerland) with animals kept warm on a heat mat during the operation. After intubation (with an 18-gauge arterial catheter), an abdominal approach to the rat heart was performed as previously described by Huikeshoven at al. (2000). Slight modification was made during the incision of the diaphragm, i.e. it was cut with scissors along the midline (1/3 of the incision included the central tendinous part and 2/3 involved the muscular part). The animals were linked to a positive pressure respirator (SAR 830, CWE Inc, USA) set on 56 breaths/min with the inspiratory pressure set between 8 and 10 cm/H_2O. The thin pericardium was torn apart so that the surface of the heart could be clearly exposed.

Tracer application technique was based on the method of Corbett et al. (1999), but using a different tracer. Shortly, a patch loaded with 0.1 μg of FB powder (EMS-Chemie, Gross-Umstadt, Germany; gift of Dr C. Anderson, University of Melbourne) was applied to only one of four chambers of the heart (left atrium or ventricle, right atrium or ventricle) in all rats from a specific group (Fig. 1A, B). All patches were placed on the ventral surfaces of the chambers. The patch was made of Micropore surgical tape (TM, St. Paul, USA) impregnated with silicone spray on its outer side (in order to prevent tracer leakage) and had the central quarter of its surface covered by a thin layer of FB powder on its 'sticky' surface. The size of the patch was 4 mm^2 and the FB powder covered only its middle section (Fig. 1B, C). Following its application to the surface of the heart, the patch was fixed in place by applying 1 μl of instant tissue glue (Super Bond, Henkel, Ireland) over the edge of the patch with the aid of a micropipette. The diaphragm was then sutured, starting with the muscular part, working towards the central tendon of the diaphragm. Before the final suture was tightly closed, air was expelled from the pleural cavity by full re-expansion of both lungs. The rats were removed from the respirator and extubated following the recovery of spontaneous breathing. Muscles and skin of the abdominal wall were sutured, and the rats were closely monitored in a warm environment during the recovery period that lasted between 3 and 5 h.

2.2. Tissue preparation

One week after the application of tracer, the animals were re-anaesthetized with the combination of anaesthetics described above, and perfused through the ascending aorta via the left ventricle with saline followed by 300 ml of Zamboni's fixative (4% paraformaldehyde and 15% picric acid in 0.01 M phosphate buffered saline (PBS) at pH 7.4). The heart, lungs and spinal cord, as well as, the nodose and dorsal root ganglia (C6-T8) were removed bilaterally and post-fixed for 2 h in the same fixative used for perfusion. Afterwards, tissues underwent long washes in 0.01 M PBS. Sections of lung, heart and spinal cord were examined under the microscope using UV settings to evaluate the patch position and to check for leakage. To confirm labeling of the heart-projecting neurons, ganglia were also examined using the same filter settings. DRGs were examined only in order to see the specific distribution of heart-projecting neurons. All ganglia were transferred to 30% sucrose overnight for cryoprotection, snap-frozen and stored at −80 °C until processing.

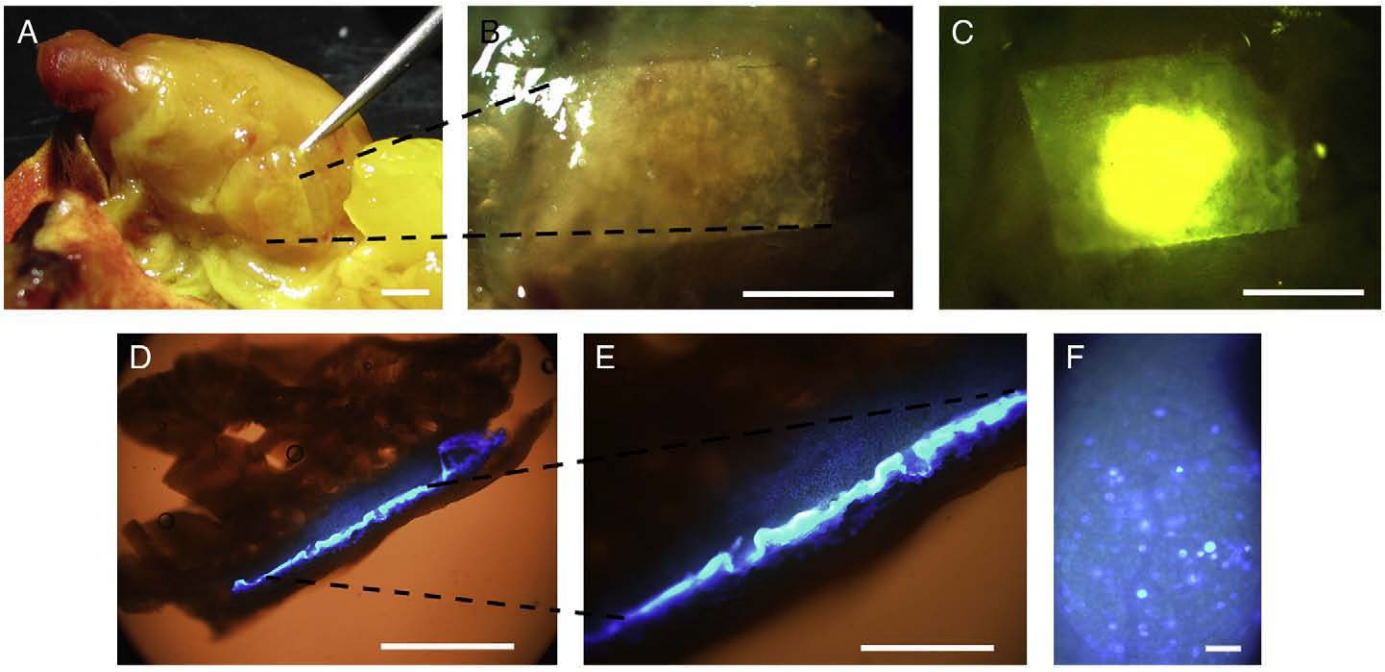

Fig. 1. Visualization of the patch-attachment area on the left atrium using normal light (A and B) and UV light setting (C, D and E) and demonstration of retrograde labeling of neurons. The patch is attached to the middle of the left atrium (A and B) and FB fluorescence is limited to its center (C). Cross sectioning of the patch area demonstrates its firm attachment and intense fluorescence penetrating the atrial wall (D and E). Numerous retrogradely labeled neurons in whole-mount preparation of nodose ganglion (F). Scale bar = 2 mm on A, 1 mm on B–D, 400 μm on E and 100 μm on F.